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Original Article

Sequences and Phylogenetic Analysis of Envelope (E) Gene of Iranian Infectious Bronchitis Virus Isolates in Iran

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Abstract

Background and Aims: Infectious bronchitis virus (IBV) is the etiological agent of infectious bronchitis (IB), which is an acute and highly contagious disease of the respiratory and sometimes the urogenital tracts of chickens. In Iran, the disease has been identified in chicken flocks with serological and virus isolation methods. Gene 3c (E) encode envelop (small membrane) protein. For envelope protein tree functions has been determined:1) Pivotal roles in virion assembly and morphogenesis 2) Induction of apoptosis and 3) Ion channel activity.

Materials and Methods: In the present study, the E gene of genome fragment of 9 IBV field isolates from Iranian poultry farms were sequenced and compared with sequences from non-Iranian origin.

Results: Results indicate that nucleotide homology among these isolates with E genes was between 80.1% to 100%. The results indicated that, we had 3 clades of IBV based on E gene in Iran: 4/91Like, Massachusetts and Gray Like.

Conclusion: Most Iranian isolates were located in 4/91 clade. In conclusion, the present study has demonstrated that the circulating IBV strains in commercial flocks in Iran based on E gene were genetically diverse and underwent continuing evolution.

Keywords: Infectious Bronchitis; Envelope Gene; Iran, Phylogenetic study

Introduction

Infectious bronchitis virus (IBV) is the etiological agent of infectious bronchitis (IB), which is an acute and highly contagious disease of the respiratory andsometimes the urogenital tracts of chickens causing tracheal rales, sneezing, coughing, a poor weight gain and reduced feed efficiency in broilers and a decline in egg production and egg shell quality in layers (1, 2).

It is economically important to the poultry industry due to the high morbidity and production losses associated with the disease. There is no specific treatment for IBV. Prevention and control are through the use of modified live or killed vaccines (1, 3).

IBV is a prototype member of the Coronaviridae family, genus Coronavirus. Its genome is a single-stranded, positive-sense linear RNA molecule of 27.6 kb.Coronaviruses are enveloped positive single-stranded RNA viruses, members ofthe order *Nidovirales* (2, 4). Their genome is 27–31kb in length and is

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composed in its 5-proximal two-thirds of two large open reading frames (ORFs), ORF1a and ORF1b, encoding the replicasecomplex. Genes encoding the structural proteins, i.e. spike (S), envelope (E), membrane (M) and nucleocapsid (N) proteins, are downstream of the ORF1b. The genes in the IBV genomeare arranged in the following order: 5'-polymerase-SE-M-N-3', with a few non-structural genes interspersed among these genes (4).

The 3c gene encodes the envelope protein, which plays an important role in budding and assembly of virion (5). The third (S, M) critical membrane-bound constituent of the virion is the small envelope (E) protein. E was only belatedly recognized as a viral structural protein, owing to its size (ca. 10 kDa) and its very low abundance relative to the M, N, and S proteins. E proteins are well conserved within each of the three groups of coronaviruses, but they show very limited homology across the different groups. All E proteins have the same general structure: a short (7- to 9-amino-acid) hydrophilic amino terminus preceding a large (21- to 29-amino-acid) hydrophobic region, followed by a large hydrophilic carboxy terminus making up one-half to two-thirds of the mass of the molecule (4, 6, 7).

In Iran, the disease has been identified in chicken flocks with serological and virus isolation methods. Introduction of new serotype was reported by following researchers which showed the existence of new serotype 4/91 in Iran.Recently, it was demonstrated that there exist some newly emerging IBV isolates in Iran.In spite of the use of three different vaccines (H120, MA5, attenuated 4/91) in poultry farms in Iran, outbreaks have been observed with high mortality in broiler farms. Since outbreaks of IBV still occur in vaccinated flocks and the virus strains isolated are frequently different from serotypes of the vaccine strains used (8-10).

In previous molecular studies on Iranian IBVisolates,S, M,N and 3' UTR gene of theses have been sequenced and analyzed (11-13). In the present study, the E geneof genome fragment of 9 IBV field isolates fromIranian poultry farms were sequenced and compared with sequences from non-Iranian origin.

Methods

Samples

Tissue samples from suspected respiratory syndrome outbreaks in different provinces of Iranin broiler farms were collected. Trachea, lung and kidneys were obtained from 3 to 4 birds in each farm and transferred to the laboratory on ice. The flocks were vaccinated with H120,4/91 and MA5 vaccine. Two different strains of IBV vaccines (H120, Razi) and (4/91, Intervet) were used as positive control.

Virus Isolation

Ten-day-old SPF embryonated chicken eggs were inoculated according to American Association of Avian Pathologists procedures. These eggs were incubated at 37 C for up to 2 days, embryonic death was monitored, and then allantoic fluid was collected under routine conditions and the presence of viruses was determined by RT _PCR and sequencing.(Data has not been shown) .The allantoic fluids containing virus were harvested and stored at −70 °C until use.

