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# Molecular Characterization of Infectious Bursal Disease Virus Isolated from Naturally Infected Broiler Chickens in Erbil, Iraq

**Ahmed I. Ahmed***

*Animal Resource Department, College of Agriculture Engineering science, Salahaddin University, Erbil, Iraq*

***Correspondence**:
ahmed.ahmed1@su.edu.krd

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## A B S T R A C T

The infectious bursal disease (IBD) is a highly contagious and immunosuppressive disease of broiler chickens and the development of a new genetic variant of the virus is responsible for major economic losses in the poultry industry. For this purpose, it was essential to isolate the molecular characterization of the virus from vaccinated broiler in Erbil, Iraq. Clinically, the infectious bursal disease is characterized by high mortality (10-15%) with hemorrhagic lesions on the breast and thigh muscles, hemorrhagic and edematous bursa of diseased chickens. In this study, the Bursa of Fabricus (BF) samples were collected between June 2018 and January 2019. Histopathological changes of the bursal sections showed existence of the cystic vacuolation of the lymphoid follicles with leukocytes infiltration as pathognomic features for IBD virus infection; and homogenates samples inoculated in chorioallantoic-membrane showed mortality in the second passage with varying degrees of hemorrhages. Agar gel precipitation test (AGPT) was positive with specific antisera. Reverse transcription polymerase chain reaction (RT-PCR) and nucleotide sequence analysis of five fragments in the hypervariable region of *VP2* gene revealed transition and transversion changes. Among the five recent IBD virus isolates, the rate of identity was approximately 99% as compared with the very virulent IBD virus from Iran (ID: DQ785171.1). Phylogenetic analysis revealed that the five isolates were closely related to the Asian group with a different percentage ranged from 98-99% while it was 97% in the European group. The local isolate of the virus was registered in the Genebank under the accession number MN48052.1. In conclusion, the isolated IBDVs belong to a very virulent group. In addition, this study demonstrates the spread of this virulent virus to poultry industries in Erbil, Iraq. Further widespread surveys could help in delivering more information on the virus variability and might assist in designing novel vaccines for this pathogen.

**Keywords**: Infectious bursal disease virus, *VP2* gene, agar gel precipitation test, RT-PCR, chorioallantoic membrane

## INTRODUCTION

Infectious bursal disease (IBD) is an important immunosuppressive disease of broiler chickens, and it causes depletion “premature B lymphocytes” in the bursa of Fabricius. It is caused by IBD virus (IBDV) which is responsible for the major economic losses in the poultry industry worldwide (1-3). The virus is a double-stranded

RNA virus that belongs to the genus *Avibirnavirus* of the family *Birnaviridae* (4). The genome of the IBDV is divided into two segments A and B. There is a large open reading frame 1 (ORF1) in segment A that encodes for a polyprotein which auto-catalytically splices into the viral proteins: VP2 (48 kDa), VP3 (32–35 kDa) and VP4 (24 kDa) that encode for viral protease (2, 5). Furthermore, the virus has two serotypes, namely serotypes 1 and 2. Serotype 1 virus is pathogenic in chickens and causes lytic infection of immature B lymphocytes (6, 7). The incubation period is short, and clinical signs of the disease are recognized within 2 to 3 days after exposure; usually occurs in 3 to 6-week old chickens (8). Antigenic variants and hypervirulent strains of IBDV are the causes of high mortality. Factors like the virulence, dose of the strain, breed, age of the chickens, maternal immunity state and passive immunity may be related to mortality too (9). The IBD is caused by the serotype 1 viruses that can yield several forms, and have commonly been observed as subclinical, classic virulent, antigenic variant or very virulent cases (8).

In Iraq, previous studies (10-13) stated that IBDV infection in commercial and non-commercial poultry flocks resulted in a high mortality rate and outbreaks. To control IBD in Iraq, this requires performing research at the molecular level to characterize the circulating field strains of IBDV. This would decrease the spread of virulent strains in vaccinated flocks as this would help in selecting appropriate and effective vaccine strains (14). The use of molecular techniques for the detection and identification of IBDV strains has increased in recent years. The *VP2* gene is commonly studied because it encodes for the major protective epitopes, contains determinants for pathogenicity, and is highly variable among strains (15, 16).

