Turkish Journal of Veterinary & Animal Sciences

Volume 43 | Number 2 Article 8

1-1-2019

Sequence characterization of full-length S

YEGANEH YOUSEFI

MOHAMMAD REZA BASSAMI

GHOLAM ALI KALIDARI

MOHAMMAD MEHDI GHAHRAMANI SENO

TÜBİTAK

Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Research Article

Turk J Vet Anim Sci
(2019) 43: 235-243

doi:10.3906/vet-1808-19

Sequence characterization of full-length S_1 gene of infectious bronchitis viruses isolated from poultry farms in Khorasan Razavi, Iran

Yeganeh YOUSEFI[1], Mohammad Reza BASSAMI[†], Gholam Ali KALIDARI[2], Mohammad Mehdi GHAHRAMANI SENO[1,*]

[1]Department of Basic Sciences, School of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran
[2]Department of Clinical Sciences, School of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran

Received: 06.08.2018 • **Accepted/Published Online:** 04.03.2019 • **Final Version:** 04.04.2019

Abstract: Infectious bronchitis virus (IBV) causes heavy economic losses in chicken farms worldwide. Like many other viral diseases, vaccination is the gold standard approach in controlling and preventing the spread of IBV. However, IBV is continuously evolving and new strains of this virus emerge that, due to variable antigenic cross-reactivity, may reduce or nullify the protective effect of vaccination programs. Therefore, advance knowledge of the IBV strain(s) circulating in a region will be very useful in deciding which commercial vaccine(s) would be most effective. In this line, we analyzed the nucleotide sequence of the full-length S_1 gene of IBV viruses isolated from broiler and layer flocks in the northeast of Iran. Three to five birds from each of 19 suspected flocks were used to collect tissue samples and six individual virus isolates were obtained from these tissue samples. Homology analyses using S_1 gene nucleotide sequence data revealed that three out of our six isolates were closest to QX-like IBV strains and the rest had the best homology with Variant 2-like (IS/1494) strains. Phylogenetic analyses grouped our isolates into two clusters, one that included Chinese QX isolates and another formed of Variant 2-like isolates mainly reported in Middle Eastern countries. Our study shows that QX and Variant 2-like strains are major IBV viruses circulating in broiler and layer flocks of the northeast of Iran, an important fact that needs to be considered for vaccination programs.

Key words: Infectious bronchitis virus, infectious bronchitis virus, IBV-QX, IS/1494/06-like, S_1 gene

1. Introduction

Infectious bronchitis (IB) is an infectious viral disease prevalent among domestic fowl. This disease is caused by a member of the genus *Gammacoronavirus* known as infectious bronchitis virus (IBV). IBV infects chickens of various ages and initially replicates in the upper respiratory tract of the infected bird, but it may later spread and infect various parts of the enteric and urinary tracts including the kidneys, gonads, and gastrointestinal tract (1,2). Based on the age at infection and the immunity state of the farm, IBV in commercial chicken farms can cause various problems (3). The infection in 1-day-old chicks results in emergence of false layers later in life, while IBV infections in broiler farms cause immediate emergence of respiratory symptoms and the broilers' growth is adversely affected (3). Layer flocks infected with IBV show respiratory distress along with reduction in both egg quantity and quality (4).

The IBV genome is composed of a single-stranded, positive-sense RNA known to incur mutation and recombination during reproduction (3,4). Therefore, emerging new variants have become a continuous challenge that adversely affect the preventive efficiency of vaccination programs, mainly due to the poor cross-immunity among different serotypes of IBV (4).

The IBV genome codes for four structural proteins: the membrane glycoprotein (M), the envelope protein (E), the nucleocapsid protein (N), and the spike glycoprotein (S) (5). Posttranslational cleavage of the S glycoprotein generates two protein subunits named S_1 and S_2 (6). The S_1 subunit includes epitopes that determine the IBV serotype-specific antigens and induce virus-neutralizing antibodies in the host; hence, S_1 is widely used to classify IBV variants infecting poultry flocks around the world (7). High-resolution genome sequencing is more informative in determining the IBV variants and as such is now extensively practiced. The S_1 protein of IBV is a validated antigenic determinant and therefore its nucleotide sequence is used as a quick proxy in identifying new IBV variants that may challenge vaccination protocols (7).

Here we report the corresponding genomic sequence of the S_1 protein in IBV cases isolated from broiler and layer farms in the province of Khorasan Razavi in Iran.

* Correspondence: mgseno@um.ac.ir

The sequence data were subsequently used to evaluate the molecular relationship between the isolated IBV strains and the IBV strains circulating around the world, including the vaccinal strains.

2. Materials and methods

2.1. Sampling

Between 2015 and 2016, three to five suspected birds with the age range of 24 days to 20 months from each of 19 suspected flocks (broilers and layers) across Khorasan Razavi Province were used to collect tissue samples. Kidneys, cecal tonsils, and cystic oviducts were used for sampling and the samples from each flock were pooled. Six positive isolates belonging to six separate farms were identified.