RNA extraction

The Viral RNA was extracted from the allantoic fluid using *ExiPrep*™ Viral RNA Kit from Bioneer, Korea according to the manufacturer's instructions. For each RNA extraction, 200 µl allantoic fluid was used and RNA was eluted in 50 µl elution buffer. Extracted RNA was used immediately for cDNA synthesis or stored at -70˚C for later use.

RT-PCR

The cDNA synthesis was conducted using MMLV reverse transcriptase (200 U), random hexamers, RT- bufferand10 mM dNTP mixture in a total reaction volume of 20 (VIVANTIS TECHNOLOGIES, Malaysia). The thermo profile for the reverse transcription was 42°C for 45 min, 95°C for 5 min. The cDNA was stored at –20°C until use. The reaction mixture (50 µl) contained 5 µl of cDNA, 15 p moles of forward (CTG GTC AGC AAG TRR TWC AAM AAA TCA G) and reverse primers (GTT CAC AYT TAG CAA GCC ACT GRC C) (4 µl), and

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VIVANTIS master mix (VIVANTIS TECHNOLOGIES, Malaysia). The amplification protocol was: One step of denaturation at 95°C for 3 min, 35 cycles of 94°C /45 sec,56°C /45 sec , and 72°C/70 sec, and final extension at 72°C for 10 min.

Agarose Gel Electrophoresis

The RT-PCR and nested PCR products were electrophoresed on a 1% agarose gel in Tris-acetate-EDTA buffer (40 m *M*of Tris and 2 m *M*of EDTA, with a p H value of 8.0) containing ethidium bromide for 45 min at 100 V and visualized under ultraviolet light.

PCR product clean up and sequencing

The amplified products were purified from agarose gel using PCR AccuPrep® PCR Purification Kit 50 reactions (BioneerCo., Korea). Purified PCR products were used as a template for sequencing on an Applied Biosystems 373S automated DNA sequencer GenBank database accession numbers: M41 (DQ834384), H120 (FJ888351), IBN (EF602439), Connecticut 46 (FJ904717), Holte (GU393336), 4/91Like (EU526388), Delaware 072 (GU393332), Beaudette (AJ311317), MHVA59 (NC_001846), TGEV(AJ271965), SARS (NC_004718), Armidale (DQ490205), Cal1995 (FJ904714), UK/68/84 (X60712),V3-02(DQ490217), TCV (UE022526), Gray (GU393334).

Gene Bank Accession Number

The sequences are deposited in the GenBank through Bankit system with the accession numbers JQ231282- JQ231289.

Results

The E gene with full length was obtained for the nine IBV isolates. BLAST software and Megalign programs were used to determine the sequence similarity of the E gene from the 9 IBV isolates. The length of E gene nucleotide of IBV isolates was between 288-330 nt. (Most Isolates contain321nt.The Most of Iranian IBV isolates contains 106 amino acid (AA) as the same number as most of IBV strains isolated elsewhere .The range of AA length were between 95- 109. As shown in figure 1. Results indicated that nucleotide homology among these isolates with E genes was using dye terminator cycle sequencing chemistry (Perkin-Elmer/Applied Biosystems, Foster City, CA, U.S.A.). Purified PCR products were sequenced from both directions.

Sequence and phylogenetic analyses

Sequence assembling, editing and analyses were made using BioEdit version 7.0.9.0 and the Seqman program of DNA STAR lasergene5.03. Distance based neighbour-joining and character based Neighbor Joining phylogenetic trees were generated using the Molecular Evolutionary Genetics Analysis (MEGA) software v.5.0. The neighbour-joining algorithm was implemented with the Kimura2 parameter model using a transition-to-translation ratio of 2.0. The robustness of the groupings was assessed by bootstrap resampling of 1000 replicate trees. Sequences used for comparison or phylogenetic analysis in this study were obtained from the following between 80.1% to 100%.The percent calculated was based on amino acid is between 77.8%- 100 %. The nucleotide and the amino acid identity of Iranian IBV with other reference IBVs are listed in figures 2, 3.In the phylogenetic tree analysis with references of IBV strains; it is of interest that the Iranian IBV strains were clustered into tree different branches (Figure 4). More detailed results of phylogenetic finding will be discussed.

