TÜBİTAK

Turkish Journal of Veterinary and Animal Sciences

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

Short Communication

Turk J Vet Anim Sci
(2023) 47: 91-99

doi:10.55730/1300-0128.4272

New insights on epidemiology of infectious bronchitis virus in Iran by comparing two genotyping methods

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Received: 14.03.2022 • **Accepted/Published Online:** 07.12.2022 • **Final Version:** 10.02.2023

Abstract: Different genotypes of infectious bronchitis virus (IBV) in poultry are circulating worldwide. The most efficient classification system for IBV genotypes is based on the complete sequencing of S1 gene, and the target organ can be trachea, kidney, oviduct, lung, esophagus, proventriculus, intestine, liver, spleen, bursa, cecal tonsils, cloaca, and testis. In Iran, IBV genotyping is usually performed by partial sequencing of S1 gene, and the trachea is often selected for sampling. This study applies a different genotyping method, and compares the results with the routine method of genotyping. Samples were collected from the trachea and kidneys of 50 broiler flocks with respiratory symptoms. The presence of IBV was confirmed by a real-time RT-PCR analysis targeting the 5'UTR region of the genome. Genotype of positive samples was determined by two methods of partial S1 gene sequencing and genotype-specific primers. In the real-time RT-PCR test, 88% and 90% of tracheal and kidney samples showed positive results, respectively. When the S gene was sequenced, Variant 2 (GI-23) (68.18%), 793/B (GI-13) (22.73%), Massachusetts (GI-1) (6.82%), and QX (GI-19) (2.27%) were detected in the tracheal samples, whereas QX (GI-19) and Massachusetts (GI-1) were not diagnosed in the kidney samples. In the genotype-specific method, IBV genotypes Variant 2, 793/B, Massachusetts, and QX were detected in both tracheal and kidney samples. The results of genotype-specific method also demonstrated that 70% of tracheal samples and 62% of kidney samples were infected with a single IBV genotype, while 30% of tracheal samples and 38% of kidney samples were coinfected with different IBV types. When comparing two genotyping methods, the use of genotype-specific primers was superior to partial S1 gene sequencing, not only in detecting different IBV types, but also in efficiently applying them in both trachea and kidney.

Keywords: Avian Infectious Bronchitis, Iran, Genotyping, Diagnosis, Specific primers

1. Introduction

Infectious bronchitis is a highly contagious disease of chicken that can affect the upper and lower respiratory tract, kidneys, and genitourinary system. The causative agent is infectious bronchitis virus (IBV), which causes significant economic losses in the poultry industry. IBV belongs to the genus gammacoronavirus and the family *Coronaviridae*. Its genome is a single-stranded linear RNA with an approximate length of 27.6–32 kb that sequentially encodes 5'UTR, replicase gene 1a, replicase gene 1b, spike, gene 3a/b, envelope, membrane, gene 5a/b, nucleocapsid, and 3'UTR [1]. The spike (S)-glycoprotein plays the main role in the attachment of the virus to the host cell and the tissue tropism of the virus. It is the main target of neutralizing antibodies and determines the specificity of serotypes [2]. The S-glycoprotein is divided into two parts, S1 and S2, after translation. The high mutation and recombination rate of IBV has led to the emergence of new variants [3]. Phylogenetic analysis based on the sequencing of IBV S1 gene has revealed the existence of seven genotypes (GI-GVII), consisting of 35 genetic lineages, as well as recombinants between lineages [4]. Methods of IBV detection include virus isolation, tissue culture, immunohistochemistry, and in situ hybridization. Reverse transcription-polymerase chain reaction (RT-PCR) and the real-time RT-PCR test have been successful in detecting various RNA viruses [5]. The real-time RT-PCR based on the protocol of Callison et al. [6] (5'UTR) was used to detect IBVs circulating in Iran. At that time, a protocol developed by Worthington et al. [7] based on amplification of a hypervariable region of S1 gene followed by sequencing was used to characterize IBVs.

Several IBV genotypes have been reported from Iran. Six genotypes (Massachusetts (Mass), 793/B, QX, Variant 2 (IS -1494 like), IR1, IR2) were identified in the first comprehensive study [8]. The next study showed the

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presence of different genotypes with different prevalence rates [9]. Several studies showed an increase in Variant 2 genotype and a decrease in the frequency of the other genotypes [8,9,10]. This study was conducted to answer the question of whether such an increased prevalence of Variant 2 compared to other genotypes is real or due to other genotypes not being diagnosed if Variant 2 was present, by using two different genotyping methods.