The IBDV continues to impact the poultry industry in Iraq. This study aimed to find out the molecular characterization of IBDV locally isolated from broiler chicken outbreaks between June 2018 and 2019 in Erbil using an RT-PCR, routine isolation, and sequence analysis of the hypervariable region (HVR) part of the *VP2* gene, along with histopathological examination.

## Materials and Methods

### Sample Collection

Diseased broiler chicken cases were sent to the laboratory section of YARA-Barz Company. These cases were suffering from high mortality and acute phase of IBD. The study was carried out between june2018 and January 2019 when all selected flocks were vaccinated with the live attenuated lasota vaccine strain (Intervet) via drinking water. After necropsy of the bursa of fabricus (BF), lesions was revealed as mild hemorrhage, edema, and hypertrophic bursae. These samples were collected and subjected to a rapid test for IBDV antigen detection using Antigen Rapid IBDV Ag Test kits (Bionote company, Korea). The BF samples (2-5 of each flock) were collected from 25 broiler flocks aged 21-30 days from deferent districts of (A): Bardarash-Duhok, (B): Barzan-Erbil, (C): Khabat-Erbil, (D): TaqTaq-Erbil and (E): Chamchamal-Sulaymaniyah, Iraq. The BF was aseptically removed and collected properly in 50 ml sterile plastic Falcon tube and stored in a freezer (-18 °C) and finally 52 samples were chosen. The BF samples were organized according to sub district Valleys after thawing frozen organs at room temperature; and pooled according to subdistricts.

### Virus Isolation on Embryonated Chicken Eggs

The pooled BF samples were chopped into fine pieces corresponding to 2 gm, and a homogenization process was performed separately as described by researchers (18,19,20). Homogenates of BF were mixed in lysis buffer (pH 8.0) and Streptomycin and Penicillin (10000 mg/mL and 10000 IU, respectively-Gibco company) were added. The samples were next centrifuged at 14000 x g in 4 °C for 20 min then the supernatant was collected carefully; filtered through a 0.22 µM filter and divided into two equal suspension of 1 mL each. One hundred µL from the supernatant fluid of each sample was inoculated on the chorioallantoic membrane (CAM) of local 9-10 day-old embryonated chicken eggs (ECEs) from Aya hatchery in Erbil. Three serial passages were performed. Then the inoculated eggs were incubated at 37 °C with candling daily. The inoculated eggs with severe lesions of hemorrhage on the CAM were harvested after 96 hours post-inoculation (21). Non-inoculated eggs were always involved as a negative control.

### Agar Gel Precipitation Test (AGPT)

AGPT test was carried out according to (22). The test was done to determine the presence of IBDV using standard specific reference antisera, purchased from Animal Health Research Institute, Netherlands, against positive viral antigen from CAM homogenate of inoculated ECE. The collected BF was also prepared as an antigen based on Hirai et al. instructions (23) to confirm the isolation of IBD viruses.

### Viral RNA Extraction

Total RNA was extracted from five pooled homogenate samples using Quick-RNA™ Viral Kit (Catalog No. R1034 & R1035 ZYMO RESEARCH, USA). One hundred µL of homogenate part of each sample was mixed well with 100 µL RNA Shield™ (2× concentrate). The procedure was applied according to the manufacturer's protocol.

### Reverse Transcription Reaction

Four µL of the purified RNA was subjected for reverse transcription procedures using Prime ScriptTM RT reagent Kit (Perfect Real Time) (Catalog. No. RR037A, Takara Bio

Inc, Japan). This kit was used for the reverse transcription reaction, and it contained PrimeScriptTM RTase for efficient cDNA template synthesis, RT-PCR reagent, and SYBR® Premix. The RT products were diluted and then stored at -20 °C for further PCR reactions.

A set of primers designated by Shakor (24) was used for the subsequent sequence analysis using forward and reverse PCR primers for the amplification of a hypervariable region (HVR) of *VP2* gene sized 620 bp [Forward primer, 5'-CAGGTGGGGTAACAACAATCA-3', and Reverse primer, 5'-CGGCAGGTGGAACAATG-3'].

## PCR Conditions

The RT-PCR reactions were carried out according to the manufacturer's instructions using Maxime PCR PreMix kit (i-Taq) 20 µl run PCR (Catalog No. 25025, iNtRON Biotechnology Inc., Korea). Specific primers were used for gene amplification. After amplification, 5 µL of PCR products were run on gel electrophoresis and visualized after staining. Sequencing of the gene was performed by Macrogen (Korea). Sequence alignment and homology search was conducted using the Basic Local Alignment Search Tool (BLAST) program, which is available at the National Center for Biotechnology Information (NCBI), and BioEdit program. Phylogenetic trees were created using the neighbor-joining algorithm based on the pairwise nucleotide differences in the (HVR) of *VP2*, method available in MEGA6 (25), employing the maximum composite likelihood model of nucleotide substitution and 1000 bootstrap replications.