Discussion

Infectious bronchitis (IB) is currently one of the importantcontagious diseases in poultry production. It not onlycauses mortality in birds, but also causes the chicken to bemore sensitive to other pathogens such as E. coli. It canalso interfere with the immunization of NDV lentogenicvaccine strain such as Lasota. Immunization failure hasfrequently occurred and caused severe economic losses inrecent years in Iran. In Iran, IB was first reported in 1994, and IBVs were found nearly all over the country in the following years. Recombination in coronavirus is generally believed to occur via template switching mechanism (14).Gene 3 of IBV strains contained three ORFs, 3a, 3b, and 3c. Gene 3c and gene 5b were relatively more conserved than the other genes. Gene 3c

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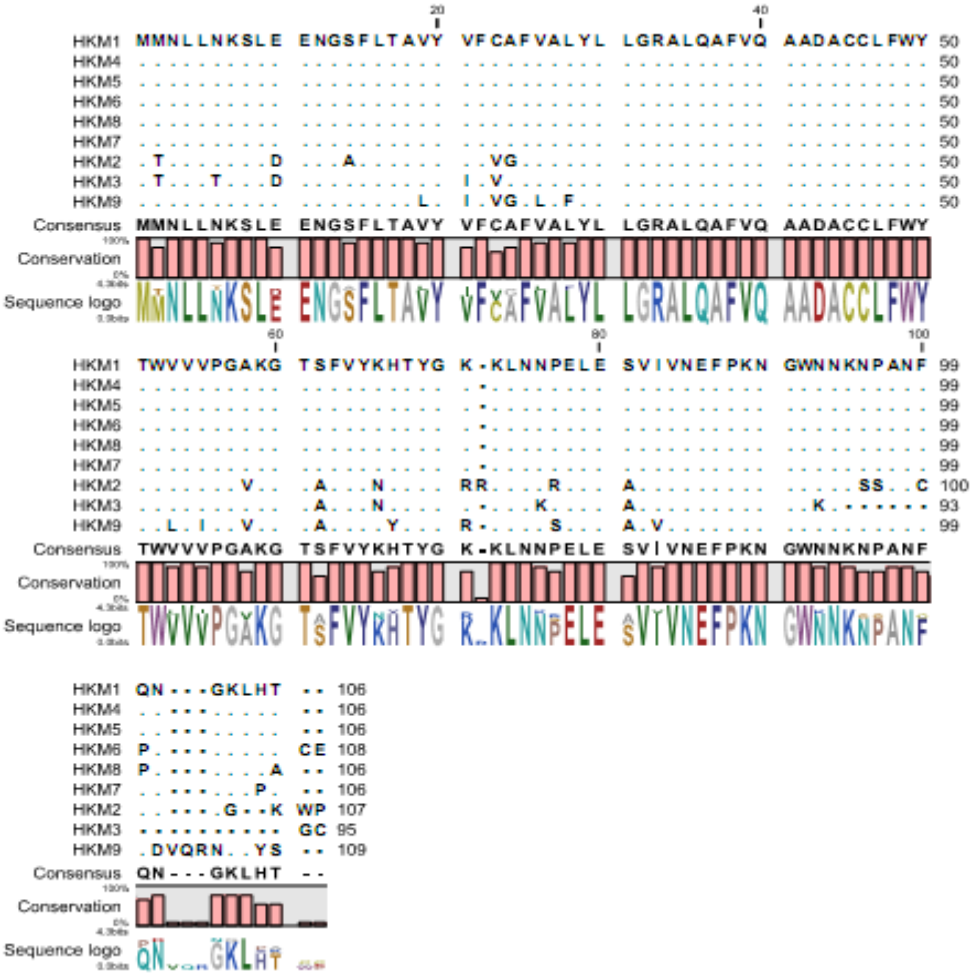


Fig. 1. Amino acid alignment of full length of Envelops protein of nine Iranian Infectious Bronchitis Virus strains (CLC Main workbench).

(E) encode envelop (small membrane) protein. For envelope protein has been determined tree functions:1) Pivotal roles in virion assembly and morphogenesis 2) Induction of apoptosis and 3) Ion channel activity (15). Hitherward, very few studies have been carried out on E gene of corona viruses and most research were on E gene of other corona viruses. In recent years, isolation and sequence analysis of IBV strains in Iran were frequently reported, and the results showed that there are multiple genotypes of IBV circulated in Iran (16, 17).