2.2. Virus isolation

Collected tissue samples from the suspected birds were homogenized in PBS (10% w/v) containing penicillin (10,000 IU/mL), gentamicin (1000 mg/mL), and nystatin (5 mg/mL) using a mortar and pestle. The homogenates were then centrifuged at 1000 × *g* for 15 min at room temperature and the supernatants were transferred to –20 °C until used. To grow and isolate viruses, 0.1–0.2 mL of each homogenate was used to inoculate 9- to 11-day-old embryonated eggs. We used five embryonated eggs for each tissue sample. The inoculated eggs were incubated at 37 °C and 60% relative humidity and checked for viability of the embryos twice daily by candling. Embryo death during the first 24 h post inoculation (PI) was considered nonspecific, but the mortalities after that period were considered to be caused by the virus. The embryos that died at 2–6 days PI were used to harvest their chorioallantoic fluids. Inoculated embryos that survived past 6 days of inoculation and those that survived 4 days past the second passage were first incubated at 4 °C for a whole day before harvesting their chorioallantoic fluid. The collected chorioallantoic fluids were stored at –80 °C until used for passaging in the embryonated eggs or for RNA extraction. The dead embryos were grossly examined for the effects of the virus.

2.3. RNA extraction and cDNA synthesis

An RNA isolation kit-III kit (Cat # S-1020-1, DENA Zist, Iran) was used to extract RNA from allantoic fluid according to the manufacturer's protocol. An AccuPower CycleScript RT PreMix kit (Cat # K-2045, Bioneer, Korea) was used to prepare cDNA using the extracted viral RNAs according to the manufacturer's protocol.

2.4. Primers, PCR, and sequencing

The details of the primers that were used to amplify the genomic region coding for the S_1 subunit are provided in Table 1. These primers have already been used by other researchers to amplify overlapping segments of the S_1 subunit (8). For PCR amplification the AccuPower PCR PreMix kit (Cat. # K-2012, Bioneer, Korea) was used under the following conditions: 95 °C/10 min, 35 cycles (95 °C/1 min, 54 °C/45 s, 72 °C/1 min), 72 °C/10 min.

After gel electrophoresis confirmation, the PCR products were sent to Macrogen Company (Korea) for purification and sequencing. Both forward and reverse primers were used for the sequencing procedure.

2.5. Sequence and phylogenetic analysis

The sequence data were analyzed, aligned, and assembled using CLC Main Workbench 5 software (http://www.qiagenbioinformatics.com/products/clc-genomics-workbench/). The phylogenetic trees were constructed using MEGA7 software (9). The maximum-likelihood method with 1000 bootstraps was used to construct the trees. The other S_1 sequences used in this study were obtained from the NCBI GenBank (10).

3. Results

3.1. Clinical and postmortem findings

Table 2 shows the details of the six IBV isolates, named IR/IBV/01, IR/IBV/02, IR/IBV/03, IR/IBV/07, IR/IBV/10, and IR/IBV/11, used for molecular analysis in this study. The samples used to isolate these viruses were obtained from six different infected farms that included two broilers and four layers farms. The infected birds showed respiratory clinical signs sometimes accompanied by nephritis symptoms such as pale kidneys, severe dehydration, and occasional sediments of urate in the kidney tubules.

The infected embryos inoculated with the viral isolates showed gross anatomical abnormalities such as stunting, curling, ruffled feathers, and dwarfing.

3.2. Molecular analysis of full-length S_1 gene

The genomic sequence of the S_1 subunit of the *S* gene encompassing about 1800 nucleotides was used for our

Table 1. The sequences and details of the primers used in this study.

Primers	Sequence (5' → 3')	Amplicon size	Reference
SF 1-1	GCCAGTTGTTAATTTGAAAAC	980 bp	Pohuang et al. (8)
SR1-1	TAATAACCACTCTGAGCTGT	980 bp	Pohuang et al. (8)
SF 1-2	ACTGGCAATTTTTCAGATGG	1065 bp	Pohuang et al. (8)
SR 1-2	AACTGTTAGGTATGAGCACA	1065 bp	Pohuang et al. (8)

sequence analysis of IBV isolates. Alignment analysis using the NCBI BLAST tool (10) revealed that three of the isolates, IR/IBV/03, IR/IBV/07, and IR/IBV/10, had the highest (99%) nucleotide homology with the QX strains while the other three isolates, IR/IBV/01, IR/IBV/02, and IR/IBV/11, showed 94% nucleotide homology with the IS/1494-like strains. Furthermore, pairwise comparison of the amino acid translations of the sequenced data displayed 76.64%–99.83% homology between our isolates, indicating different strains circulating in the region.

To develop a systematic view of the antigenic relationship between IBV isolates in our region and some other prevalent and important IBV isolates in the world, a pairwise amino acid comparison in the CLC Main Workbench 5 software was performed (Table 3). For this purpose, the amino acid translations of our sequence data were used to compare with the amino acid sequences from the following IBV strains deposited in GenBank: QX (AF193423), Variant 2 (AF093796), Variant 2/06 (JX027070), TR8 (KP259312), IS/1494/06 (EU780077), IS/720/99 (AY091552), IS/885 (AY279533), KR605489 (Massachusetts), and 793B (AY544778). Based on this analysis, at the amino acid level the S_1 subunits of the IR/IBV/03, IR/IBV/07, and IR/IBV/10 isolates were closest to the QX standard strain (AF193423) (~95.4%) and IR/IBV/01, IR/IBV/02, and IR/IBV/11 isolates showed the closest amino acid identity to the TR8 isolate (KP259312) (93%–94%) (Table 3).

