Genetic Analysis of Field Isolates of Infectious Bursal Disease Virus in Iraqi Farms

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ABSTRACT

Sixty samples of bursa of Fabricius were collected from broiler chickens suspected to be infected with infectious bursal disease virus (IBDV) in different areas of Iraq for molecular evaluation. The extracted nucleic acid was amplified using reverse transcriptase polymerase chain reaction (RT-PCR) targeting genes of segment A (*Vp*2, *Vp*3, *Vp*4 and *Vp*5 genes) and segment B (*VP*1 genes). The products of amplification were sent to Korea for sequencing using Sanger method. The sequencing analysis of the IBDV from the Iraqi isolates revealed that each gene had different transition and transversion (nonsense and missense of point mutation) compared to reference genes. The phylogenetic tree analysis showed that the *VP*2 of segment A of the Iraqi samples was similar to that of an Egyptian strain with 96%similarity, the polypeptide *VP*2-3-4 of segment A of the Iraqi samples was similar to those of a Chinese strain with 99% similarity and the *VP*5 of segment A was similar to that of Chinese strain with 99% similarity. However, the phylogenetic tree analysis showed that the *VP*1 of segment B had 95% similarity with that of a Chinese strain.

Keywords: IBDV, Conventional RT-PCR, *VP* genes, Sequence analysis, Iraq

Introduction

Infectious bursal disease virus (IBDV) is a double stranded RNA (dsRNA) virus, which belongs to *Avibirnavirus* genus in the *Birnaviridae* family. The virus has two segmented genome segments, A (large segment) and B (small segment), within a nonenveloped and icosahedral capsid. IBDV is the causative agent of an immunosuppressive disease in young chickens (1). Each segment encodes viral proteins (*VP*), segment A encodes *VP*2, *VP*3, *VP*4, and *VP*5 and segment B encodes *VP*1. Each viral protein has specific functions in the viral replication. The *VP*1is RNA-dependent RNA polymerase, which is responsible for the genome replication, transcription (2), and maybe the translation of viral proteins (3). The *VP*2 is the major structural viral protein that builds the viral capsid (4) and has an antigenic domain responsible for inducing the secretion of neutralization antibodies (5). The *VP*2 and *VP*3 together play an important role in the morphogenesis and encapsidation of the viral genome (6). The *VP*4 is a viral protease (7) that affects post-translational cleavage of the polyproteins (*VP*2–*VP*4–*VP*3) and takes part in the maturation of the *VP*2 peptide (8). *VP*5 is a non-structural protein that is responsible for releasing virus progeny from infected cells both through the non-lytic mechanism during the early phase of infection (9) and the activation of cell apoptosis in the later stages by interacting with mitochondrial ionic channels (VDAC2) (10). Thereby the nature of segmented genome of the virus has natural reassortment whereby an exchange of the genetic material can occur if many viruses infect one cell at the same time. This natural reassortment of IBDV has been reported in several countries such as Asia (11), South America (12), North America (13), Africa (14), and Europe (15). The IBDV has serotype 1 and serotype II. The serotype II is nonpathogenic to chicken, but the serotype I is pathogenic and has several strains according to the virulence of this strain. The strains of serotype I, including the very virulent of IBDV (vvIBDV), are the most pathogenic and can cause mortality up to 100% in specific-pathogen-free

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DOI: https://doi.org/10.30539/ijvm.v44i1.931

chickens (SPF). Classical strain and variant strain are less virulent, but they are immunosuppressive (16).

In Iraq, IBDV is a major health problem in chicken and causes economic losses in birds through immunosuppression and susceptibility to other infections. Many studies confirmed the presence of this virus in Iraq in which the virus was isolated by Yousif and Nawzad (17) who diagnosed this virus from infected broilers in Sulaimania, in north region of Iraq, using PCR technique (18). Also, IBDV field strains were characterized in commercial broiler flocks in Tikrit city using RT-PCR to detect vvIBDV using a specific primer set (19) and the (20) who revealed the IBDV has antigenic drift. This means that the virus possesses antigenic variation in the *VP*2 (hydrophilic region) that preserves the neutralizing domine which is responsible for binding with monoclonal antibodies and this revealed that the vaccine should be prepared from a local strain, but the amplification of both genes (*VP*1 and *VP*2) sequencing is still not studied. Therefore, the current study was designated to investigation the virulence of field isolates of IBDV by sequencing of both genes which might responsible for virulence of field isolates that found in Iraq.

Materials and Methods

Collection of Samples

Bursa of Fabricius samples were collected from 60 broiler chickens at age 25-35 days suspected to be infected with Gumboro disease after postmortem examination. These specimens were collected from different areas of Iraq including Baghdad province (20 samples), Karbala province (5 samples), Diyala province (Baqubah) (3 samples), Wasit province (Kut) (10 samples), Al-Anbar province (Ramadi) (3 samples), Babil province (Hillah) (12 samples), Salah Aldin province (4 samples), and Al-Basrah province (3 samples). These tissues were put in test tubes and preserved at -20 °C till use.

Primers Used in Conventional PCR

The primers used for sequencing the viral genome of avian infectious bursal disease virus were designed by using the National Center for Biotechnology Information (NCBI) software. Three pairs of primers were used to amplify different regions of segment B of the virus, and four pairs of primers were used to amplify genes of segment A (*VP*2, *VP*3, *VP*4, and *VP*5). The primers are listed in Table 1.