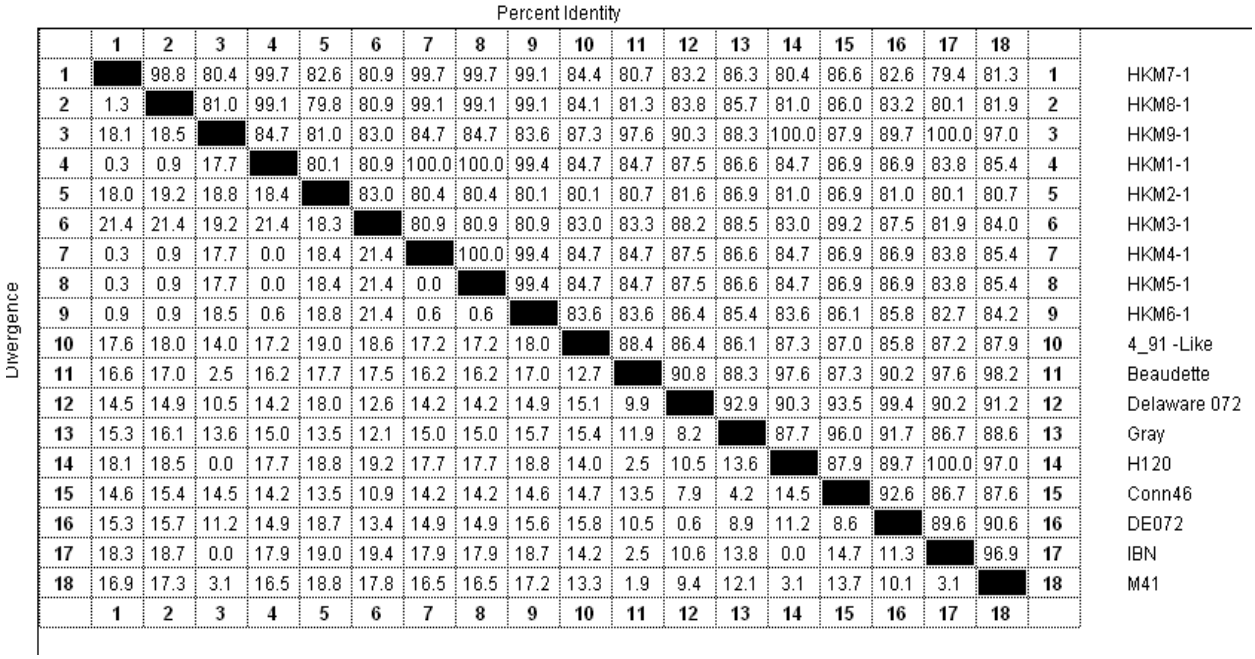
Previous studies on Iranian IBV isolates are be focused on S segment. These findings indicate the dominant serotype is 4/91.Hashemzadeh et al reported new serotype of IBV in Iran. (Unpublished Data)With regard to this subject that envelope is a conserved gene, this difference between Iranian IBV isolates based on nucleotide and amino acid is highly significant. Phylogenetic analysis by Ghahremani et al on Iranian IBV based on both S1 gene nucleotide and amino acid deduced sequences showed that isolates of Iran were classified into two genotypes Massachusetts (3 isolates) and 4/91 (7 isolates) (16).

Molecular analysis of the nucleocapsid gene and 3' untranslated region of two infectious Bronchitis Virus field isolates from Iranian poultry farms showed 90% similarity to the commonly used IBV vaccine strains, H52 and H120. However, based on phylogenetic analyses, Iranian IBVs were found to cluster separately from the IBV vaccine strains used in Iran as well as other IBVs isolated in China, Australia and the United States (17).

The results presented here (Figure2) indicated that there were 3 clades of IBV based on E gene in Iran: 4/91Like, Massachusetts and