All the previous sequences reported from Iran for the S_1 subunit partially cover this subunit. Therefore, in order to include the previously reported sequences from Iran in the analysis, in another CLC pairwise comparison we

Table 2. Some general information on the IBV strains isolated and used in this study.

Isolates	Year	Vaccinated	Nephropathogenic lesions	Chicken embryo passage	Flocks
IR/IBV/01	2016	Yes	Yes	2	Layer
IR/IBV/02	2016	No	Yes	2	Layer
IR/IBV/03	2015	Yes	Yes	2	Broiler
IR/IBV/07	2015	No	Yes	3	Layer
IR/IBV/10	2015	No	Yes	2	Broiler
IR/IBV/11	2016	Yes	Yes	2	Layer

Table 3. Pairwise comparison (percent homology) of S_1 amino acid sequences obtained from IBV strains isolated in this study and S_1 amino acid sequences from some select IBV strains, including vaccinal strains.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1.IR/IBV/01														
2.IR/IBV/02	98.7													
3.IR/IBV/03	78.19	77.26												
4.IR/IBV/07	78	77.8	99.44											
5.IR/IBV/10	78.19	77.26	99.63	99.81										
6.IR/IBV/11	100	98.7	78.19	78	78.19									
7. QXIBV	78.19	77.26	95.73	95.73	95.92	78.19								
8. IS720	85.37	84.26	77.92	77.74	77.92	85.37	77.74							
9. IS885	85.93	84.81	77.92	77.74	77.92	85.93	77.74	97.39						
10. Variant 2	87.59	86.48	78.85	78.85	79.04	87.59	78.85	82.5	82.87					
11. Variant 02/06	91.3	90.19	77.92	77.92	78.11	91.3	78.11	86.59	87.15	91.43				
12. IS1494	90.93	89.81	77.92	77.74	77.92	90.93	77.92	86.22	86.96	90.88	98.88			
13. TR8	94	93	78.29	78.11	78.29	94	78.29	86.41	86.96	91.25	98.51	98.14		
14. 793B	75.74	75.74	76.99	76.99	77.18	76.30	76.44	76.21	76.21	78.25	77.14	76.77	77.7	
15. H120	75.93	75	76.44	76.44	76.62	75.93	76.25	75.09	74.91	75.65	75.28	75.70	75.46	74.16

only used the region common between all these sequences (nucleotides 240–340), including ours. In this analysis we included S_1 sequences from some of the representative Variant 2-like strains from GenBank as well. This analysis revealed a rather high degree of homology ranging from 96.77% to 100% between the analyzed sequences (data not shown).

3.3. Alignment analysis of translated S_1 sequence

The full-length S_1 gene sequences from our isolates were translated and aligned with the amino acid sequences from some of the representative IBV strains reported around the world (Figure 1). The strains used in this analysis included QX, Variant 2-like, IS/720/99, IS/885, 793B, H120, IR-I, and IR-II strains. IR-I and IR-II are strains previously reported from Iran. This analysis showed that, based on the amino acid homology at the S_1 hypervariable region, three of our isolates, iIR/IBV/03, IR/IBV/07, and IR/IBV/10, closely replicate Chinese isolates, and the other three of our isolates, IR/IBV/01, IR/IBV/02, and IR/IBV/11, resemble strains reported from the Middle East (Figure 1). This grouping with Chinese and Middle Eastern isolates was also evident when the amino acid sequences of S cleavage sites were considered (Table 4).

3.4. Phylogenetic analysis of IBV isolates

The translated full-length S_1 gene from our isolates and those of 38 published IBV sequences retrieved from