Table 1. Primers designed for *VP*1, *VP*2 genes and segment A and B of IBDV

No.	Segment	Primer name		Product size	Sequence（5'--3'）
1	Segment B	B1	Forward	606	5'-GTCTCAAGTCCAAGGTCGGG-3'
			Reverse		5'-CGGAAGTCGACTGAACTGCT-3'
		B2	Forward	533	5'-CATGCGGGCTTTTGTTTCCA-3'
			Reverse		5'-GATGAACGTGGCTGCATTCC-3'
		B3	Forward	940	5'-CGGCGACCCAATGTTCAATC-3'
			Reverse		5'-GAGTTTCTCGGCTTCTGCCT-3'
2	Segment A	A1	Forward	739	5'-GAGCCTAGCAGTGACGATCC-3'
			Reverse		5'-GCTGTTCAGTGCTTTGGGTG-3'
		A2	Forward	568	5'-CACCCAAAGCACTGAACAGC-3'
			Reverse		5'-TTGAACTCTGATGCGGCCAT-3'
		A3	Forward	677	5'-ACTCCCTGGTGGCGTTTATG-3'
			Reverse		5'-GGATCGTCACTGCTAGGCTC-3'
		A4	Forward	1000	5'-CGTCCCGTCACACTAGTAGC-3'
			Reverse		5'-AAGCATTGAGGGCTCCTGTC-3'

Prior to running conventional reverse transcriptase PCR (RT-PCR), total RNA was extracted using total RNA mini extraction kit (Gene aid Corporation) from the specimens of bursa of Fabricius. Then EnergicScript® first strand cDNA synthesis kit was used to convert the viral RNA by reverse transcriptase into cDNA. This reaction involved the addition of 1 µl of RTase, 1 µl of random primers, 3 µl of DEPC-ddH_2O, 10 µl of 2x RT buffer, and 5 µl of RNA sample. The PCR reaction tube (total reaction volume of 25 µl) composed of 5 µl of cDNA sample, 12.5 µl of Go Taq® Green Master Mix (Promega), 1 µl of each of forward and reverse primers as well as 5.5 µl of RNase-free water. The PCR conditions were one cycle for initial denaturation for 5 minutes at 94℃, followed by 35 cycles of denaturation for 45 seconds at 95℃, annealing for 30 seconds at 62℃, extension for 45 seconds at 72℃, extra extension for10 minutes at 72℃, and holding at 4℃. To confirm PCR products, agarose gel (2%) was made using Tris-Borate EDTA (TBE) buffer (Biotech). Then, the cDNA samples along with DNA ladder (100-1500 bp) were loaded, and the electrophoresis was run at 10 V/cm for 40 min to separate the cDNA products already stained with Ethidium bromide (Biotech). Eventually, the cDNA bands were visualized using UV transilluminator.

Sequencing

The products of amplification of the viral cDNA were sent to Korea (Macrogen) for sequencing using Sanger method. Later, both BLAST (http://blast.ncbi.nlm.nih.gov) and Mega 6 software were used to analyze the obtained nucleotide sequences.

Bioinformatics Analysis of the VP Proteins

Raptorx software was used to analyze *VP* proteins of the viral isolates.

Phylogenetic Analysis

The phylogenetic relationships of the IBDV isolates were analyzed using the Mega 6 software.

Results

Molecular Detection of the IBDV

The gel electrophoreses revealed bands of the expected sizes of the genes amplified by conventional PCR (Figure 1).

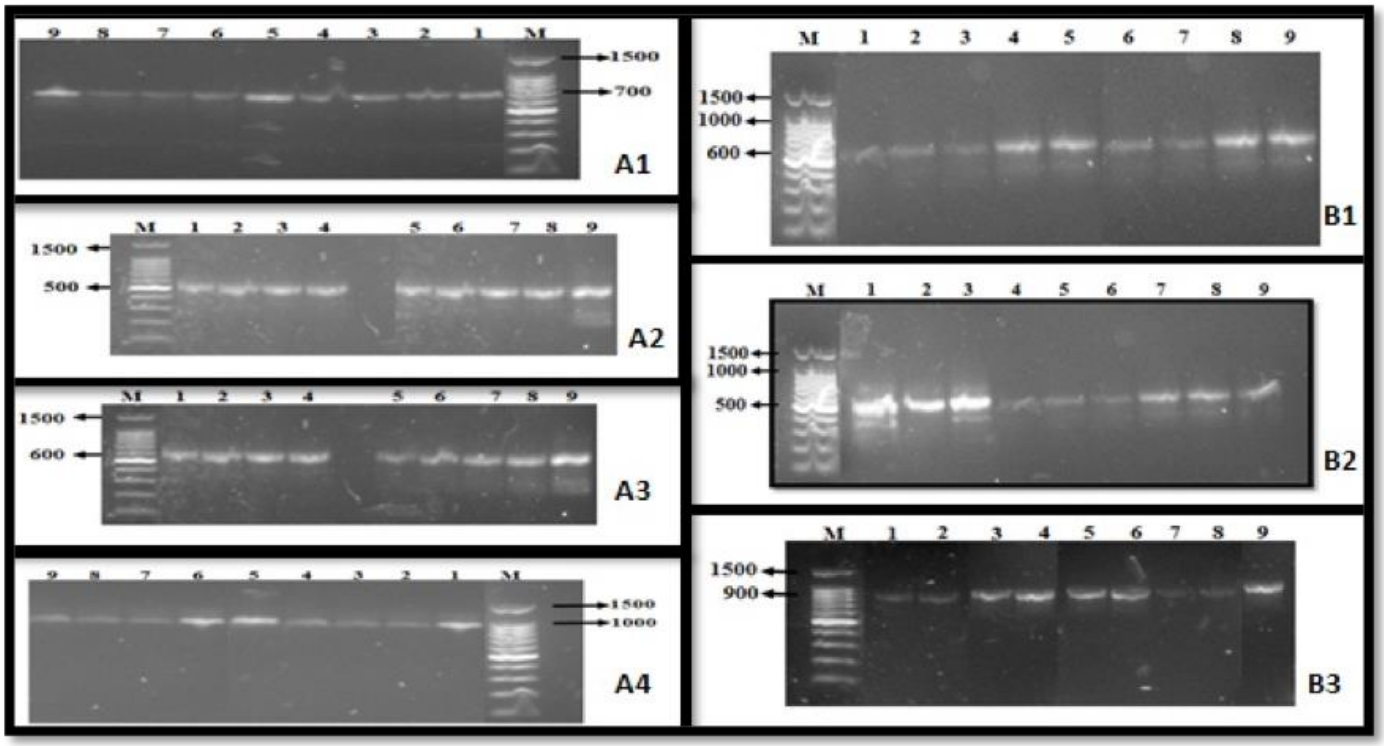


Figure 1. Agarose gel electrophoresis shows bands of the expected sizes of the amplified segment A (A1, 2, 3, 4) and segment B (B1, 2, 3). Lane M= Ladder, Lane 1 and 2= samples from Baghdad, Lane 3= samples from Karbala, Lane 4= samples from Diyala, Lane 5= samples from Wasit, Lane 6= samples from Al-Anbar, Lane 7= samples from Babylon, Lane 8= samples from Saladin, Lane 9= samples from Basrah