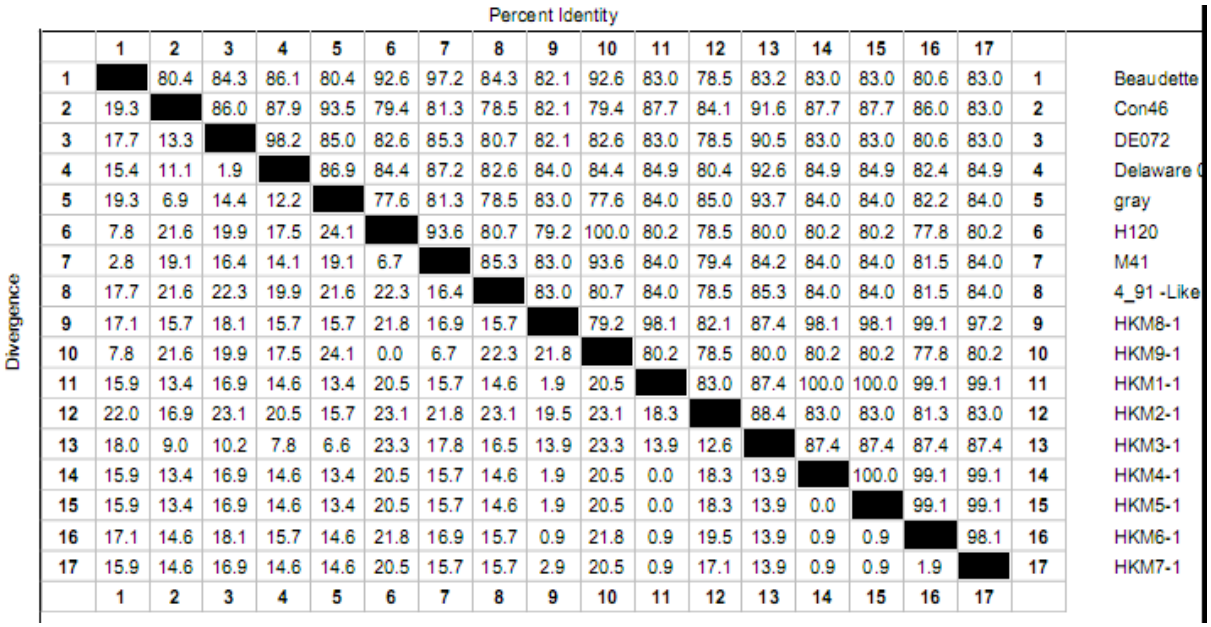
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	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18		
1		98.8	80.4	99.7	82.6	80.9	99.7	99.7	99.1	84.4	80.7	83.2	86.3	80.4	86.6	82.6	79.4	81.3	1	HKM7-1
2	1.3		81.0	99.1	79.8	80.9	99.1	99.1	99.1	84.1	81.3	83.8	85.7	81.0	86.0	83.2	80.1	81.9	2	HKM8-1
3	18.1	18.5		84.7	81.0	83.0	84.7	84.7	83.6	87.3	97.6	90.3	88.3	100.0	87.9	89.7	100.0	97.0	3	HKM9-1
4	0.3	0.9	17.7		80.1	80.9	100.0	100.0	99.4	84.7	84.7	87.5	86.6	84.7	86.9	86.9	83.8	85.4	4	HKM1-1
5	18.0	19.2	18.8	18.4		83.0	80.4	80.4	80.1	80.1	80.7	81.6	86.9	81.0	86.9	81.0	80.1	80.7	5	HKM2-1
6	21.4	21.4	19.2	21.4	18.3		80.9	80.9	80.9	83.0	83.3	88.2	88.5	83.0	89.2	87.5	81.9	84.0	6	HKM3-1
7	0.3	0.9	17.7	0.0	18.4	21.4		100.0	99.4	84.7	84.7	87.5	86.6	84.7	86.9	86.9	83.8	85.4	7	HKM4-1
8	0.3	0.9	17.7	0.0	18.4	21.4	0.0		99.4	84.7	84.7	87.5	86.6	84.7	86.9	86.9	83.8	85.4	8	HKM5-1
9	0.9	0.9	18.5	0.6	18.8	21.4	0.6	0.6		83.6	83.6	86.4	85.4	83.6	86.1	85.8	82.7	84.2	9	HKM6-1
10	17.6	18.0	14.0	17.2	19.0	18.6	17.2	17.2	18.0		88.4	86.4	86.1	87.3	87.0	85.8	87.2	87.9	10	4_91 -Like
11	16.6	17.0	2.5	16.2	17.7	17.5	16.2	16.2	17.0	12.7		90.8	88.3	97.6	87.3	90.2	97.6	98.2	11	Beaudette
12	14.5	14.9	10.5	14.2	18.0	12.6	14.2	14.2	14.9	15.1	9.9		92.9	90.3	93.5	99.4	90.2	91.2	12	Delaware 072
13	15.3	16.1	13.6	15.0	13.5	12.1	15.0	15.0	15.7	15.4	11.9	8.2		87.7	96.0	91.7	86.7	88.6	13	Gray
14	18.1	18.5	0.0	17.7	18.8	19.2	17.7	17.7	18.8	14.0	2.5	10.5	13.6		87.9	89.7	100.0	97.0	14	H120
15	14.6	15.4	14.5	14.2	13.5	10.9	14.2	14.2	14.6	14.7	13.5	7.9	4.2	14.5		92.6	86.7	87.6	15	Conn46
16	15.3	15.7	11.2	14.9	18.7	13.4	14.9	14.9	15.6	15.8	10.5	0.6	8.9	11.2	8.6		89.6	90.6	16	DE072
17	18.3	18.7	0.0	17.9	19.0	19.4	17.9	17.9	18.7	14.2	2.5	10.6	13.8	0.0	14.7	11.3		96.9	17	IBN
18	16.9	17.3	3.1	16.5	18.8	17.8	16.5	16.5	17.2	13.3	1.9	9.4	12.1	3.1	13.7	10.1	3.1		18	M41
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18		

Fig. 2. Nucleotide identities and divergences of IBV E gene Sequences. Comparison of the nine Iranian IBV field isolates and nine reference IBV strains of different serotypes (Obtained from Gene bank).