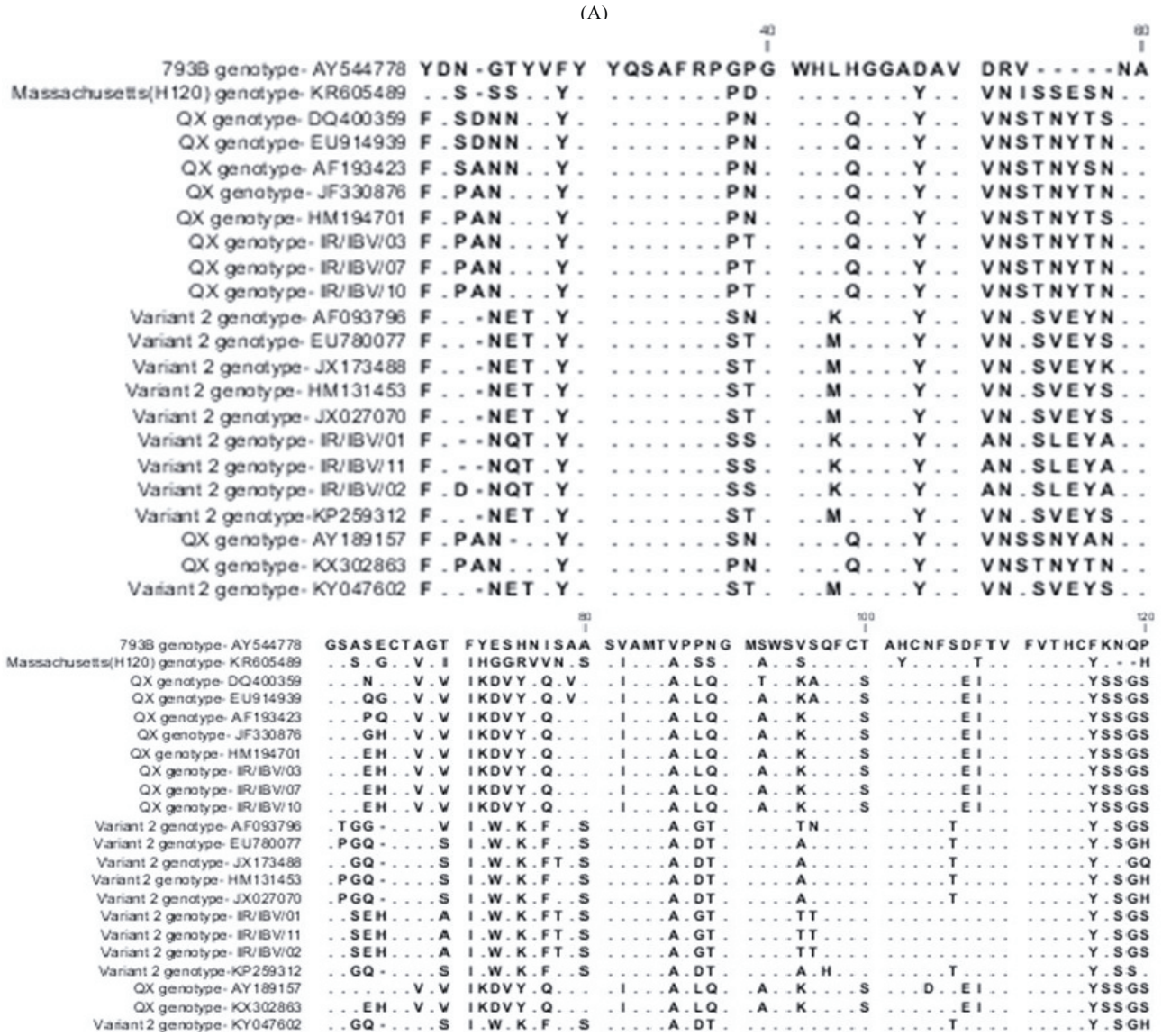

Figure 1. Multiple alignment of the IBV hypervariable regions. The amino acid sequences from the IBV strains isolated in this study and those of some representative IBV strains were used for this analysis (see Section 3 for details).

(B)