2. Materials and methods

In 2017, tracheal and kidney tissue specimens were collected from 50 broiler flocks from eight provinces of Iran (Isfahan (8 flocks), Khorasan (8 flocks), Kurdistan (7 flocks), Mazandaran (7 flocks), Azerbaijan (5 flocks), Qazvin (5 flocks), Khuzestan (5 flocks), and Semnan (5 flocks). The chickens received a combination of Mass-type and 793/B-type IBV vaccines at one day of age. However, the birds showed clinical signs of respiratory disease suspicious for IBV. Five birds were selected from each flock to have their trachea and kidneys sampled (total 500 tissue samples). Five tracheal samples from each flock were pooled. In addition, five kidney samples from each flock were pooled so that in the end there was one pooled kidney sample and one pooled trachea sample from each of the 50 flocks. The pooled samples were homogenized in PBS and stored at –70^{0}C for RNA extraction.

RNA was extracted using CinnaPure-RNA (Sinaclone, Iran) according to the manufacturer's protocol. cDNA was synthesized using RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, Burlington, Canada) according to the product manual [11].

All samples were subjected to a real-time RT-PCR which amplified the 5′UTR conserved region of IBVs of any genotypes [6].

Genotype of samples, which gave positive results in the real-time RT-PCR, were determined by amplification and sequencing of partial S1 gene. A nested PCR, using SX1, SX2, SX3, and SX4 primers, which their sequences shown in Table 1 was applied [7]. Initial PCR was performed in a 20 µL volume in a mixture containing 2 µL of distilled water, 2 µL of the primers SX1 and SX2, 3 µL of cDNA, and 13 µL of Sinaclon 2x PCR master mix (Sinaclon, Iran). The amplification reaction was performed in an Eppendorf Master Cycler gradient thermocycler (Eppendorf, Hamburg, Germany) for an initial denaturation period at 94 °C for 2 min, and 35 cycles of denaturation at 94 °C for 15 s, annealing at 58 °C for 30 s, and at 72 °C for 30 s. The final step was performed at 72 °C for 10 min. For the nested PCR, a 1-µL aliquot of a 1:100 dilution of the first-round amplicons was subjected to the second round of amplification using the primers SX3 and SX4, and the same cycling procedures. The amplicon size was 390 bp. The RT-PCR products obtained from nested PCR using SX1, SX2, SX3, and SX4 primers were sent for Sanger sequencing to Bioneer Company (Bioneer, Korea). Multiple sequence alignments were performed with ClustalW, and a phylogenic tree was constructed with MEGA 7 software using the neighbor-joining method with 1000 bootstrap replicates to assign confidence levels to branches. The obtained sequences were aligned and compared with reference strains available at gene databases based on NCBI BLAST.

Table 1. Specific primers used for IBV genotyping.

No	Primer name	Sequences (5-->3)	Application	Product size (bp)	Reference
1	Var2 F	CCGTTTGTGTAAAGGTGTTTATATTG	Specific detection	283	In this study.
	Var2 R	TATGGCACCAGATGTGTCTAGG			
2	QX F	CAGTTTTTGTCACACATTGTTATAGT	Specific detection	659	In this study.
	QX R	GGTAAGACCCATACATAAAATCACT			
3	Mass F	CTCTTCATCTGGGTGTACTGTT	Specific detection	100	[30]
	Mass R	AGACAAAGCCATACCTGATGAC			
4	D274 F	CCGTAACCAAGAGAGACGGAAAGTGAG	Specific detection	227	In this study.
	D274 R	GTAAGTAATGCTACCCCTAACACAG			
5	793/B F	GTTGGTTAACATCTTCACAG	Specific detection	163	In this study.
	793/B R	GGTCTTATTACTAATGTAACTGAAG			
6	SX1	CACCTAGAGGTTTG T/C T A/T GCAT	Sequencing	390	[7]
	SX2	TCCACCTCTATAAACACC C/T TT			
	SX3	TAATACTGG C/T AATTTTTCAGA			
	SX4	AATACAGATTGCTTACAACCACC			