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17		
1		80.4	84.3	86.1	80.4	92.6	97.2	84.3	82.1	92.6	83.0	78.5	83.2	83.0	83.0	80.6	83.0	1	Beaudette
2	19.3		86.0	87.9	93.5	79.4	81.3	78.5	82.1	79.4	87.7	84.1	91.6	87.7	87.7	86.0	83.0	2	Con46
3	17.7	13.3		98.2	85.0	82.6	85.3	80.7	82.1	82.6	83.0	78.5	90.5	83.0	83.0	80.6	83.0	3	DE072
4	15.4	11.1	1.9		86.9	84.4	87.2	82.6	84.0	84.4	84.9	80.4	92.6	84.9	84.9	82.4	84.9	4	Delaware 0
5	19.3	6.9	14.4	12.2		77.6	81.3	78.5	83.0	77.6	84.0	85.0	93.7	84.0	84.0	82.2	84.0	5	gray
6	7.8	21.6	19.9	17.5	24.1		93.6	80.7	79.2	100.0	80.2	78.5	80.0	80.2	80.2	77.8	80.2	6	H120
7	2.8	19.1	16.4	14.1	19.1	6.7		85.3	83.0	93.6	84.0	79.4	84.2	84.0	84.0	81.5	84.0	7	M41
8	17.7	21.6	22.3	19.9	21.6	22.3	16.4		83.0	80.7	84.0	78.5	85.3	84.0	84.0	81.5	84.0	8	4_91 -Like
9	17.1	15.7	18.1	15.7	15.7	21.8	16.9	15.7		79.2	98.1	82.1	87.4	98.1	98.1	99.1	97.2	9	HKM8-1
10	7.8	21.6	19.9	17.5	24.1	0.0	6.7	22.3	21.8		80.2	78.5	80.0	80.2	80.2	77.8	80.2	10	HKM9-1
11	15.9	13.4	16.9	14.6	13.4	20.5	15.7	14.6	1.9	20.5		83.0	87.4	100.0	100.0	99.1	99.1	11	HKM1-1
12	22.0	16.9	23.1	20.5	15.7	23.1	21.8	23.1	19.5	23.1	18.3		88.4	83.0	83.0	81.3	83.0	12	HKM2-1
13	18.0	9.0	10.2	7.8	6.6	23.3	17.8	16.5	13.9	23.3	13.9	12.6		87.4	87.4	87.4	87.4	13	HKM3-1
14	15.9	13.4	16.9	14.6	13.4	20.5	15.7	14.6	1.9	20.5	0.0	18.3	13.9		100.0	99.1	99.1	14	HKM4-1
15	15.9	13.4	16.9	14.6	13.4	20.5	15.7	14.6	1.9	20.5	0.0	18.3	13.9	0.0		99.1	99.1	15	HKM5-1
16	17.1	14.6	18.1	15.7	14.6	21.8	16.9	15.7	0.9	21.8	0.9	19.5	13.9	0.9	0.9		98.1	16	HKM6-1
17	15.9	14.6	16.9	14.6	14.6	20.5	15.7	15.7	2.9	20.5	0.9	17.1	13.9	0.9	0.9	1.9		17	HKM7-1
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17		

Fig. 3. Amino Acid identities and divergences of IBV E gene Sequences. Comparison of the nine Iranian IBV field isolates and nine reference IBV strains of different serotypes (Obtained from Gene bank).

Gray Like. Most Iranian isolates were located in 4/91 clade. It is very close to results from phylogenetic analysis of S segment. These clades are very close to uk/68/84 and Pheasantcorana virus origin Strains.HKM2 & HKM9which are classified in Massachusetts group and HKM3islocated in Gray group.

Mardani *et al* (2008) sequencedthe7.5 kb of the genomes of 17 Australian IBV strains. Envelope gene nucleotide and amino acid identities were different between classical and novel genotype (Classical: Nuc (87%-97%), AA (84.6%-97.3%) Novel: Nuc (92.1%-9.6%), AA (90.9%-94.6%)) (18).