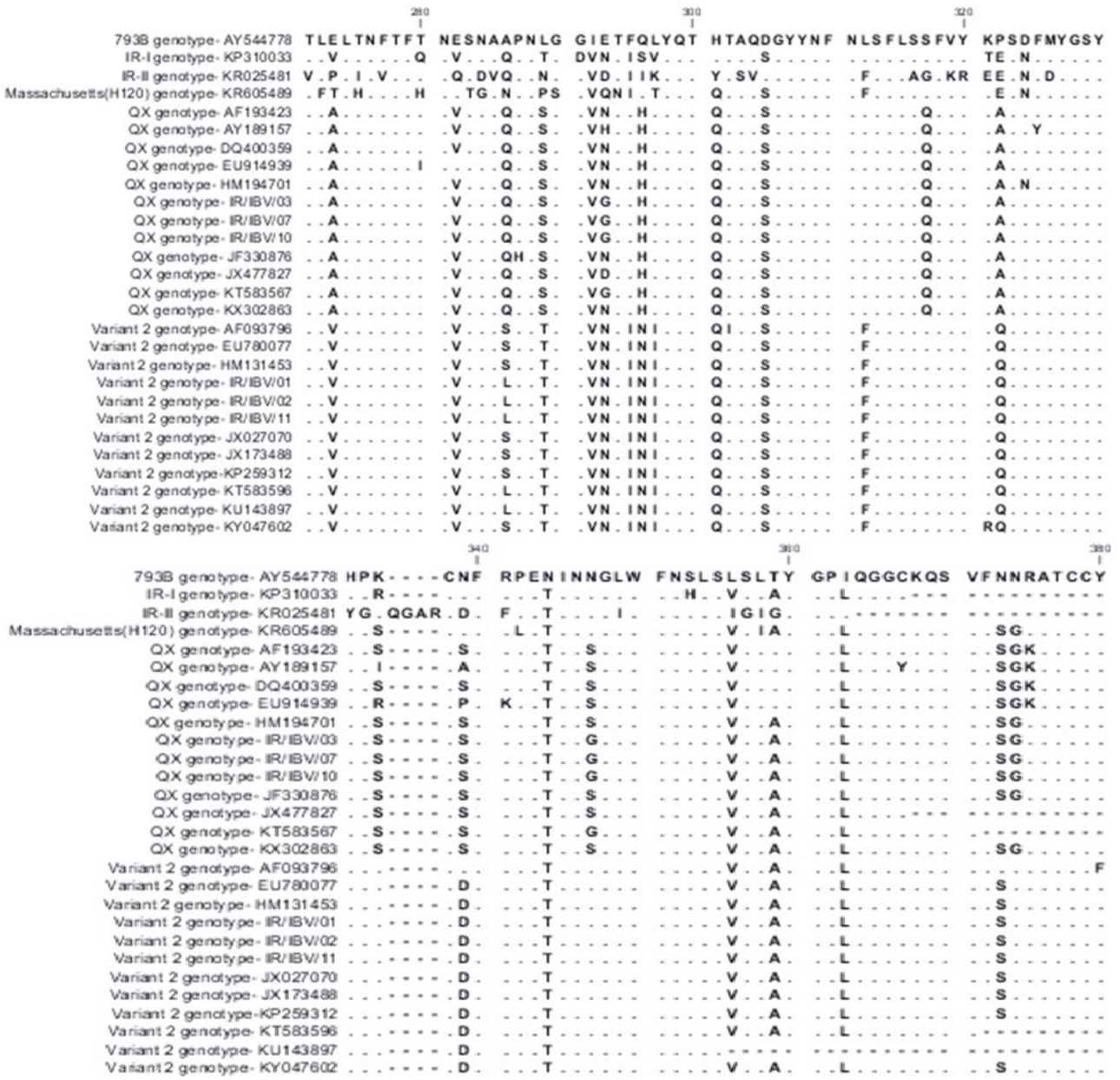

Figure 1. (Continued).

GenBank (10) were used for phylogenetic analysis using MEGA7 software. This analysis showed that three of our isolates, IR/IBV/03, IR/IBV/07, and IR/IBV/10, belong to the QX genotype and the other three, IR/IBV/01, IR/IBV/02, and IR/IBV/11, have common origin with Variant 2-like genotypes (Figure 2).

A pairwise analysis in CLC Main Workbench 5 using the same amino acid sequences used for phylogenetic analysis showed 88.75%–100% and 86.67%%–98.88% homology between the QX and Variant 02-like genotypes, respectively (data not shown).

Most of the available S_1 gene sequence data for Iranian isolates do not cover the full length of the gene. Therefore, we included the available sequence data for Iranian isolates in a separate phylogenetic analysis where we used the S_1 sequence data common to all available Iranian isolates plus the corresponding sequences reported in the Middle East and reference sequences for the QX strain (Figure 3). Similar to previous reports from Iran, this analysis indicated that our IR/IBV/01, IR/IBV/02, and IR/IBV/11 isolates were closely related to IBV isolates reported from the Middle East, though they were grouped in a separate

Table 4. The amino acid specifications of the hypervariable regions and cleavage sites of IBV strains isolated in this study plus those of some selected representative IBV strains (QX isolates: HM194701, JF330876, KX302863; Variant 2-like isolates: KY047602, KP259312; IS/720/99 isolate: AY091552), including vaccinal strains (Z83979 (793B), KR605489 (H120)).

Strains	Substitutions					**Insertions**		**Cleavage site**s
	38	54	72–75	107, 108	295–297	24	141, 142	
Z83979	G	F	YESH	HV	FQL	-	--	RRSRR
KR605489	P	S	HGGR	DT	IQT	-	--	Not available
IR/IBV/03	P	T	KDVY	EI	FHL	A	--	HRRRR
IR/IBV/07	P	T	KDVY	EI	FHL	A	--	HRRRR
IR/IBV/10	P	T	KDVY	EI	FHL	A	--	HRRRR
HM194701	P	T	KDVY	EI	FHL	A	--	HRRRR
JF330876	P	T	KDVY	EI	FHL	A	--	HRRRR
KX302863	P	T	KDVY	EI	FHL	A	--	HRRRR
IR/IBV/01	S	S	YWSK	DF	INI	-	KG	RRTRR
IR/IBV/02	S	S	YWSK	DF	INI	-	KG	RRTRR
IR/IBV/11	S	S	YWSK	DF	INI	-	KG	RRTRR
KY047602	S	S	YWSK	DF	INI	-	--	RRTRR
KP259312	S	S	YWSK	DF	INI	-	--	RRTRR
AY091552	S	S	GWSK	DF	INL	-	--	RRTRR