Sequence Analysis of Segment A

Sequence analysis of PCR products obtained from the *VP*2 gene showed that three out of nine PCR amplicons of the *VP2* gene amplified using primer A1 showed nucleotide changes, and two out of nine belong to A2 gene were showed positive sequence for *VP5* in segment A. But the A3 had two out of nine PCR product positive for *VP*2-3-4 of segment A and the A4 gene had two out nine of PCR product positive for *VP*2.

The sequence analysis revealed the presence of changes in nucleotide sequences in different regions of the gene compared to the known reference gene sequences of the IBDV. There was either transition or transversion (missense or nonsense). These nucleotide changes made the Iraqi IBDV a unique isolate in the region from which it was isolated. These changes are listed as numbers in Table 2.

Table 2. Nucleotide changes of the sequences amplified from segment A

Sample No.	Region of isolate	Sequence ID	Identity strain	Primer used	Transition		Transversion	
					Missense	Nonsense	Missense	Nonsense
1.	Baghdad*	AF159218.1	96%	A1 (*VP*2)	5		5	
					4	1	5	0
2.	Diyala	HG974563.1	97%		17		6	
					16	1	4	2
3.	Karbala	AF159218.1	97%		11		0	
					0	11	0	0
4.	Al-Anbar	AF092943.1	99%	A2 (*VP*5)	1		1	
					0	1	1	0
5.	Wasit	AF322444.1	99%		3		1	
					1	2	1	0
6.	Baghdad	AF092943.1	98%	A3 (*VP*2-4-3)	2		0	
					0	2	0	0
7.	Babil	AY628217.1	99%		1		0	
					0	1	0	0
8.	Saladin	AF159218.1	98%	A4 (*VP*2)	9		2	
					0	9	0	2
9.	Babil	LM651365.1	98%		12		1	
					0	12	1	0

*This sample was submitted to NCBI under the accession number MK507831.1

Sequence Analysis of Segment B

Sequence analysis for the PCR products of the *VP*1 gene showed that two out of nine PCR amplicons and six out of nine belonged to B2 gene showed positive sequence. But the B3 had 4 out of 9 PCR products were positive the sequence analysis which revealed the presence of change in different regions of nucleotide sequences with different percentages compared to other global known IBDV. There was either transition or transversion (missense or nonsense). These changes in nucleotide sequences made the Iraqi IBDV a unique isolate in the region from which it was isolated. These changes are listed as numbers in Table 3.

VP Protein Characteristics of the IBDV

Results of bioinformatics analysis of the secondary structure of VP2, VP2-3-4, and VP5 in segment A and *VP1* in segment B are shown in Figure 2. These structures were found to be composed of alpha-helix, beta-sheet, and coiled coil. The viral proteins revealed a ribbon diagram of protein structure included amino acids.

Table 3. Nucleotide changes of the sequences amplified from different regions of the *VP*1gene on segment B

Sample No.	Region of isolate	Sequence ID	Identity strain	Primer used	Transition		Transversion	
					Missense	Nonsense	Missense	Nonsense
1.	Baghdad	KU234529.1	94%	B1 (*VP*1)	15		4	
					0	15	1	3
2.	Wasit	EU595678.1	95%		10		8	
					1	9	7	1
3.	Babil	EU595676.1	94%	B2 (*VP*1)	11		8	
					0	11	3	5
4.	Saladin	EU595676.1	97%		5		0	
					0	5	0	0
5.	Al-Anbar	KU234529.1	94%		11		8	
					1	10	5	3
6.	Diyala	KU234529.1	93%		9		5	
					4	5	4	1
7.	Karbala	EU595678.1	98%		2		2	
					1	1	1	1
8.	Al-basrah	EU595677.1	95%		8		4	
					0	8	0	4
9.	Al-Basrah	AJ295026.1	99%	B3 (*VP*1)	3		0	
					0	3	0	0
10.	Wasit	AJ295026.1	99%		2		0	
					0	2	0	0
11.	Saladin	MF969108.1	99%		2		2	
					1	1	2	0
12.	Baghdad*	MF969108.1	99%		1		0	
					0	1	0	0