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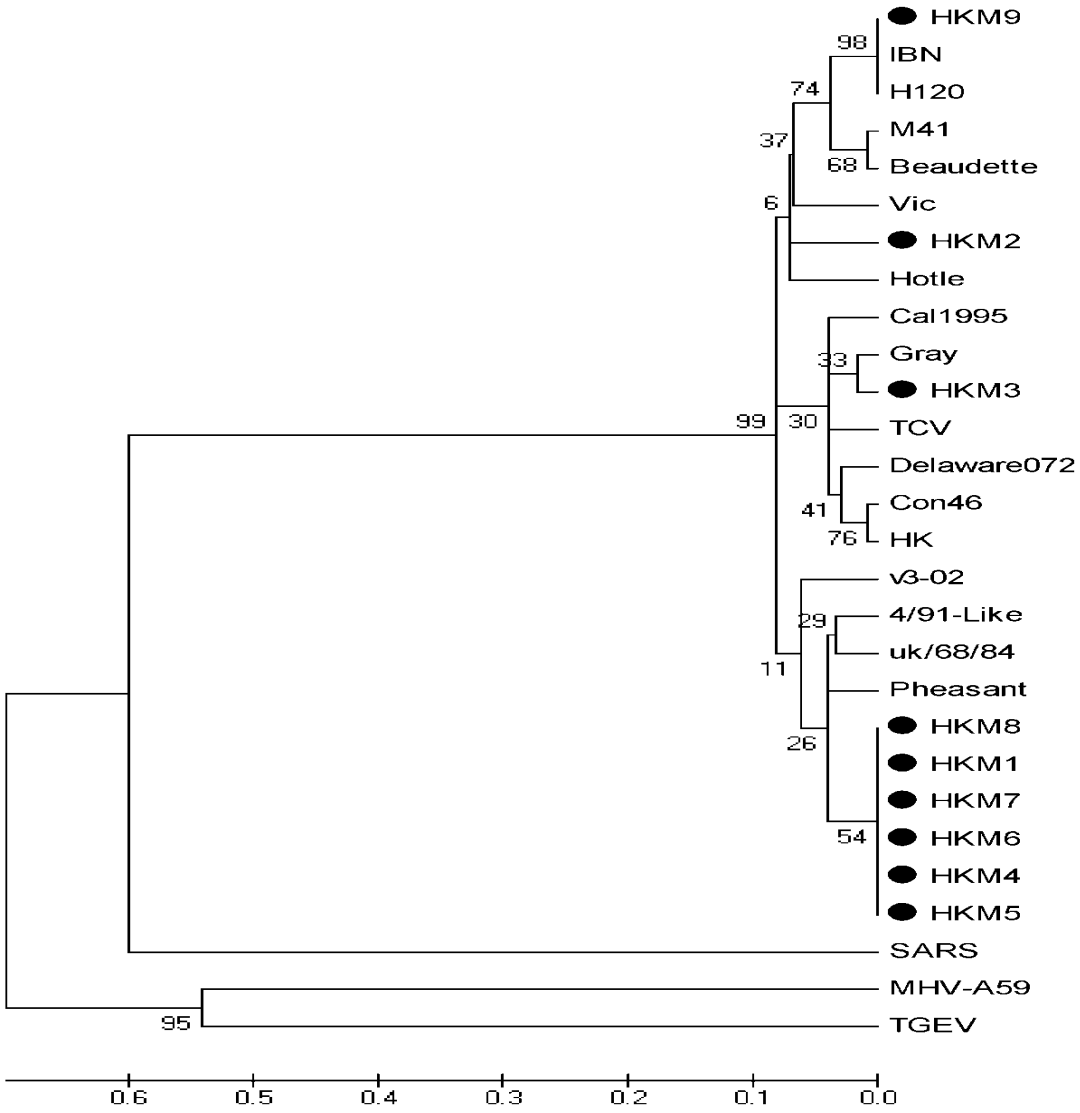


Fig. 4. Amino Acid Based Phylogenetic relationships of E genes of IBVs isolated from Iran. The Phylogenetic tree was generated using Neighboring Joining model with MEGA (version 5.1 beta). Numbers below branches indicate bootstrap value percentages from 1000 replicates, bootstrap values. Horizontal distances are proportional to the minimum number of Amino Acid differences required to join nodes. The vertical lines are for spacing branches and labels. Analysis was based on complete open reading frames of all gene segments. The scale bar represents the distance unit between sequence pairs. The viruses characterized in this report are indicated as Black Circle. The sequences obtained from Gene Bank.

In study of Book *et al* on comparisons of envelope through 5B sequences of infectious bronchitis , results indicate that the 5b open reading frame (ORF) having the greatest identity (94-99%) while the identities of the E, 5a and M ORFs ranged from 87 to 100% (19). Ammayappan *et al* sequenced whole genome of An infectious bronchitis virus Arkansas DPI (Ark DPI) virulent strain Among structural genes, S1has 97% identity with Ark 99; S2 has 100% identity with JMK and 96% to Conn; 3b 99%, and 3C to N is 100% identical to Conn strain. Ark DPI strain has high similarity (91%) with TCV based on E gene (20). Complete genome sequence analysis of a predominant infectious bronchitis virus (IBV) strain in China has been done. There are 699nt in gene 3, encoding non-structural protein3a, 3b and structural protein E with 174nt, 192nt and 327 nt, respectively. E

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protein was also the most similar to S14 and LDT3 with 95.5%identity.In phylogenetic analysis, strains grouped with most Chinese isolates into one Sub cluster (21). In complete genome sequence analyses of H120 strain (Zhang *et al*, 2010) , reveal that H120 E gene has nucleotide identities with other IBV strains between 73.9%-97.3%.There were three ORFs in gene 3 including 3a (174nt), 3b (195nt), and 3c (330nt),encoding proteins of sizes 57, 64, and 109 aa in length, respectively. Also, in the trees based on structural genes of S1, S2, E, M, H120 grouped with the Mass strains into one clade (14). The secondary structure of IBV E protein was predicted by using TMHMM Server v.20 through online submission http://www.cbs.dtu.dk/services/TMHMM), and the results were collected and analyzed by comparing the data manually. As secondary structure prediction, tree types of structures have been detected trough. The HKM3 has a different morphology. For more precise evaluation more biophysics and biochemistry should be carried out.

Therefore, it is safe to conclude that 4.91 like strains were predominant strains based on E gene that circulating in commercial chicken farms in Iran. Although that new genotype has been entered. In conclusion, the present study has demonstrated that the circulating IBV strains in commercial flocks in Iran based on E gene had were genetically diverse and underwent continuing evolution. Regarding the developing of turkey and pheasant breeding in Iran, Authors suggest that some researches and studies have been done and designed on Turkey and pheasant corona viruses. As high adverse effect of IBV on Iranian poultry industry for more detailed information and recombinant analysis, whole genome sequencing of Iranian IBV gene will been done.