## RESULTS AND DISCUSSION

Infectious bursal disease virus still imposes threats in poultry industries worldwide due to improper uses of vaccines and lack of vaccine evaluation (11, 12). Investigations of clinical signs and postmortem lesions were done upon receiving diseased broiler chickens in the laboratory section. The studied flocks were suffering from clinical signs such as ruffled feather; watery diarrhea with high mortality 10-15% within 2-3 days. The postmortem lesions of sacrificed diseased chickens showed hemorrhages on breast and thigh muscles, hemorrhagic and edematous BF, petechial hemorrhages in proventriculus mucosa. Equivalent lesions were also observed by Sara and Arafa (26).

Histopathological findings of BF stained sections of sacrificed naturally infected chicken with field IBDV showed cystic vacuolation of the lymphoid follicles with leukocytes infiltration (Figure1 a and b). Bursal lymphoid follicles were atrophied with inter-follicular edema, follicular necrosis and lymphoid depletion with a cyst-like space containing eosinophils (Figure 1 c). The severity of lesions in the BF is an indicator and related to the degree of pathogenicity of the virus in naturally infected chickens, These observations were also indicated by Raji (27), and BF changes with lymphoid follicles depletion in this study were similar to those previously reported due to infection with very virulent IBDV (11, 28).

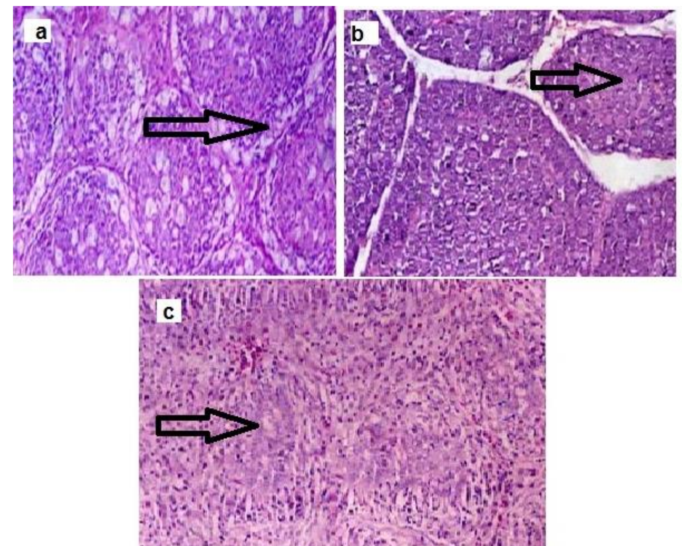


**Figure 1.** Histopathologic sections of bursae fibrisous from chicken naturally infected with IBDV (H&E 100×): a. Vacuolation of cystic lymphoid follicles and depletion with leukocytes infiltration. b. Atrophied bursal lymphoid follicles with inter-follicularedema. c. Complete follicular necrosis with cyst-like space containing eosinophils

## Viral Isolation and Confirmation

Data of five bursa homogenates of the pooled samples were inoculated in CAM and the result showed mortality in a second passage with a varying degree (mild to severe) of hemorrhages on CAM and edema on the skin of embryos (Table 1). The isolation of IBDV on CAM was emphasized and positive results with specific antisera in AGPT were revealed. It was well known that *VP2* was the antigenic determinant and commonly used for the molecular characterization of IBDV (3, 29); *VP2* was also used for the identification and analysis of IBDV variants (30, 31).

The second half of homogenizes from five pooled samples was the RNA extraction, which was subjected to RT-PCR. The RT-PCR is a molecular tool frequently applied for IBD diagnosis (32) where fragments of the genome (essentially the hypervariable *VP2* region) have to be amplified followed by sequencing analysis and phylogenetic tree comparisons. This actually represented the valuable molecular alternative for the classification of IBDV strains (33).

The amplified fragments were visualized on gel electrophoresis after staining of DNA by Red stain (Figure 2). The steps of DNA extraction were achieved according to Vogelstein and Gillespiet (34). Then, the purified amplified fragments were sent to Macrogen (Korea) to perform sequencing using the same primers used for amplification.