*This sample was submitted to NCBI under the accession number MK507832.1

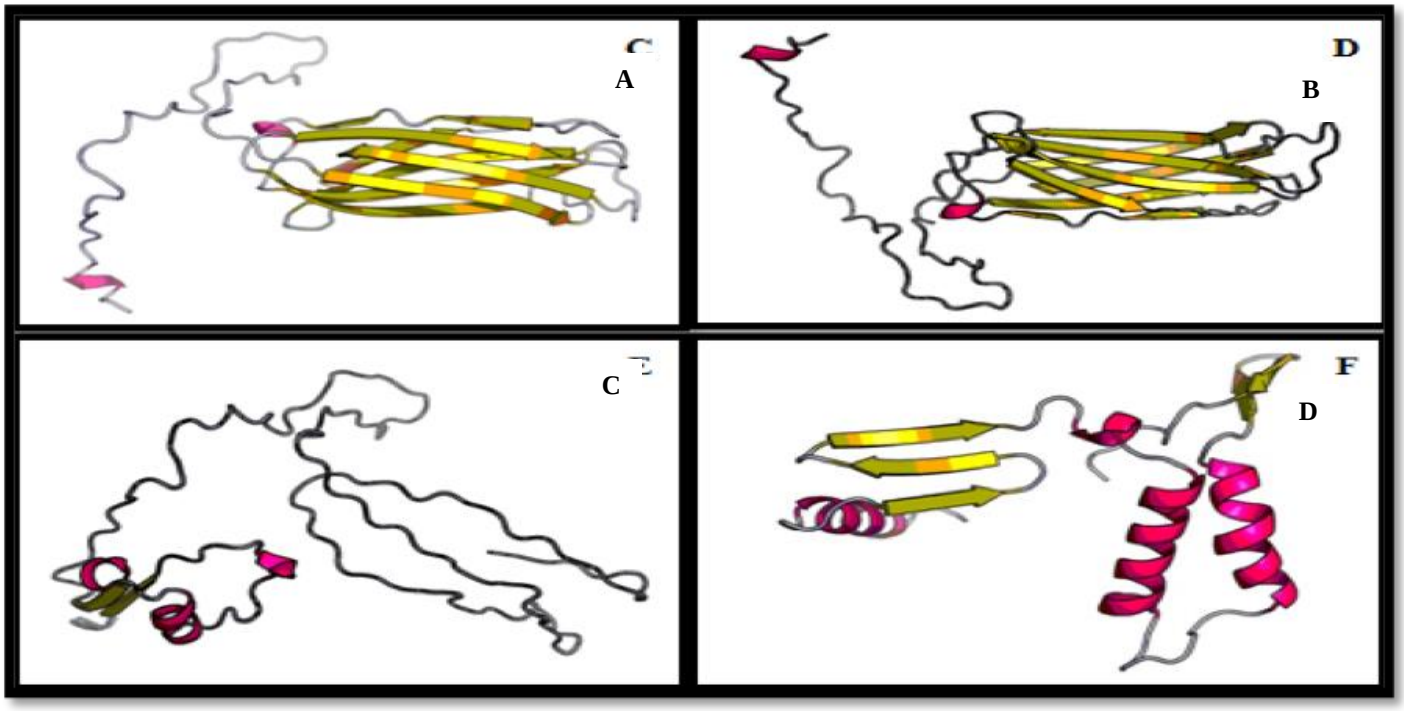
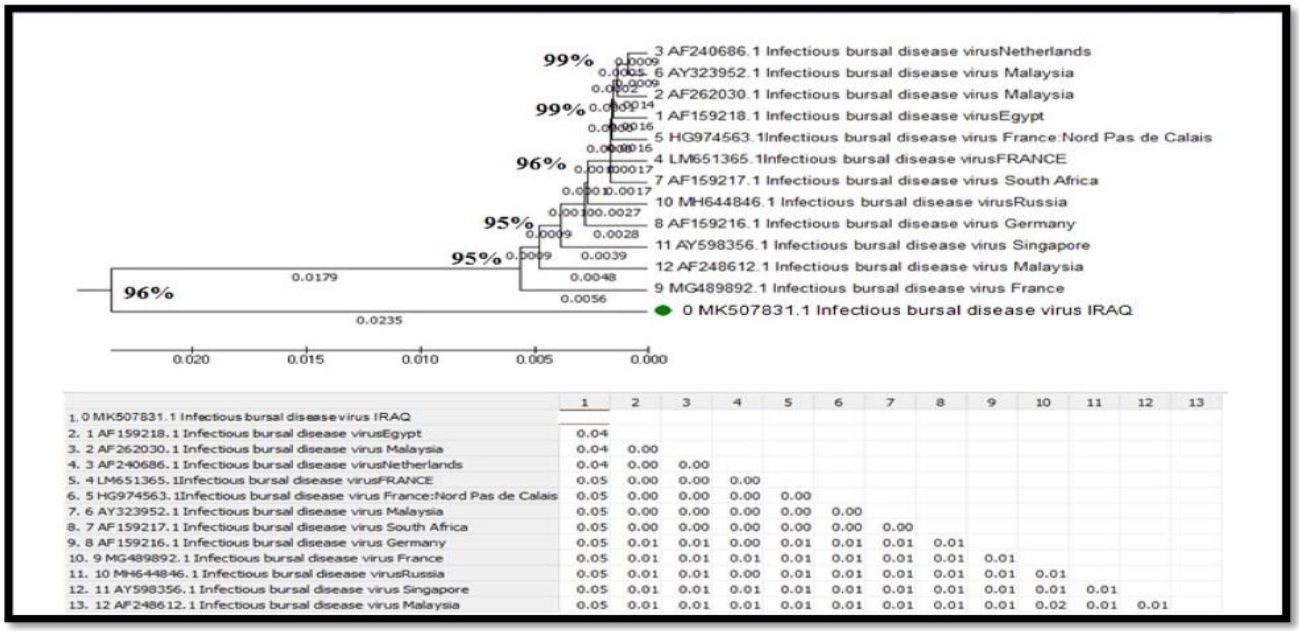


Figure 2. *VP* protein analysis: A) *VP*2 in segment A, B) *VP*2-3-4 in segment A, C) *VP*5 in segment A and D) *VP*1 in segment B for IBDV. Raptorx software was used for drawing structure protein (alpha-helixes, beta-sheets, and coils) of amino acid