This study manifests the importance of continuing surveillance and complete sequencing of old and new IBV strains in order to better prepare for next epidemic or pandemic outbreaks of IBV infections in commercial flocks.

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References

1. Jordan FTW, Pattison M. Poultry diseases: Bailliere Tindall; 2001.
2. Cavanagh D. Coronavirus avian infectious bronchitis virus. Vet Res. 2007;38(2):281-97.
3. Saif YM, Barnes HJ. Diseases of poultry. 12th ed. Ames, Iowa: Blackwell Pub. Professional; 2008.
4. Fields BN, Knipe DM, Howley PM. Fields virology. 5th ed. Philadelphia ; London: Wolters Kluwer Health / Lippincott Williams & Wilkins; 2007.
5. Bos EC, Luytjes W, van der Meulen HV, Koerten HK, Spaan WJ. The production of recombinant infectious DI-particles of a murine coronavirus in the absence of helper virus. Virology. 1996;218(1):52-60.
6. Lim KP, Xu HY, Liu DX. Physical interaction between the membrane (M) and envelope (E) proteins of the coronavirus avian infectious bronchitis virus (IBV). Adv Exp Med Biol. 2001;494:595-602.
7. Corse E, Machamer CE. Infectious bronchitis virus envelope protein targeting: implications for virus assembly. Adv Exp Med Biol. 2001;494:571-6.
8. Haghighat-Jahromi M, Asasi K, Nili H, Dadras H, Shooshtari AH. Coinfection of avian influenza virus (H9N2 subtype) with infectious bronchitis live vaccine. Arch Virol. 2008;153(4):651-5.
9. Seyfi Abad Shapouri MR, Mayahi M, Assasi K, Charkhkar S. A survey of the prevalence of infectious bronchitis virus type 4/91 in Iran. Acta Vet Hung. 2004;52(2):163-6.
10.Shoushtari AH, Toroghi R, Momayez R, Pourbakhsh SA, others. Ninetythree B type, the Predominant Circulating Type of Avian Infectious Bronchitis Viruses 1999-2004 in Iran: a retrospective study. Archives of Razi. 2009;63(1):1-5.
11.Mardani K, Noormohammadi AH, Ignjatovic J, Browning GF. Naturally occurring recombination

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between distant strains of infectious bronchitis virus. Arch Virol. 2001;155(10):1581-6.
12.Akbari Azad G, Marandi MV, Aminae HK. Molecular analysis of three Iranian isolated belonged to 793/B serotype of infectious bronchitis viruses. J Vet Res. 2007;62:69-80.
13.Haqshenas G, Asasi K, Akrami H. Isolation and molecular characterization of infectious bronchitis virus, isolate Shiraz 3. IBV, by RT-PCR and restriction enzyme analysis. Iranian J Vet Res University of Shiraz. 2005;6:9-15.
14.Zhang Y, Wang HN, Wang T, Fan WQ, Zhang AY, Wei K, et al. Complete genome sequence and recombination analysis of infectious bronchitis virus attenuated vaccine strain H120. Virus Genes. 2010;41(3):377-88.
15.Liu DX, Yuan Q, Liao Y. Coronavirus envelope protein: a small membrane protein with multiple functions. Cell Mol Life Sci. 2007 Aug;64(16):2043-8.
16.Ghahremani N, Fard MHB, Shoushtari H, Momayez R, Sheikhi N, Khoshzahmat A, et al. Molecular Analysis of Infectious Bronchitis Viruses Isolated in Iran from 1998-2008. Journal of Animal and Veterinary Advances. 2011;10(22):2961-7.
17.Majdani R, Mardani K, Morshedi A, VasfiMarandi M, Talebi A. Molecular analysis of the nucleocapsid gene and 3' untranslated region of two infectious Bronchitis Virus field isolates from Iranian poultryfarms. International Journal of Veterinary Research. 2011;5 (1): 53-8.
18. Mardani K, Noormohammadi AH, Ignjatovic J, Browning GF. Naturally occurring recombination between distant strains of infectious bronchitis virus. Arch Virol. 2010;155(10):1581-6.
19. Brooks JE, Rainer AC, Parr RL, Woolcock P, Hoerr F, Collisson EW. Comparisons of envelope through 5B sequences of infectious bronchitis coronaviruses indicates recombination occurs in the envelope and membrane genes. Virus Res. 2004;100(2):191-8.
20. Ammayappan A, Upadhyay C, Gelb J, Jr., Vakharia VN. Complete genomic sequence analysis of infectious bronchitis virus Ark DPI strain and its evolution by recombination. Virol J. 2008;5:157.
21. Liu XL, Su JL, Zhao JX, Zhang GZ. Complete genome sequence analysis of a predominant infectious bronchitis virus (IBV) strain in China. Virus Genes. 2009;38(1):56-65.

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