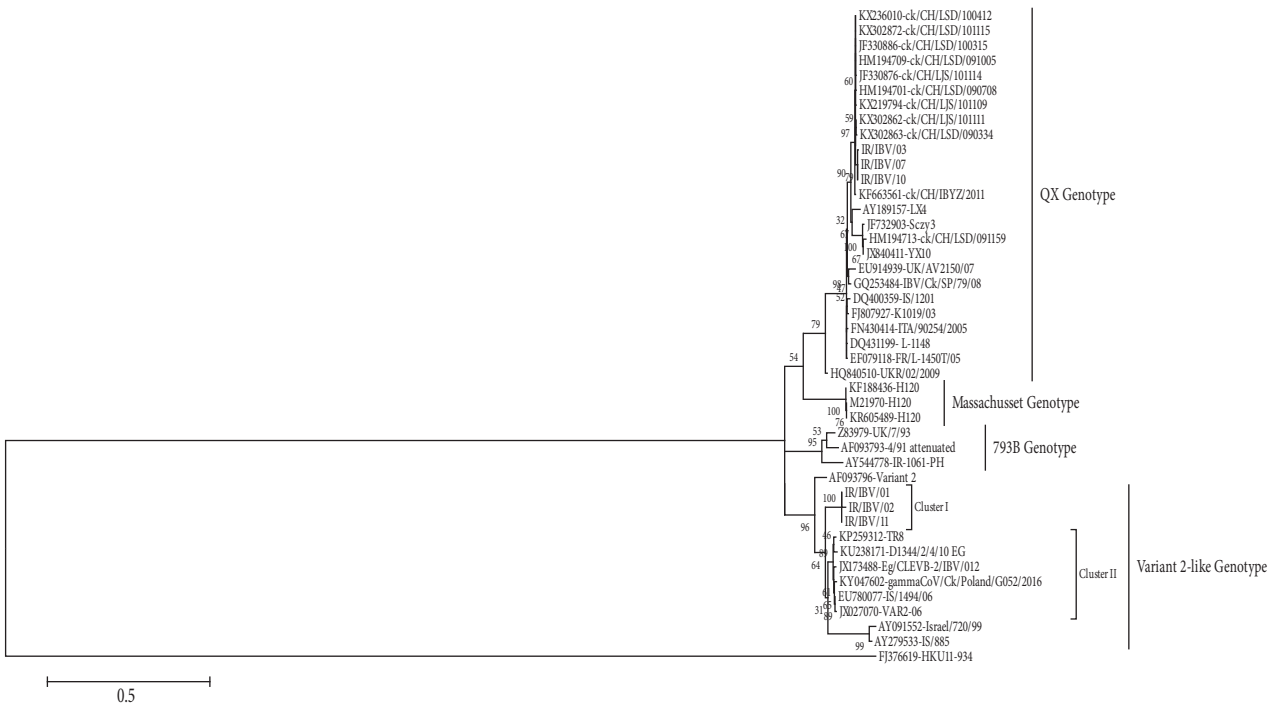

Figure 2. The phylogenetic tree was prepared by using S_1 amino acid sequences. The S_1 amino acid sequences from IBV strains isolated in this study and those from some other representative and reference IBV strains were used to infer relationships using phylogenetic analysis. Maximum likelihood with 1000 bootstraps approach was used for this analysis.