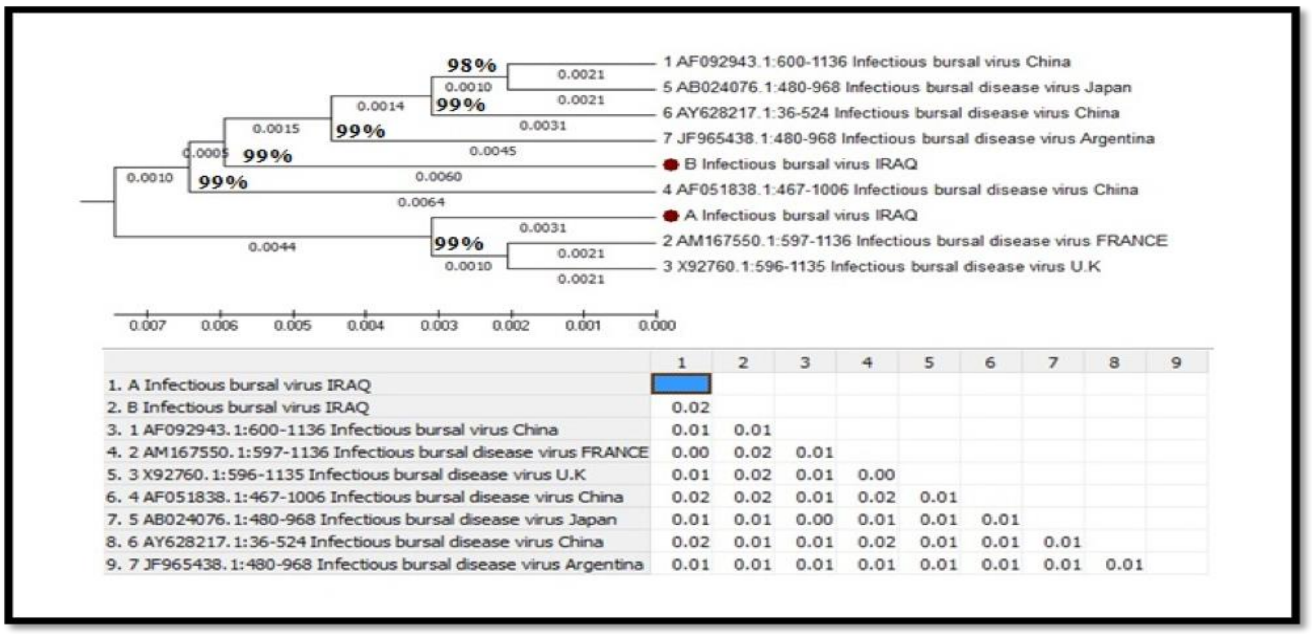
Phylogenetic Tree Analysis

The phylogenetic analysis showed that five isolates having *VP*2 were similar to the Egyptian strain with an identity of 96% and a French strain with an identity of 95% (Figure 3). But the phylogenetic analysis of genes *VP*2, *VP*3, *VP*4, and *VP*5 showed that two isolates were similar to a Chinese strain with an identity of 99%, and another Chinese strain with an identity of 98%. An Australian strain was identical with 99% to *VP*5 (Figures 4 and 5). Moreover, the phylogenetic analysis of segment B (*VP*1) revealed that six isolates were similar to the Chinese strain with an identity of 95% and German strain with an identity of 95% (Figure 6).



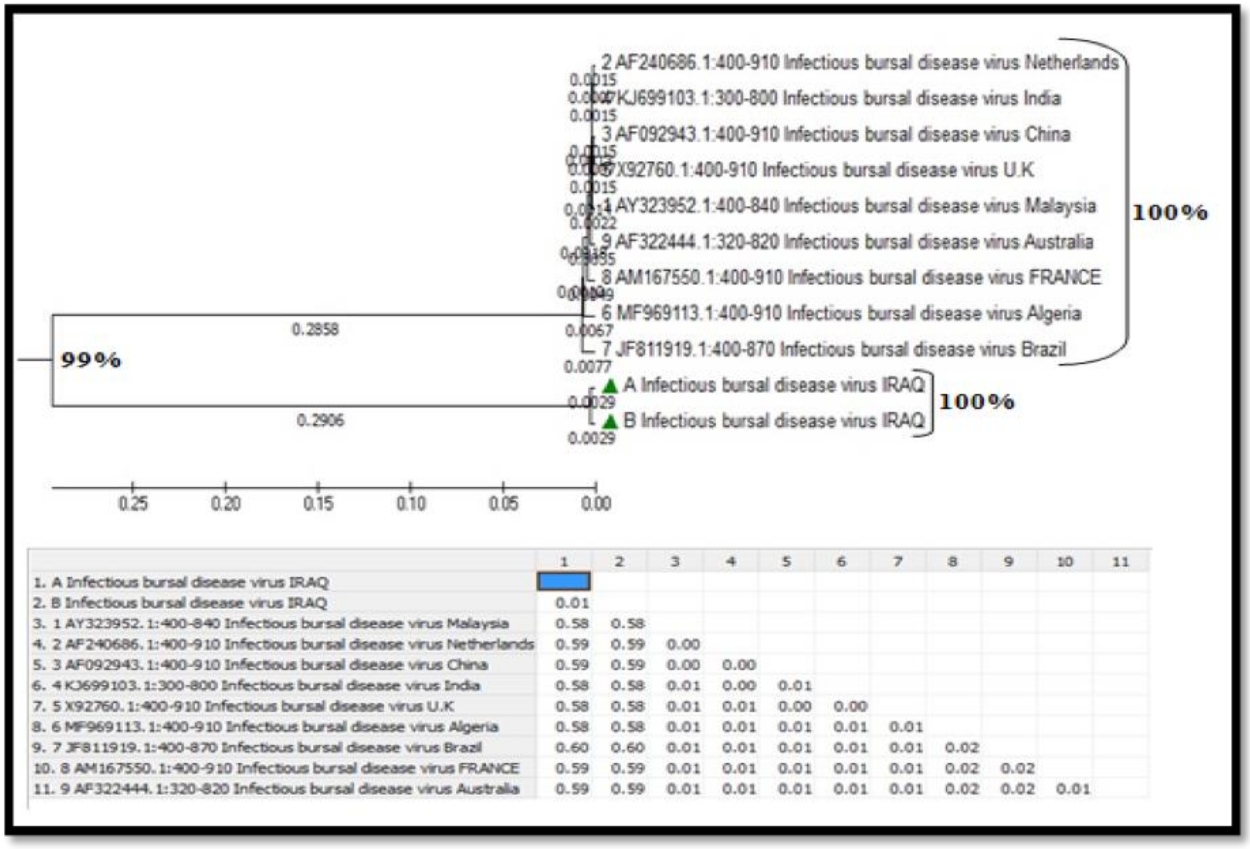
	1	2	3	4	5	6	7	8	9	10	11	12	13
1. 0 MK507831.1 Infectious bursal disease virus IRAQ													
2. 1 AF159218.1 Infectious bursal disease virusEgypt	0.04												
3. 2 AF262030.1 Infectious bursal disease virus Malaysia	0.04	0.00											
4. 3 AF240686.1 Infectious bursal disease virusNetherlands	0.04	0.00	0.00										
5. 4 LM651365.1Infectious bursal disease virusFRANCE	0.05	0.00	0.00	0.00									
6. 5 HG974563.1Infectious bursal disease virus France:Nord Pas de Calais	0.05	0.00	0.00	0.00	0.00								
7. 6 AY323952.1 Infectious bursal disease virus Malaysia	0.05	0.00	0.00	0.00	0.00	0.00							
8. 7 AF159217.1 Infectious bursal disease virus South Africa	0.05	0.00	0.00	0.00	0.00	0.00	0.00						
9. 8 AF159216.1 Infectious bursal disease virus Germany	0.05	0.01	0.01	0.00	0.01	0.01	0.01	0.01					
10. 9 MG489892.1 Infectious bursal disease virus France	0.05	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01				
11. 10 MH644846.1 Infectious bursal disease virusRussia	0.05	0.01	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.01			
12. 11 AY598356.1 Infectious bursal disease virus Singapore	0.05	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01		
13. 12 AF248612.1 Infectious bursal disease virus Malaysia	0.05	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01	