The sequence analysis of five positive amplified fragments is shown in Table 2. The nucleotide sequences of the fragments from *VP2* genes of IBDV were analyzed and

**Table 1.** Isolated IBDV from pooled samples and confirmation by AGPT

| Samples | Code of pooled samples | Lesions in inoculated ECE eggs | | | Results of AGPT |
|---|---|---|---|---|---|
| | | Passage 1 | Passage 2 | Passage 3 | |
| Duhok (Bardarash) | A | +++ | ++ | Nd | Positive |
| Erbil (Barzan) | B | +++ | ++ | Nd | Positive |
| Erbil (Khabat) | C | +++ | + | Nd | Positive |
| Erbil (Taq Taq) | D | +++ | + | Nd | Positive |
| Sulaimania (Chamchamal) | E | +++ | ++ | Nd | Positive |

+ = Mild lesions; ++ = moderate lesion; +++ = severe lesions of ECE; Nd = not done

compared with homologous sequences using BLAST. The nucleotide composition of the fragment revealed a number of substitutions (transversion and transition) (Table 2), indicating that there was a mutation in the genome that, could, increased the pathogenicity of the virus. The isolates were 99% identical to the extremely virulent IBDV (ID: DQ785171.1) previously isolated from Iran by Razmyar et al. (35).

The nucleotide sequences of five isolates differed in several positions. The changes revealed that isolate A had no amino acid change while the amino acid changed from threonine to serine and leucine to phenylalanine in isolate B. In addition to the latter, the isolates C and D showed changing the amino acid from glycine to alanine whereas, the isolate E showed changing the amino acids phenylalanine to leucine, serine to glycine, and asparagine to serine (Table 2). This came with what Xu and his team (36) stated that the slight difference in the HVR region of *VP2* gene resulted in immune evasion and outbreaks of IBDV in field conditions.

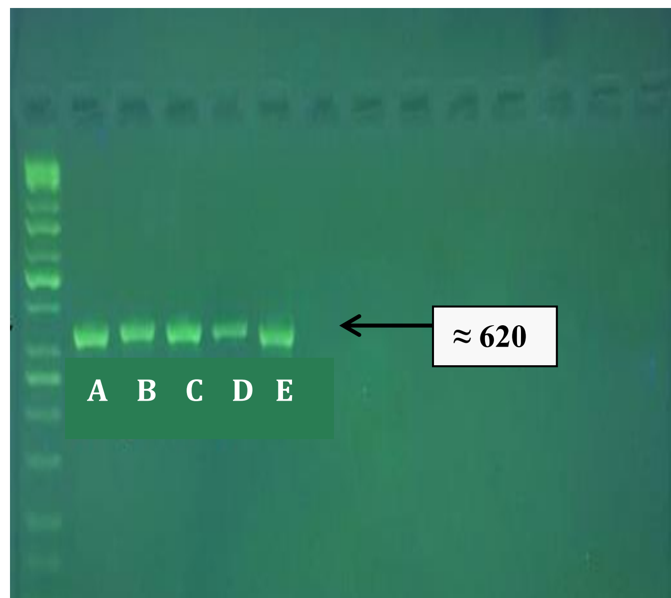


**Figure 2.** Gel electrophoresis of A= Duhok, B= Barzan-Erbil, C= Khabat-Erbil, D=TaqTaq-Erbil and E=Sulaymaniyah. Lane 1: DNA ladder (1000 bp), Lanes 2, 3, 4, 5, and 6: PCR products with the band sizes of approximately 620 bp stained by Red stain