In parallel with genotyping based on partial S1 gene sequencing, all IBV positive samples were examined by specific primers detecting different genotypes of Mass, 793/B, Variant 2, and QX. The amplification reactions were performed in a final volume of 25 µL, using a 2X ready-to-use master mix containing 2 mM 123 MgCl2, 0.2 mM each dNTP, 10X PCR buffer, 2 U Taq DNA polymerase (Sinaclon, Iran), and 1µL 10 pmol concentration of each primer (Sinaclon, Iran). Sequences of primers are depicted in Table 1. Var2 F and Var2 R primer set were used in a PCR targeting Variant 2 genotype. QX F and QX R, Mass F and Mass R, D274 F and D274 R, 793/B F and 793/B R were primer pairs which were used to amplify QX genotype, Mass genotype, D274 genotype, and 793/B genotype, respectively. Thermal conditions were the same for each of the four PCRs including initial denaturation at 95 °C for 5 min, then 35 cycles of 95 °C for 30 s, 60 °C for 30 s, 72 °C for 30s, and a final step at 72 °C for 7 min.

3. Results

Real-time RT-PCR results with 5'UTR primers showed the presence of IBV in all 50 flocks, even within one organ. Virus was detected in the trachea of 44 flocks (88%), and in the kidneys of 45 flocks (90%); 39 flocks (78%) had both tracheal and renal infection.

Gene sequencing was performed (Figure 1). Four IBV genotypes were identified in the tracheal samples: Variant 2 (68.18%), 793/B (22.73%), Mass (6.82%), and QX (2.27%). However, Variant 2 (75.56%) and 793/B (24.44%) genotypes were detected in the kidney samples (Table 2). According to phylogenetic analysis, Variant 2 samples showed the greatest homology with viruses detected in Iran, Iraq, and Afghanistan in recent years. The QX viruses detected in this work showed high sequence similarity with viruses detected in Iran and Iraq in 2014 to 2015, and in China in 2017. Some of the 793/B viruses in this study showed close relationship to vaccine strain 4/91 (KF377577), and some of them had homologies with 793/B viruses previously detected in Iran, Pakistan, and Egypt. Mass viruses detected in this study showed homology with strains previously present in Iran and also with strain H120 (Figure 2). The prevalence of the different genotypes of Variant 2, 793/B, Mass, and QX detected with genotype specific primers in tracheal samples was 52%, 11%, 4%, and 2%, respectively. However, mixed infections were also detected in tracheal samples: Variant 2+793/B, Mass+793/B, and Variant 2+Mass+793/B in 18%, 6%, and 4% of samples, respectively. Among kidney samples, 51% of samples were infected with Variant 2 only, while 13% of samples were infected with genotype 793/B. The prevalence of the mixture of Variant 2 and 793/B was 26%, while Mass+Variant 2, Mass+Variant 2+793/B, Mass+793/B, and QX+Variant 2 were 2% (Table 2).

4. Discussion

Avian infectious bronchitis is a highly contagious disease that can spread rapidly in chicken flocks [12]. It is necessary to update our knowledge about the characterization of IBV types circulating in a given region at all times to ensure that the most effective control strategy is implemented. In Iran several studies have been conducted on IBV genotyping. In studies conducted between 2002 and 2011, mainly type-specific primers designated by Cavanagh et al. [13] were used to diagnose IBV types 793/B, Mass, and

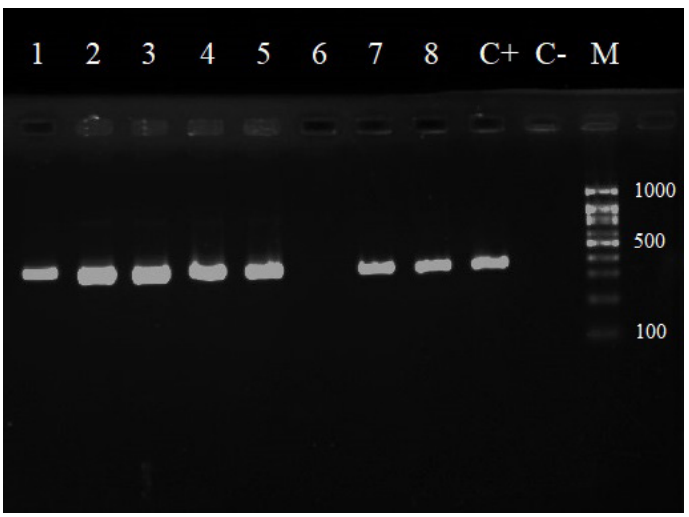

Figure 1. PCR products obtained from S1 gene amplification. Positive samples giving 390 bp band were sent for purification and sequencing. M: 100 bp DNA ladder, C-: Negative control, C+: Positive control, 1 to 8: samples.