Figure 3. Phylogenetic tree of the *VP2* gene of an Iraqi isolates submitted to NCBI under the accession number MK507831.1



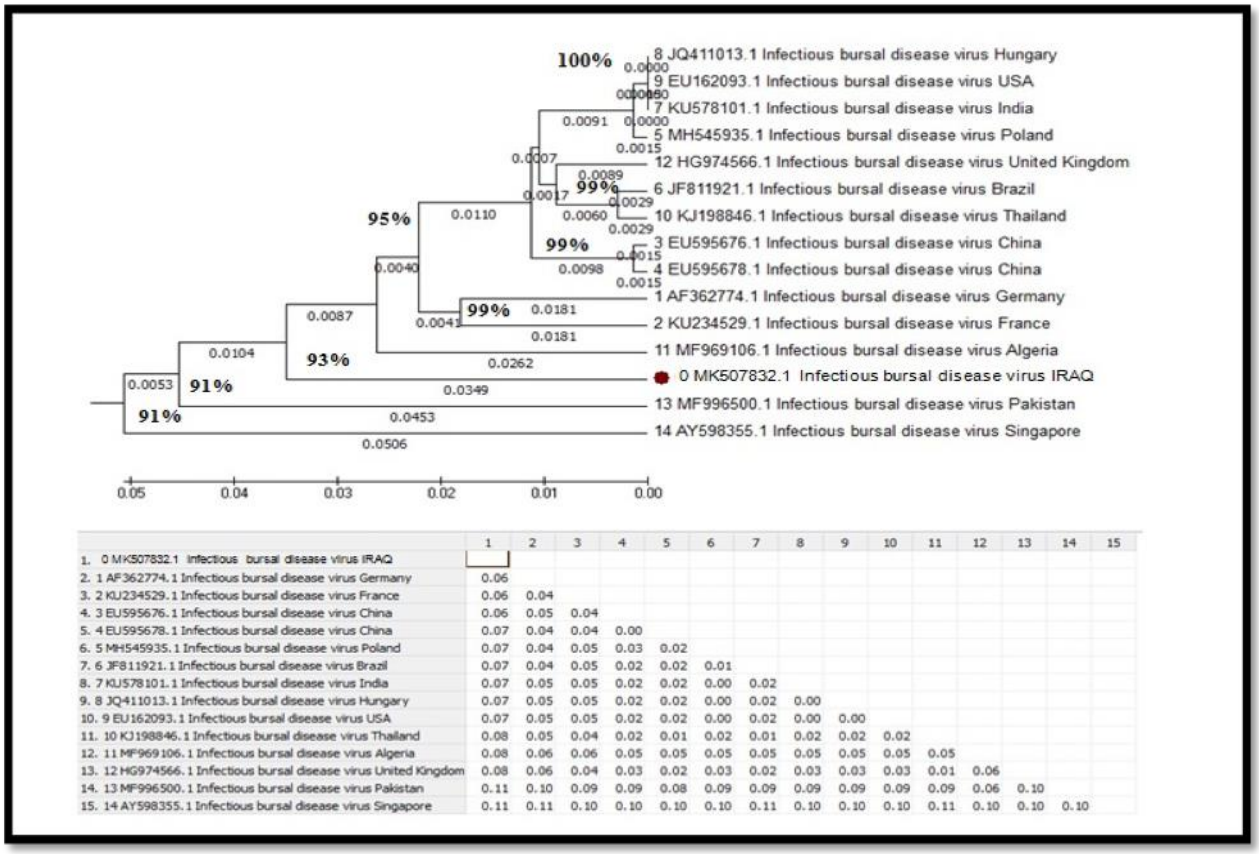
	1	2	3	4	5	6	7	8	9
1. A Infectious bursal virus IRAQ									
2. B Infectious bursal virus IRAQ	0.02								
3. 1 AF092943.1:600-1136 Infectious bursal virus China	0.01	0.01							
4. 2 AM167550.1:597-1136 Infectious bursal disease virus FRANCE	0.00	0.02	0.01						
5. 3 X92760.1:596-1135 Infectious bursal disease virus U.K	0.01	0.02	0.01	0.00					
6. 4 AF051838.1:467-1006 Infectious bursal disease virus China	0.02	0.02	0.01	0.02	0.01				
7. 5 AB024076.1:480-968 Infectious bursal disease virus Japan	0.01	0.01	0.00	0.01	0.01	0.01			
8. 6 AY628217.1:36-524 Infectious bursal disease virus China	0.02	0.01	0.01	0.02	0.01	0.01	0.01		
9. 7 JF965438.1:480-968 Infectious bursal disease virus Argentina	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	