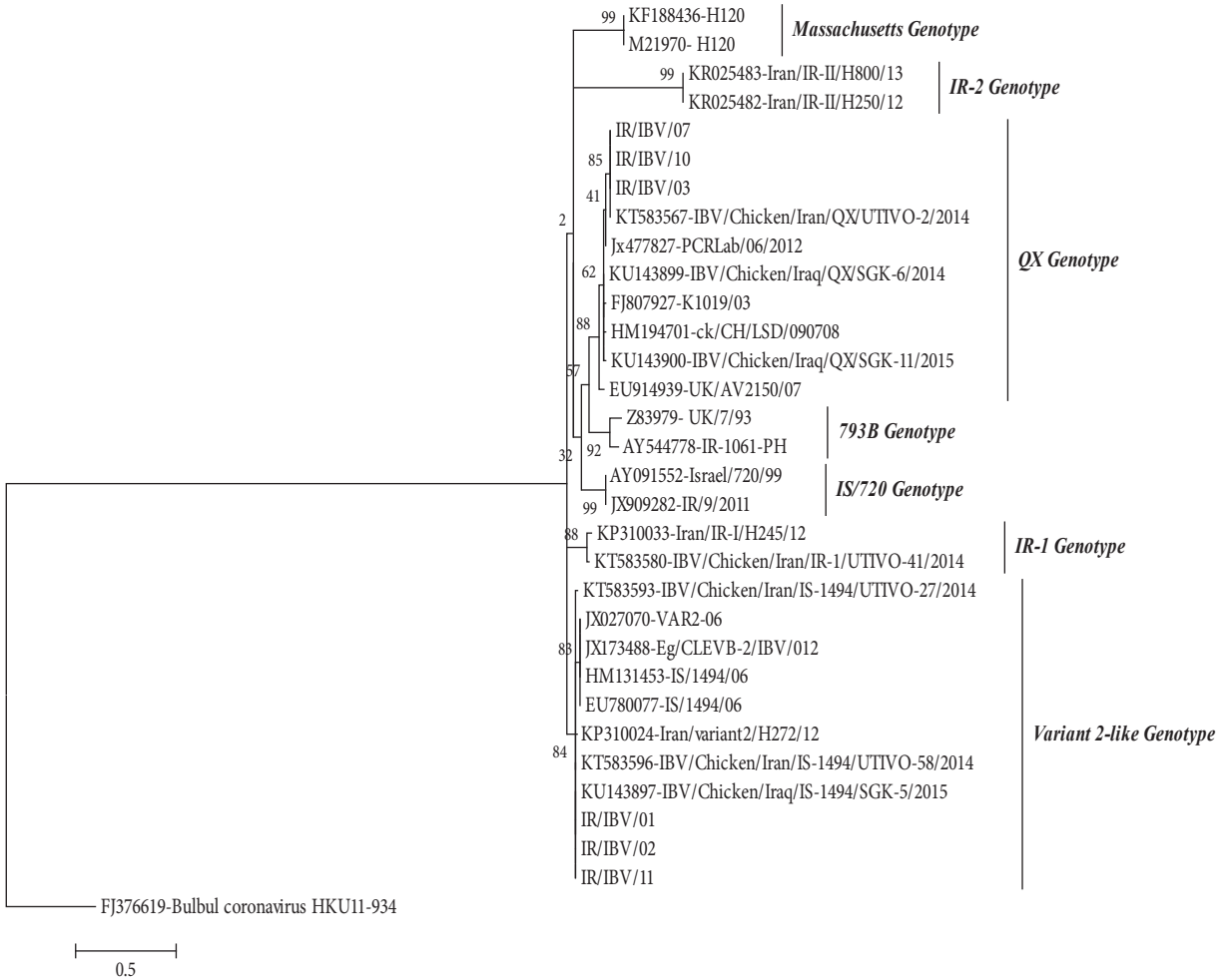

Figure 3. The phylogenetic tree was prepared by using partial amino acid sequence of S_1. Only the amino acid sequences of the S_1 subunit that were commonly present in the IBV strains isolated in this study and some other representative and reference IBV strains including those reported from Iran and Iraq were used to infer relationships using phylogenetic analysis. Maximum likelihood with 1000 bootstraps approach was used for this analysis.

cluster. Our other three isolates, IR/IBV/03, IR/IBV/07, and IR/IBV/10, which in the initial phylogenetic analysis (Figure 3) clustered with the QX-like genotypes, grouped with the Chinese reference sequence. This cluster also contained IBV isolates previously reported from Iran.

4. Discussion

Various strategies, such as continuous alteration of antigenic determinants, help animal viruses to successfully evade the host's immune system reaction. Vaccination based on using pathogens' antigenic determinants to train host immune systems is the gold standard approach to protect food animals against pathogenic viruses (7). However, the protective effect of vaccination diminishes or is even nullified by the antigenic changes resulting from the genetic variations commonly happening in the genome of many pathogenic viruses (5).

Hence, comprehensive knowledge of viruses' genotypes prevalent in each geographic region plus continuous surveillance for detection of emerging genotypes is necessary for having a successful and healthy farming plan.

IBV is an avian pathogen that incurs a huge economic burden for poultry farmers around the world (2). The IBV genomic region that includes *S* genes, and specifically the S_1 gene, has been considered a main region coding for antigenic determinants (7). Reports from around the world show that the S_1 gene has endured continuous nucleotide variations, many of which translate into amino acid changes resulting in antigenic shifts (11). Accordingly, various genotypes, so called Variant 1 (12), Variant 2 (13), Variant 2-like (14), QX (15), and QX-like (5), are being reported for IBV from various geographic regions around the world. Of note, the IBV strains used for commercial vaccines are considerably different from these variants with

regard to the amino acid sequences of their S_1 subunits. For instance, considering the S_1 amino acid sequence, Variant 2 and QX strains isolated in Iran showed ~82.44%, and ~78.6% homology to the Massachusetts vaccine strains, respectively. These figures for the 793B vaccine strain sum up to 83.5% and 85.1%, respectively (16–18).

The S_1 gene includes regions that comparatively display high rates of sequence variations (19). These regions are called hypervariable regions and are routinely used by investigators to determine the IBV genotype. Since other parts of S_1 that do incur variations as well are also important in antigenicity, we sequenced the full-length S_1 gene in 6 isolates from samples collected in the northeast of Iran.

Our molecular analysis of the sequence data that included pairwise and multiple alignment and phylogenetic analyses showed that two different strains, Variant 2-like and QX-like, are circulating in the region (Figures 2 and 3). Previous investigations have shown that Variant 2-like strains, first reported in the Middle East from Israel in 2001, are the most prevalent IBV strains in Middle Eastern countries, including Jordan (14), Iraq (20), Egypt (14), Saudi Arabia (14), Oman (14), Lebanon (14), and Kuwait (14). Furthermore, Variant 2-like strains have also been reported in Turkey (21,22).

Our pairwise alignment based on the amino acid sequence of the full-length S_1 showed that our Variant 2-like strains are closest (94% homology) to strains isolated from Turkey (Table 3).

The IBV QX strain was first reported in China in 1997 (15). Later, QX-like strains were reported from Russia and some other European and Asian countries (5). Although QX-like strains have been reported from some Middle Eastern countries including Iran, this strain is much less frequent than the Variant 2- like strains in Iran (16).

Three of our isolates clustered with the QX strains reported from China, based on the amino acid sequence of full-length S_1 (Figures 2 and 3). The pairwise alignment of the amino acid sequences showed great homology (~99%) between our QX-like isolates and those reported from China, but this homology was lower for QX-like strains reported from Iraq (data not shown). This may indicate a different origin for our QX-like strains than those in Iraq. Furthermore, our QX-like isolates showed a higher homology with the Chinese QX-like strains in the amino acid sequences of their S protein cleavage sites (Table 4). It is believed that the sequence of cleavage site correlates with geographic distribution (6), and therefore, this may indicate for a possible common origin for some of the QX-like strains reported from China and our QX-like isolates.

Since the available S_1 sequence from Iranian isolates covers part of the S_1 gene, in a separate analysis we included only the sequence common between our isolates and the Iranian reports. We also included in this analysis reference sequences of QX strains and a few sequences from isolates in Middle Eastern countries, and also vaccinal strains. The phylogenetic analysis run on these amino acid sequences again showed that our QX-like isolates are closer to those reported from China (Figure 2).