Table 2. The summary of IBV genotyping of positive real-time IBV samples from broiler flocks based on using genotype-specific primers and the partial S1 gene sequencing. Rows highlighted with light gray showed negative results in 5´UTR real-time PCR and none of genotyping methods was conducted on their samples.

Tissue	Kidney					Trachea				
Methods	Specific primers				Partial S1 gene	Specific primers				Partial S1 gene
Flock no	QX	Variant 2	Massachusetts	793/B		QX	Variant 2	Massachusetts	793/B	
1										793/B
2					Variant 2					Variant 2
3					Variant 2					Variant 2
4					Variant 2					Variant 2
5					Variant 2					Variant 2
6					Variant 2					Variant 2
7					Variant 2					Variant 2
8					Variant 2					Variant 2
9					793/B					Variant 2
10										Variant 2
11					793/B					793/B
12					793/B					
13					Variant 2					Variant 2
14					Variant 2					Variant 2
15					Variant 2					
16					793/B					
17					Variant 2					
18					793/B					QX
19										Variant 2
20					Variant 2					Massachusetts
21					Variant 2					
22					Variant 2					793/B
23					793/B					793/B
24					Variant 2					793/B
25					Variant 2					Massachusetts
26					Variant 2					Variant 2
27					Variant 2					Variant 2
28					Variant 2					Variant 2
29					Variant 2					Variant 2
30					Variant 2					Variant 2
31					Variant 2					Variant 2
32										Variant 2
33					Variant 2					793/B
34					793/B					793/B
35					Variant 2					Variant 2
36					Variant 2					Variant 2
37					793/B					793/B

Table 2. (Continued).

38					Variant 2					Variant 2
39					793/B					Variant 2
40					Variant 2					Variant 2
41					Variant 2					
42										793/B
43					793/B					Massachusetts
44					793/B					Variant 2
45					Variant 2					Variant 2
46					Variant 2					793/B
47					Variant 2					Variant 2
48					Variant 2					Variant 2
49					Variant 2					Variant 2
50					Variant 2					Variant 2

D274. Depending on the type of primers used (Mass or 793/B type-specific primers), their results announced the presence of 793/B or Mass or both IBV types in Iranian poultry farms [14–17]. Later, researchers found five new genotypes (IS720, Variant 2, QX, IR-1, and IR-2) in Iran by RT-PCR of S1 gene and sequencing [8–10]. The results revealed that the prevalence of Variant 2 genotype of IBV progressively increased from 7% during a survey period from 2010 to 2014 [8], to 20% during a survey period from 2015 to 2017 [10], and to a prevalence of 85% in 2017 [18]. In Iran, Mass and 793/B vaccine strains are available, and various IB vaccination programs have been implemented. Recently, 793/B vaccines (4/91, IB88, iBird) were used in combination with Mass-type vaccines in day-old chicks. It has been shown that the use of two or more live attenuated IBV vaccines can produce extensive protective immunity against heterologous serotypes [19]. Habibi et al. [20] stated that the best crossprotection against IBV Variant 2 is 69%, when a combination of Mass and 793/B vaccines is used.

The current situation raises the question of whether the prevalence of Variant 2 genotype has actually increased and the prevalence of the other genotypes has decreased as a result of mass vaccination; or whether this is because the other genotypes were not diagnosed even though Variant 2 was present. To answer these questions, in the present study, we compared two different methods of genotyping (one, based on partial sequencing of S1 gene, and the other based on the use of four genotype-specific primers) to find a more sensitive method for diagnosing IBVs currently circulating in Iranian farms. The present study also includes a recent report on the molecular epidemiology of IBV in Iran in 2017.

Various methods are used for the detection and genotyping of IBVs. For IBV detection, conserved regions of the genome (5'UTR, polymerase, membrane, nucleoprotein, and 3' UTR) are usually targeted in a RT-PCR or real-time RT-PCR [3,6,21,22]. For genotyping, RT-PCR methods with specific primers followed by sequencing, or RT-PCR /RLFP on the spike gene are commonly used [10,13,23]. Genotype-specific primers has a disadvantage of not detecting new genotypes. On the other hand, primers amplifying S1 gene has a disadvantage of not amplifying IBVs due to the high mutation rate in this part of the genome. In virus isolation, which is considered the reference standard for IBV diagnosis, a disadvantage is that egg-adapted variants usually replicate faster and in higher titers, so that other IBV types may be missed in sequencing.