Figure 4. Phylogenetic tree of *VP2*-3-4 of two Iraqi isolates



	1	2	3	4	5	6	7	8	9	10	11
1. A Infectious bursal disease virus IRAQ											
2. B Infectious bursal disease virus IRAQ	0.01										
3. 1 AY323952.1:400-840 Infectious bursal disease virus Malaysia	0.58	0.58									
4. 2 AF240686.1:400-910 Infectious bursal disease virus Netherlands	0.59	0.59	0.00								
5. 3 AF092943.1:400-910 Infectious bursal disease virus China	0.59	0.59	0.00	0.00							
6. 4 KJ699103.1:300-800 Infectious bursal disease virus India	0.58	0.58	0.01	0.00	0.01						
7. 5 X92760.1:400-910 Infectious bursal disease virus U.K	0.58	0.58	0.01	0.01	0.00	0.00					
8. 6 MF969113.1:400-910 Infectious bursal disease virus Algeria	0.58	0.58	0.01	0.01	0.01	0.01	0.01				
9. 7 JF811919.1:400-870 Infectious bursal disease virus Brazil	0.60	0.60	0.01	0.01	0.01	0.01	0.01	0.02			
10. 8 AM167550.1:400-910 Infectious bursal disease virus FRANCE	0.59	0.59	0.01	0.01	0.01	0.01	0.01	0.02	0.02		
11. 9 AF322444.1:320-820 Infectious bursal disease virus Australia	0.59	0.59	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.01	

Figure 5. Phylogenetic tree of *VP*5 gene of two Iraqi isolates



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. 0 MK507832.1 Infectious bursal disease virus IRAQ															
2. 1 AF362774.1 Infectious bursal disease virus Germany	0.06														
3. 2 KU234529.1 Infectious bursal disease virus France	0.06	0.04													
4. 3 EU595676.1 Infectious bursal disease virus China	0.06	0.05	0.04												
5. 4 EU595678.1 Infectious bursal disease virus China	0.07	0.04	0.04	0.00											
6. 5 MH545935.1 Infectious bursal disease virus Poland	0.07	0.04	0.05	0.03	0.02										
7. 6 JF811921.1 Infectious bursal disease virus Brazil	0.07	0.04	0.05	0.02	0.02	0.01									
8. 7 KU578101.1 Infectious bursal disease virus India	0.07	0.05	0.05	0.02	0.02	0.00	0.02								
9. 8 JQ411013.1 Infectious bursal disease virus Hungary	0.07	0.05	0.05	0.02	0.02	0.00	0.02	0.00							
10. 9 EU162093.1 Infectious bursal disease virus USA	0.07	0.05	0.05	0.02	0.02	0.00	0.02	0.00	0.00						
11. 10 KJ198846.1 Infectious bursal disease virus Thailand	0.08	0.05	0.04	0.02	0.01	0.02	0.01	0.02	0.02	0.02					
12. 11 MF969106.1 Infectious bursal disease virus Algeria	0.08	0.06	0.06	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05				
13. 12 HG974566.1 Infectious bursal disease virus United Kingdom	0.08	0.06	0.04	0.03	0.02	0.03	0.02	0.03	0.03	0.03	0.01	0.06			
14. 13 MF996500.1 Infectious bursal disease virus Pakistan	0.11	0.10	0.09	0.09	0.08	0.09	0.09	0.09	0.09	0.09	0.09	0.06	0.10		
15. 14 AY598355.1 Infectious bursal disease virus Singapore	0.11	0.11	0.10	0.10	0.10	0.10	0.11	0.10	0.10	0.10	0.11	0.10	0.10	0.10	

Figure 6. Phylogenetic tree *VP1* gene of an Iraqi isolate submitted to NCBI under the accession number MK507832.1

Discussion

Infectious bursal disease (IBD) or (Gumboro disease) is a highly contagious disease of young chickens caused by infectious bursal disease virus (IBDV) (21). The sequencing analysis of PCR products for segment A and segment B showed changes in different regions of nucleotide sequences with different percentages compared to other globally known IBDV. These results revealed the exchange of amino acid at different positions in segment A (*VP*2, *VP*2-3-4, and *VP*5) and segment B (*VP*1) may be leading to changing of viral antigenic properties or viral replication and viral characteristics strains. These results are in agreement with (22, 23) who revealed the mutation at different positions leading to exchange of amino acid in hydrophilic part or hydrophobic part of *VP*2, therefore, resulted in the vvIBDV has tolerated antigenic properties or resulted in lack of a neutralizing monoclonal antibody to vvIBDV strains.

The results of conformation of protein in sequencing analysis showed the secondary structure of protein consists of helical, β-sheet, and coil or ribbon model of IBDV proteins including segment A (*VP*2, *VP*2-3-4, and *VP*5) and segment B (*VP*1). This conservation of secondary structure is an important parameter in the evaluation of the structural model of viral proteins. These were substitution mutations which either transition or transversion (nonsense or missense) that lead to change in amino acid sequence. The transition is a point mutation which changes the purine nucleotide to another purine nucleotide (Adenine to Guanine) or (Guanine to Adenine) or changes the pyrimidine nucleotide to another pyrimidine nucleotide (Cytosine to Thymine) or (Thymine to Cytosine) that means these changes (transition) are 2 out of 3 single nucleotide polymorphisms (24). The transversion is a point mutation which changes the purine (2 rings) nucleotide (Adenine or Guanine) into pyrimidine (1 ring) nucleotide (Thymine or Cytosine) and vice versa and occurs spontaneously (25). These point mutations that occurred within protein coding region of a gene and classified into nonsense mutations (synonymous substitutions) or missense mutation (non-synonymous substitutions). The nonsense mutations which do not alter amino acid sequence (silent mutation) while the missense mutations which alter amino acid sequences of a protein resulted in modified amino acid (26). The phylogenic tree analysis showed the *VP*1 of segment B of Iraqi detected strain under accession number (MK507832.1) was similar to the China strain (under accession number EU595676.1) with 95% homology.