**Table 2.** Transitions and transversions of nucleotide change percentage of *VP2* fragment

| No of sample | Type of substitution | Location | Nucleotide change | Amino Acid change | Predicted effect | Sequence ID | Identities | Source |
|---|---|---|---|---|---|---|---|---|
| A | Transversion<br>Transition<br>Transversion | 152<br>161<br>248 | GTC>GTG<br>CTT>CTC<br>GCC>GCG | Valine> Valine<br>Leucine > Leucine<br>Alanine> Alanine | Nonsense<br>Nonsense<br>Nonsense | DQ785171.1 | 99% | IBDv VP2 gene |
| B | Transversion<br>Transition<br>Transition | 214<br>261<br>275 | ACC>AGC<br>CTC>TTC<br>AAT>AAC | Threonine> Serine<br>Leucine>Phenylalanine<br>Asparagine>Asparagine | Missense<br>Missense<br>Nonsense | DQ785171.1 | 99% | IBDv VP2 gene |
| C | Transition<br>Transversion<br>Transversion | 161<br>248<br>349 | CTT>CTC<br>GCC>GCG<br>GGT>GCT | Leucine>Leucine<br>Alanine>Alanine<br>Glycine>Alanine | Nonsense<br>Nonsense<br>Missense | DQ785171.1 | 99% | IBDv VP2 gene |
| D | Transition<br>Transversion<br>Transversion | 344<br>361<br>362 | AAA>AAG<br>GGG>GCC<br>GGG>GCC | Lysine>Lysine<br>Glycine>Alanine<br>Glycine>Alanine | Nonsense<br>Missense<br>Missense | DQ785171.1 | 99% | IBDv VP2 gene |
| E | Transition<br>Transversion<br>Transition<br>Transition | 138<br>359<br>384<br>418 | TTT>CTT<br>GCG>GCT<br>AGT>GGT<br>AAC>AGC | Phenylalanine>Leucine<br>Alanine>Alanine<br>Serine> Glycine<br>Asparagine>Serine | Missense<br>Nonsense<br>Missense<br>Missense | DQ785171.1 | 99% | IBDv VP2 gene |

A phylogenetic analysis tree resulting from the analysis of the nucleotide sequences showed the relationship between the nucleotide sequence of this field isolates of IBDV and sequence divergence of different isolates in countries of origin stated elsewhere around the globe and available in GenBank (Table 4). The Phylogenetic trees revealed that the sequences of five isolates defined a different percentage of 99% similarity to Iran, Pakistan, India, and China, while 97% similarity to other viruses (Table 3, Figure 3). This comparison showed a very virulent IBDV (vvIBDV) circulated in the globe including Erbil, Iraq means that those isolates belong to Asian group. The variant of this part of the genome is critical, the nucleotide sequences of HVR are a major neutralizing antigenic domain and this region has been widely used for diagnostics and typing as variant, classic or very virulent among serotype-1 strains of IBDV (37). Regarding this change, the isolated vvIBDV may infect the vaccinated chickens due to this variation in the immunogenic profile (38). Several researchers have previously studied HVR of the *VP2* gene using pairs of the primer (33). The flocks considered in this study are at risk and have no information on the serological evaluation post-vaccination, as well as IBDV has the capability to break over high titers of maternal antibodies and causes disease; even the high levels of neutralizing antibodies that were produced by classical vaccine strains may not yield a sufficient protection against vvIBDVs due to this change. Similar findings were mentioned by Jenberie et al (39). Our result determines that the genetic changes in HVR of the *VP2* gene should be considered for assessment of virulence of IBD virus and diagnosis; and similar results outcome were also reported by other researchers (11, 12, 15).

In the last decade, the IBDV has showed gene alteration, and new variants have developed vvIBDVs in the field. Results of our phylogenetic analysis was based on the hyper variable region of *VP2* nucleotide sequences established the divergent relationships between the previously and recently isolated field IBDVs. Sequence analysis of the hyper variable region of the *VP2* gene, typically is used for alignment and phylogenetic analysis of the full genome or at least the entire *VP2* sequence would provide better molecular and functional comparisons. Based on our findings, we concluded that the five recently isolated IBDV of the present study belong to a very virulent group. Moreover, this study demonstrates the spread of vvIBDVs in poultry farms in Erbil, Iraq, and is registered in GenBank as (IBDV isolate AIAE80 VP2 (*VP2*) gene, partial cds; GenBank: MN480452.1. Furthermore, complete surveys would provide more information on the variability, distribution and phylogenetic affiliations of different isolates of IBDVs in Iraq.