Lin et al. [24] reported a combination of PCR and RLFP for amplification of the S2 portion of six classical North American and nine Japanese IBV isolates. They were able to identify IBV isolates from the field, four of which were novel. Cavanagh et al. [13] designated genotype-specific primers and Mass, 793/B, D274, and D1466 were successfully detected. Keeler et al. [25] reported the specific-primer-PCR method was able to amplify IBV serotypes 793/B, Ark, JMK, Delaware, Mass, Conn, and California.

Phylogenetic analysis revealed that Variant 2 and QX genotypes from this study are closely related to viruses previously reported from Iran, Iraq, and Afghanistan. Uncontrolled trade of birds between Iran and neighboring countries may explain these sequence similarities. The type 793/B and Mass IBVs detected in this work had similarities to the corresponding vaccine strains, which is plausible given the routine vaccination programme in Iranian poultry farms. Prevalence results obtained from tracheal samples collected in this work, showed that Variant 2 was the most dominant genotype, and 793/B

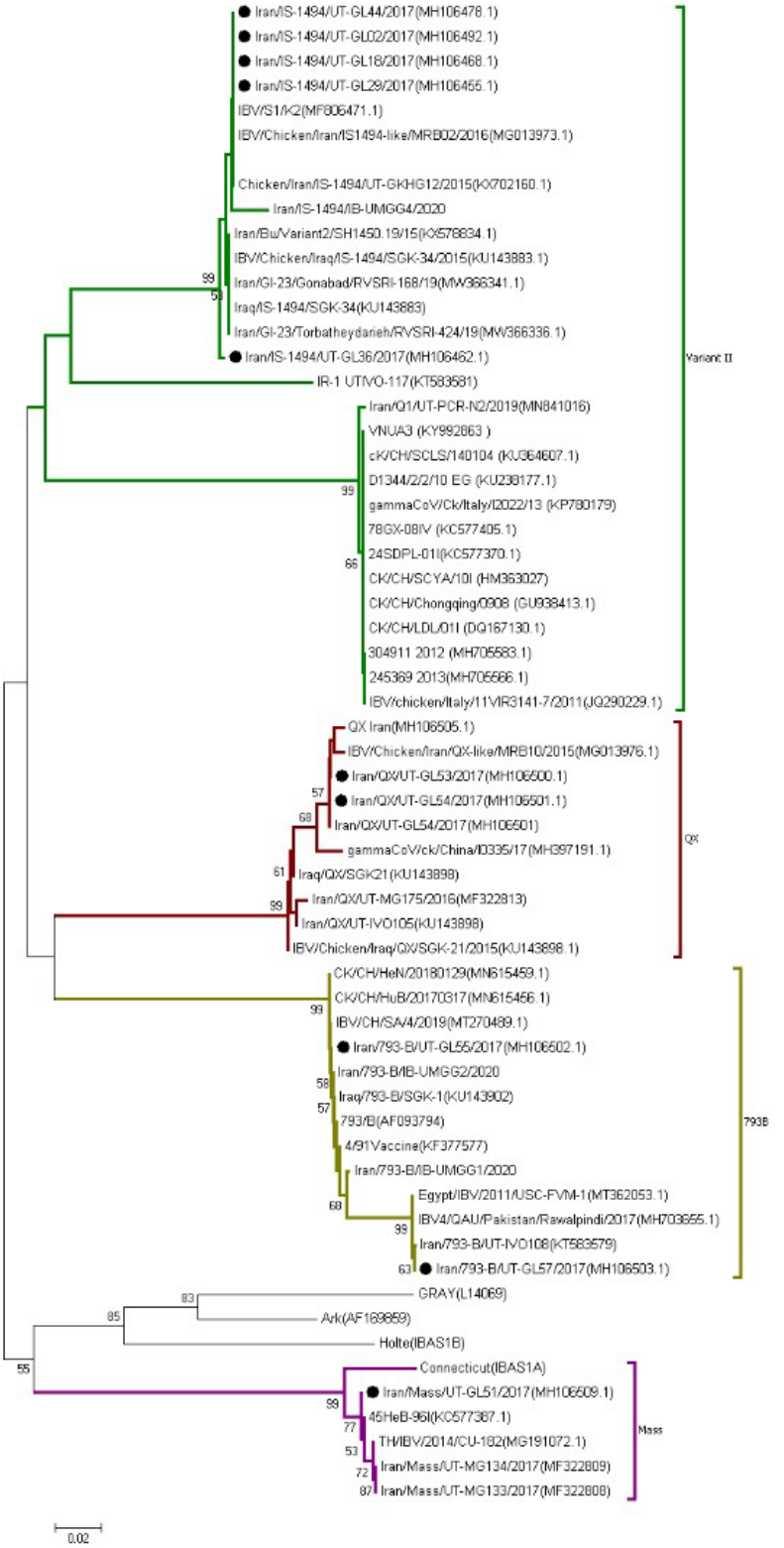

Figure 2. Phylogenetic tree based on a partial sequence of S1 gene, showing the relationship between the Iranian strains and other IBV strains. The neighbor-joining method was used with the Kimura 2-parameter substitution model and 1000 bootstrap replicates to assign confidence level to the branches of the phylogenetic tree. Some of the viruses identified in the current study along with accession numbers are indicated by black circles. The vertical lines are for spacing branches and labels. The scale bar represents the distance unit between sequence pairs. The sequences were obtained from GenBank.

was the second most common genotype, while Mass and QX were detected at lower incidence. In a comprehensive study conducted in Iran between 2010 and 2014, Variant 2 was most frequently detected and QX was next, whereas Mass and 793/B had the lowest prevalence with a slight difference from QX [8]. In two other studies, conducted from 2014 to 2015, and from 2015 to 2017, Variant 2 was the most frequently detected genotype, and 793/B was the second most common IBV type. QX and Mass were next [9,10]. The results of this work are relatively similar with recent studies on IBV epidemiology in Iran. The lower proportion of QX identified in this work may be an indication of the efficacy of heterologous vaccination against this genotype in Iran, which is consistent with crossprotection studies [26].