Our work shows the presence of IBV sequence variants in the northeast of Iran that have not only deviated from the commercial IBV strains but also show considerable differences from most prevalent strains in the region. Taking into account the continuous decline in protection efficacy of commercial vaccines (7,23), a direct and effective collaboration between clinicians, molecular virologists, and vaccine providers is necessary for developing dynamic vaccination strategies tailored to the patterns observed in the field.

References

1. Uenaka T, Kishimoto I, Sato S, Animas S, Ito T, Otsuki K, Cook JKA. Intracloacal infection with avian infectious bronchitis virus. Avian Pathol 1998; 27: 309-312.
2. Cavanagh D. Coronavirus avian infectious bronchitis virus. Vet Res 2007; 38: 281-297
3. Cavanagh D, Naqi S. Infectious bronchitis. In: Saif YM, Barnes HJ, Glisson JR, Fadly AM, McDougald LR, Swayne DE, editors. Diseases of Poultry. 11th ed. Ames, IA, USA: Iowa State University Press, 2003. pp. 101-119.
4. Cook JK, Jackwood M, Jones R. The long view: 40 years of infectious bronchitis research. Avian Pathol 2012; 41: 239-250.
5. Jackwood MW. Review of infectious bronchitis virus around the world. Avian Dis 2012; 56: 634-641.
6. Jackwood MW, Hilt DA, Callison SA, Lee CW, Plaza H, Wade E. Spike glycoprotein cleavage recognition site analysis of infectious bronchitis virus. Avian Dis 2001; 45: 366-372.
7. De Wit J, Cook JK, Van der Heijden HM. Infectious bronchitis virus variants: a review of the history, current situation and control measures. Avian Pathol 2011; 40: 223-235.
8. Pohuang T, Chansiripornchai N, Tawatsin A, Sasipreeyajan J. Sequence analysis of S_1 genes of infectious bronchitis virus isolated in Thailand during 2008–2009: identification of natural recombination in the field isolates. Virus Genes 2011; 43: 254-260.
9. Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Mol Biol Evol 2016; 33: 1870-1874.
10. NCBI Resource Coordinators. Database resources of the national center for biotechnology information. Nucleic Acids Res 2016; 44: 7-19.
11. Kusters J, Niesters H, Lenstra J, Horzinek M, Van Der Zeijst B. Phylogeny of antigenic variants of avian coronavirus IBV. Virology 1989; 169: 217-221.

12. Abdel-Moneim A, Madbouly H, Gelb J, Ladman B. Isolation and identification of Egypt/Beni-Seuf/01 a novel genotype of infectious bronchitis virus. Vet Med J Giz 2002; 50: 1065-1078.

13. Callison S, Jackwood M, Hilt D. Molecular characterization of infectious bronchitis virus isolates foreign to the United States and comparison with United States isolates. Avian Dis 2001; 45: 492-499.

14. Ganapathy K, Ball C, Forrester A. Genotypes of infectious bronchitis viruses circulating in the Middle East between 2009 and 2014. Virus Res 2015; 210: 198-204.

15. Wang Y, Wang Y, Zhang Z, Fan G, Jiang Y, Liu X, Ding J, Wang S. Isolation and identification of glandular stomach type IBV (QX IBV) in chickens. Chin J Anim Quarantine 1998; 15: 1-3.

16. Hamadan AM, Ghalyanchilangeroudi A, Hashemzadeh M, Hosseini H, Karimi V, Yahyaraeyat R, Najafi H. Genotyping of Avian infectious bronchitis viruses in Iran (2015-2017) reveals domination of IS-1494 like virus. Virus Res 2017; 240: 101-106.

17. Saadat Y, Fard MHB, Charkhkar S, Hosseini H, Shaikhi N, Akbarpour B. Molecular characterization of infectious bronchitis viruses isolated from broiler flocks in Bushehr province, Iran: 2014-2015. Vet Res Forum 2017; 8: 195-201.

18. Nabavi H, Karimi V, Ghalyanchilangeroudi A, Shateri S, Seger W, Najafi H. Phylogenetic analysis and full-length characterization of S_1 gene of IS-1494 (Variant 2) like infectious bronchitis virus isolates, Iran, 2015. Virol Rep 2016; 6: 18-24.

19. Kant A, Koch G, Van Roozelaar D, Kusters J, Poelwijk F, Van der Zeijst B. Location of antigenic sites defined by neutralizing monoclonal antibodies on the S_1 avian infectious bronchitis virus glycopolypeptide. J Gen Virol 1992; 73: 591-596.

20. Seger W, Ghalyanchi Langeroudi A, Karimi V, Madadgar O, Marandi MV, Hashemzadeh M. Genotyping of infectious bronchitis viruses from broiler farms in Iraq during 2014-2015. Arch Virol 2016; 161: 1229-1237.

21. Yilmaz H, Altan E, Cizmecigil UY, Gurel A, Ozturk GY, Bamac OE, Aydin O, Britton P, Monne I, Cetinkaya B et al. Phylogeny and S_1 gene variation of infectious bronchitis virus detected in broilers and layers in Turkey. Avian Dis 2016; 60: 596-602.

22. Kahya S, Coven F, Temelli S, Eyigor A, Carli KT. Presence of IS/1494/06 genotype-related infectious bronchitis virus in breeder and broiler flocks in Turkey. Ankara Univ Vet Fak 2013; 60: 27-31.

23. Seyfi Abad Shapouri M, 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: 163-166.