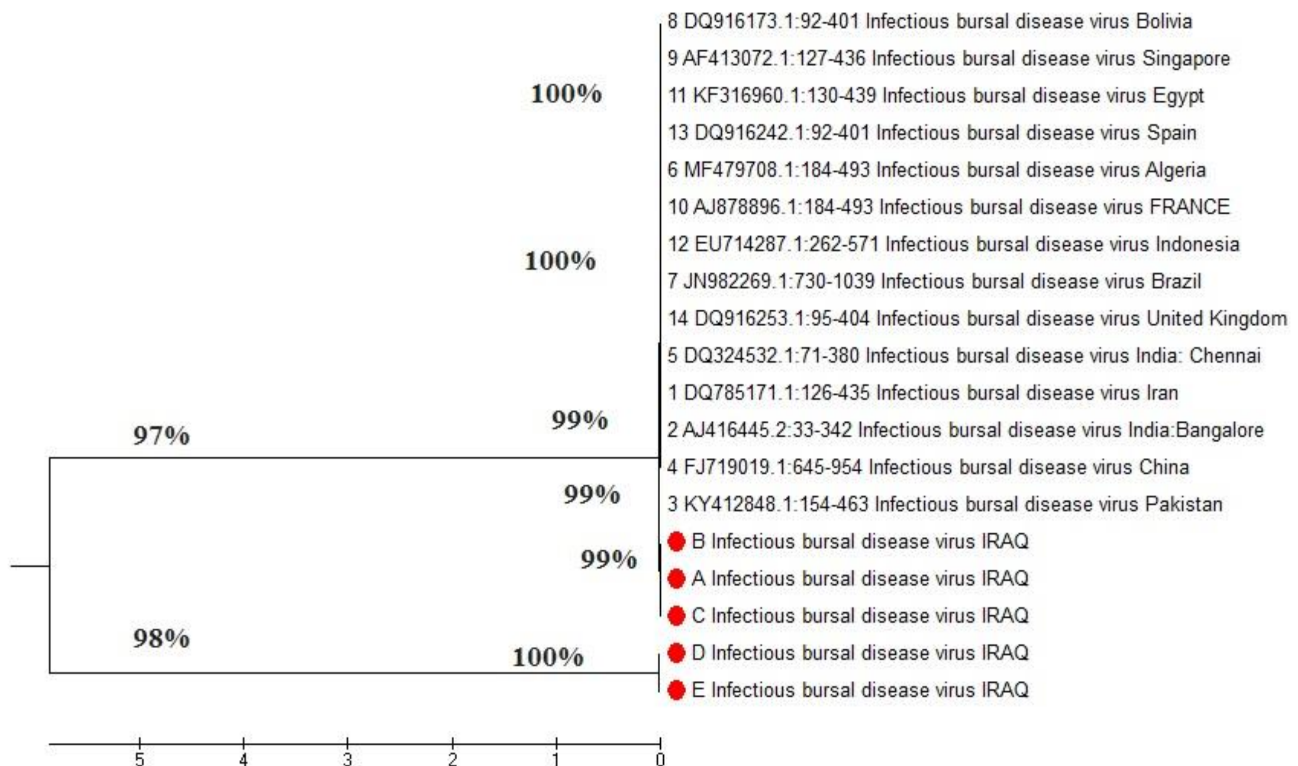


**Figure** 3. Phylogenetic tree of partial fragment of the *VP2* gene of five Iraqi field isolates of IBDV from the clinical samples compared with globally published IBDV strains. Branched distances correspond to sequence divergence. Accession numbers of the sequences