Another issue is that the target organs of all recent surveys were mainly trachea or pooled samples of trachea and kidney, whereas our study was the first work in Iran, in which IBV was detected separately in both trachea (88%) and kidney (90%). All recent data in Iran focused on the detection of the virus in a single organ or they pooled different organs together, so that comparison between different organs was not possible [8–10,16,18]. According to S gene sequencing results of the current study, the four IBV genotypes were detected in the trachea, whereas Mass and QX genotypes were not detected in the kidney. De Wit et al. [27] also showed that collection of samples from trachea and kidney is more accurate for IBV detection using S1 gene sequencing. Mass type was significantly more frequent in the trachea than in the kidney (11 times), genotypes 793/B, QX, Q1, Ark, and D274 were also more frequently detected in the trachea, while IBV types Xindadi and Italy-02 were more frequent in the kidney. Therefore, it is suggested to take samples from both the trachea and the kidney to obtain more valuable results.

In our study, Variant 2 and 793/B were detected more frequently in the kidney than in the trachea. Such higher incidence in kidney might be due to tropism of some IBV types that are latent in kidney [28].

In this study, two methods for genotyping IBV were compared. Genotype-specific primers showed high sensitivity, when each genotype-specific primer set was tested with different concentrations of the specific viral genotype, and high specificity when each genotype-specific primer set was tested with different IBV genotypes, Newcastle disease virus and influenza virus. When comparing two genotyping methods, the use of genotype-specific primers was superior to the partial S1 gene sequencing method, not only in detecting different types of IBV, but also in efficient application in both sampled organs. For example, when cocirculation of QX and Variant 2 occurred, QX was never detected with the partial S1 primer-based protocol (SX primer), which could be important for monitoring valuable herds in the first week of breeding [29]. Another example is that three different genotypes (Variant 2, Mass, and 793/B) were identified with specific primers in the trachea and kidney samples, but they were not detected by partial S1 gene sequencing. It is possible that the Variant 2 viruses have a greater tendency to bind with the SX primers or that the viral load of Variant 2 is higher than that of the other IBV genotypes in the samples. Because previous studies on the molecular epidemiology of IBV in Iran and the Middle East were usually based on spike gene sequencing, it is possible that the prevalence of some IBV genotypes is underestimated. However, the results of the current study using both genotyping methods confirmed the dominance of IBV Variant 2 in the Iranian poultry industry, and such a high prevalence may arise from the fact that the combination of Mass and 793/B vaccines does not provide adequate protection against Variant 2 [20].