These results may be interference of wild type virus with laboratory adaptation virus (vaccine strain), therefore these viruses have competitive interference (heterologous interference) or auto-interference (homologous interference) by either the first virus which competes with the second virus on components for its replication such as polymerase or translation factors or the first virus inhibit the multiplication of the second virus by destroying viral receptors. These results are consistent with those of (27) who revealed the attenuation of very virulent IBDV was due to multigenic mutations and competitive replication during *in vitro* and *in vivo* serial passages.

Moreover, the *VP*2 of segment A of Iraqi detected strain under accession number (MK507831.1) was similar to the Egypt strain (under accession number AF159218.1) with 96% homology. These results are due to antigenic drift in inside or outside of hydrophilic region and hydrophobic region of *VP*2 that leads to some sites to become more stable and others less stable. Also, the mutations of *VP*2 are responsible for viral antigenicity, pathogenicity, and tissue tropism as reported by (23, 28).

The *VP*2-3-4 of segment A of two Iraqi detected strains were closely related to the Chinese strain (under accession number AY628217.1) both with 99% homology and the *VP*5 of segment A of two Iraqi detected strains were showed a high nucleotide homology to the Chinses strain (under accession number AF692943.1) both with 99%. The shared 99% of nucleotide identity with these strains may be due to recombination event within segment A between wild type virus and attenuation virus or vaccine strain of IBDV. These results are consistent with (29) who reported that the full length of *VP*2-4-3 contained a unique residue within *VP*2 and the substitution at some position may influence polyprotein cleavage efficiency and/or protease activity. Additionally, the author found that the *VP*5 is not required for viral replication and infection but plays an important role in the release of viral progeny from infected cells.

The results of IBDV sequencing analysis, protein conformation, and phylogenetic tree may indicate that reassortment of IBDV or antigenic drift of the virus. The reassortment is a mixing of the genetic materials that occurs when two different strains of a

similar virus are infecting the same cell of a single host therefore it results in a new strain that will share properties of parental virus. The reassortment is responsible for the genetic shift in segmented RNA viruses that confer the phenotypic change for new viruses which means to form a new subtype having a mixture of the surface antigens of the two or more original strains (5). The results are in agreement with (16) who reported that the virulence of IBDV contributed to both segment A and B due to the reassortment IBDV. The reassortment is one of the mechanisms of genetic diversity characteristic of viruses with segmented genomes and the shuffling of the segment of viral genome was originating from two parental strains with distinct patho-types (very virulent and classical cell culture adapted) also investigate the very virulent/attenuated reassortment that has hold over high virulence.

But the antigenic drift is an accumulation of mutation (point mutation) within subtype that confer new stain has of virus particles cannot be inhibited by antibodies that targeted previous strains. This mechanism occurs in RNA viruses due to RNA polymerase has no proofreading mechanisms (not error correcting process) and this mechanism allows the new virus has able to escape from host immune system due to mutations occur in viral surface proteins. The present results are in agreement with (20) who found that the IBDV has antigenic drift that means the virus possesses antigenic variation in the *VP*2 hyper-variable domine (hydrophilic region) that preserved the neutralizing domine which responsible for binding with monoclonal antibodies and revealed the vaccine should be prepared from the local strain. Therefore, these results indicate that the trading of infected birds or using trading live attenuated vaccines lead to the emergence of very virulent IBDV in Iraqi farms.

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التحليل الجيني للعزلات الحقلية لفايروس لمرض الجراب الفايبريشي المعدي في الحقول العراقية

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الخلاصة

جرى في هذه الدراسة جمع 60 عينة (جراب فايبريشيا) من الدجاج اللاحم التي يشتبه اصابتها سريرياً بفيروس جراب فايبريشيا المعدي من مناطق مختلفة من العراق. استخلص الحمض النووي من العينات واستخدمت تقنية تفاعل انزيم البلمرة المتسلسل التقليدي (Conventional PCR) لتضخيم جينات القطعة A, جينات القطعة B. ارسلت نتائج تضخيم التفاعل الانف الذكر الى كوريا لغرض معرفة تتابع القواعد النتروجينية لجينات الفايروس. اظهرت نتائج تتابع قواعدالنيوكليد لجينات للعزلات الحقلية لفيروس مرض التهاب جراب فايبريشيا المعدي على احتوائها عدد من القواعد النتروجينية المختلفة عند مقارنتها مع تتابعات لجينات مرجعية مما ادى الى تغاير في تكوين الاحماض الامينية. اظهرت نتائج تحليل الشجرة الوراثية للجين *VP*2 في قطعة Aلفيروس الجراب الفايبريشي المعدي تشابه مع نفس الجين الموجود فيالعتر المصرية بنسبة تطابق 96% , وكان الجين *VP*2-3-4 في قطعة Aللفايروس مشابه للعتر الصينية بنسبة تطابق 99%, والجين *VP*5 في قطعة Aللفايروس مشابه للعتر الصينية بنسبة تطابق 99%. بينما اظهرت نتائج التحليل الوراثي لقطعة B للجين *VP*1تشابه مع العتر الصينية بنسبة تطابق 95%.

الكلمات المفتاحية:فيروس جراب فايبريشيا المعدي, تفاعل انزيم البلمرة التقليدي, تحليل التسلسل جينات *VP*, العراق