**Table 3.** Pairwise comparison of nucleotide sequence identity and homology for 14 representative IBDV strains

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. A Infectious bursal disease virus IRAQ | | | | | | | | | | | | | | | | | | |
| 2. B Infectious bursal disease virus IRAQ | 0.0211 | | | | | | | | | | | | | | | | | |
| 3. C Infectious bursal disease virus IRAQ | 0.0069 | 0.0211 | | | | | | | | | | | | | | | | |
| 4. D Infectious bursal disease virus IRAQ | 11.3467 | 11.5290 | 11.3353 | | | | | | | | | | | | | | | |
| 5. E Infectious bursal disease virus IRAQ | 11.5256 | 11.7026 | 11.5148 | 0.0247 | | | | | | | | | | | | | | |
| 6. 1 DQ785171.1:126-435 Infectious bursal disease virus Iran | 0.0104 | 0.0104 | 0.0104 | 11.6819 | 11.8510 | | | | | | | | | | | | | |
| 7. 2 AJ416445.2:33-342 Infectious bursal disease virus India:Bangalore | 0.0140 | 0.0139 | 0.0140 | 11.6715 | 11.8410 | 0.0034 | | | | | | | | | | | | |
| 8. 3 KY412848.1:154-463 Infectious bursal disease virus Pakistan | 0.0211 | 0.0282 | 0.0211 | 11.5039 | 11.6782 | 0.0174 | 0.0139 | | | | | | | | | | | |
| 9. 4 FJ719019.1:645-954 Infectious bursal disease virus China | 0.0246 | 0.0317 | 0.0246 | 11.7102 | 11.8788 | 0.0209 | 0.0174 | 0.0246 | | | | | | | | | | |
| 10. 5 DQ324532.1:71-380 Infectious bursal disease virus India: Chennai | 0.0246 | 0.0245 | 0.0246 | 11.6574 | 11.8271 | 0.0139 | 0.0104 | 0.0175 | 0.0281 | | | | | | | | | |
| 11. 6 MF479708.1:184-493 Infectious bursal disease virus Algeria | 0.0282 | 0.0281 | 0.0282 | 11.6715 | 11.8410 | 0.0174 | 0.0139 | 0.0210 | 0.0174 | 0.0104 | | | | | | | | |
| 12. 7 JN982269.1:730-1039 Infectious bursal disease virus Brazil | 0.0282 | 0.0281 | 0.0282 | 11.6998 | 11.8690 | 0.0174 | 0.0139 | 0.0210 | 0.0245 | 0.0174 | 0.0139 | | | | | | | |
| 13. 8 DQ916173.1:92-401 Infectious bursal disease virus Bolivia | 0.0282 | 0.0281 | 0.0282 | 11.6856 | 11.8550 | 0.0174 | 0.0139 | 0.0281 | 0.0174 | 0.0174 | 0.0069 | 0.0139 | | | | | | |
| 14. 9 AF413072.1:127-436 Infectious bursal disease virus Singapore | 0.0282 | 0.0281 | 0.0282 | 11.6856 | 11.8550 | 0.0174 | 0.0139 | 0.0281 | 0.0174 | 0.0174 | 0.0069 | 0.0139 | 0.0000 | | | | | |
| 15. 10 AJ878896.1:184-493 Infectious bursal disease virus FRANCE | 0.0282 | 0.0281 | 0.0282 | 11.6715 | 11.8410 | 0.0174 | 0.0139 | 0.0210 | 0.0174 | 0.0104 | 0.0000 | 0.0139 | 0.0069 | 0.0069 | | | | |
| 16. 11 KF316960.1:130-439 Infectious bursal disease virus Egypt | 0.0318 | 0.0317 | 0.0318 | 11.6856 | 11.8550 | 0.0209 | 0.0174 | 0.0246 | 0.0209 | 0.0139 | 0.0034 | 0.0104 | 0.0034 | 0.0034 | 0.0034 | | | |
| 17. 12 EU714287.1:262-571 Infectious bursal disease virus Indonesia | 0.0319 | 0.0318 | 0.0319 | 11.8451 | 12.0093 | 0.0210 | 0.0175 | 0.0246 | 0.0281 | 0.0139 | 0.0104 | 0.0104 | 0.0104 | 0.0104 | 0.0104 | 0.0069 | | |
| 18. 13 DQ916242.1:92-401 Infectious bursal disease virus Spain | 0.0318 | 0.0317 | 0.0318 | 11.6856 | 11.8550 | 0.0209 | 0.0174 | 0.0246 | 0.0209 | 0.0139 | 0.0034 | 0.0104 | 0.0034 | 0.0034 | 0.0034 | 0.0000 | 0.0069 | |
| 19. 14 DQ916253.1:95-404 Infectious bursal disease virus United Kingdom | 0.0318 | 0.0317 | 0.0318 | 11.6715 | 11.8410 | 0.0209 | 0.0174 | 0.0246 | 0.0209 | 0.0139 | 0.0104 | 0.0245 | 0.0104 | 0.0104 | 0.0104 | 0.0139 | 0.0210 | 0.0139 |

**Table 4.** Referential IBDV strain were used in this study for phylogenetic analysis of VP2 protein

| | Accession ID | Country | Isolate | Source | Compatibility |
|---|---|---|---|---|---|
| 1 | DQ785171.1 | Iran | very virulent | IBDV | 99% |
| 2 | AJ416445.2 | India: Bangalore | host protective antigen | IBDV | 99% |
| 3 | KY412848.1 | Pakistan | pathovar: 10995" | IBDV | 99% |
| 4 | FJ719019.1 | China | Tianjing07-3 | IBDV | 99% |
| 5 | DQ324532.1 | India: Chennai | hypervariable region | IBDV | 98% |
| 6 | MF479708.1 | Algeria | segment="A" | IBDV | 97% |
| 7 | JN982269.1 | Brazil | SC12 | IBDV | 97% |
| 8 | DQ916173.1 | Bolivia | Bolivia05B63 | IBDV | 97% |
| 9 | AF413072.1 | Singapore | very virulent strain | IBDV | 97% |
| 10 | AJ878896.1 | FRANCE | outer capsid protein | IBDV | 97% |
| 11 | KF316960.1 | Egypt | Very Virulent Infectious | IBDV | 97% |
| 12 | EU714287.1 | Indonesia | hypervariable region | IBDV | 97% |
| 13 | DQ916242.1 | Spain | Spain97SP2 | IBDV | 97% |
| 14 | DQ916253.1 | United Kingdom | UnitedKingdom01_1 | IBDV | 97% |

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## CONFLICT OF INTEREST

The author declare that there is no conflict of interest.

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# التوصيف الجزيئي لفايروس مرض غدة فابريشيا المعدي المعزول من دجاج التسمين المصاب طبيعيا في اربيل-العراق

**احمد ابراهيم احمد**

فرع الثروة الحيوانية، كلية هندسة العلوم الزراعية، جامعة صلاح الدين، أربيل، العراق

## الخلاصة

مرض التهاب غدة فابريشيا هو مرض معد للغاية ومثبط لمناعة دجاج التسمين، كما أن حدوث تغير جيني جديد في الفيروس مسؤول عن خسائر اقتصادية كبيرة في صناعة الدواجن. لهذا الغرض ، كان من الضروري إجراء التشخيص الجزيئي للفيروس المعزول من دجاج التسمين الملقح في أربيل-العراق. ان حقول دجاج التسمين الصابة بهذا المرض تتميز بنسبة عالية من الموت المفاجئ (10-15٪) مع وجود آفات النزف على عضلات الصدر والفخذ وكذلك نزف وخزب في جراب فابريشيا ، تم جمع عينات غدة فابريشيا النزفي والخزبي للدجاج المصاب للفترة حزيران 2018-ك الثاني 2019. أظهرت التغيرات النسيجية المرضية لغدة فابريشيا منها تجويف الكيسي للجريبات اللمفاوية مع ارتشاح الكريات البيض تعد صفات مرضية مميزة للإصابة بهذا الفايروس IBDV كما ان حقن مستحضرمتجانس من عينات في الغشاء اللقانقي المشيمي للبيض المخصب اظهرت هلاكات في التمريرة الثانية مع درجات مختلفة من النزف، وقد اظهر اختبارالوسط الجيلاتيني المرسب تفاعل موجب من خلال استخدام مصل مضاد خاص. و اجراء تقنية تفاعل سلسلة البلمرة الاني وكذلك تحليل تسلسل النيوكليوتيدات لخمسة مقاطع من منطقة عالية التغاير من جين *VP2*تبين تغييرات من نوع الانتقال والتحول في نيوكليوتيدات الجين في خمس عزلات حديثة لفايروس التهاب غدة فايبريشي ، كان معدل التماثل او التطابق حوالي 99٪ مقارنة بفيروس التهاب غدة فايبريشيا المعزول من إيران (ID: DQ785171.1). أظهر التحليل الوراثي بأن العزلات الخمس كانت متطابقة الى حد كبير مع المجموعة الآسيوية بنسبة مئوية مختلفة تتراوح بين (98-99٪) و للمجموعة الأوروبية بنسبة بنسبة (97٪). تم تسجيل العزلة المحلية لفيروس التهاب غدة فابريشيا الضاري في البنك الجيني : (MN48052.1). يمكن أن تستنتج هذه الدراسة إلى أن فايروس التهاب غدة فايبريشيا المعزول محليا ينتمي إلى مجموعة شديدة الضراوة ، فضلاً عن كشف انتشار الفايرس شديد الضراوة في صناعة الدواجن في أربيل-العراق ، ومن المفيد أيضا إجراء دراسات مسحية والتي تكمن أهميتها في توفير المزيد من المعلومات حول إنتشار هذا الفايروس الدراسات، وكذلك الحاجة الى مطابقة اللقاح مع عزلات فايروس التهاب غدة فابريشيا.

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**الكلمات المفتاحية:** فيروس جراب فايبريشيا المعدي، جين VP2، اختبارالوسط الجيلاتيني المرسب، تفاعل انزيم البلمرة الاني، الغشاء اللقانقي المشيمي للبيض