5. Conclusion

Because spike sequencing-based genotyping may not be able to detect some IBV genotypes, it is recommended that genotype-specific primers be used to detect important genotypes in the region. Apart from this, given the constant emergence of new IBV variants, continuous monitoring of IBV types and also full S1 gene sequencing is recommended.

Acknowledgment

The University of Tehran financially supported this study (Grant No. 28692/6/10).

Ethical statement

The procedure was completed according to the instructor's guide and ethical standards for treating animals at the University of Tehran (date: November 21, 2018, 2018-1971).

References

1. Peng S, Wang Y, Zhang Y, Song X, Zou Y et al. Current knowledge on infectious bronchitis virus non-structural proteins: the bearer for achieving immune evasion function. Frontiers in Veterinary Science 2022; 9: 820625-820632. https://doi.org/10.3389/fvets.2022.820625

2. Zuo L, Yan W, Song Z, Li H, Xie X et al. Design and characterization of a DNA vaccine based on spike with consensus nucleotide sequence against infectious bronchitis virus. Vaccines 2021; 9 (1): 50-61. https://doi.org/10.3390/vaccines9010050

3. Jackwood MW, Jordan BJ. Molecular evolution of infectious bronchitis virus and the emergence of variant viruses circulating in the United States. Avian Diseases 2021; 65 (4): 629-634. https://doi.org/10.1637/aviandiseases-D-21-00104

4. Houta MH, Hassan KE, El-Sawah AA, Elkady MF, Kilany WH. The emergence, evolution and spread of infectious bronchitis virus genotype GI-23. Archives of Virology 2021; 166 (1): 9-26. https://doi.org/10.1007/s00705-020-04920-z

5. Legnardi M, Tucciarone CM, Franzo G, Cecchinato M. Infectious bronchitis virus evolution, diagnosis and control. Veterinary Sciences 2020; 7: 79-85. https://doi.org/10.3390/vetsci7020079

6. Callison SA, Hilt DA, Boynton TO, Sample BF, Robison R et al. Development and evaluation of a real-time Taqman RT-PCR assay for the detection of infectious bronchitis virus from infected chickens. Journal of Virological Methods 2006; 138: 60-65. https://doi.org/10.1016/j.jviromet.2006.07.018

7. Worthington KJ, Currie R, Jones RC. A reverse transcriptase-polymerase chain reaction survey of infectious bronchitis virus genotypes in Western Europe from 2002 to 2006. Avian Pathology 2008; 37: 247-257. https://doi.org/10.1080/03079450801986529

8. Hosseini H, Fard MHB, Charkhkar S, Morshed R. Epidemiology of avian infectious bronchitis virus genotypes in Iran (2010–2014). Avian Diseases 2015; 59: 431-435. https://doi.org/10.1637/11091-041515-ResNote.1

9. Najafi H, Langeroudi AG, Hashemzadeh M, Karimi V, Madadgar O et al. Molecular characterization of infectious bronchitis viruses isolated from broiler chicken farms in Iran, 2014-2015. Archives of Virology 2016; 161: 53-62. https://doi.org/10.1007/s00705-015-2636-3

10. Hamadan AM, Ghalyanchilangeroudi A, Hashemzadeh M, Hosseini H, Karimi V et al. Genotyping of avian infectious bronchitis viruses in Iran (2015–2017) reveals domination of IS-1494 like virus. Virus Research 2017; 240: 101-106. https://doi.org/10.1016/j.virusres.2017.08.002

11. Seger W, GhalyanchiLangeroudi A, Karimi V, Madadgar O, Marandi MV et al. Genotyping of infectious bronchitis viruses from broiler farms in Iraq during 2014-2015. Archives of Virology 2016; 161: 1229-1237. https://doi.org/10.1007/s00705-016-2790-2

12. Zhang Y, Xu Z, Cao Y. Host antiviral responses against avian infectious bronchitis virus (IBV): focus on innate immunity. Viruses 2021; 13: 1698-1709. https://doi.org/10.3390/v13091698

13. Cavanagh D, Mawditt K, Britton P, Naylor C. Longitudinal field studies of infectious bronchitis virus and avian pneumovirus in broilers using type-specific polymerase chain reactions. Avian Pathology 1999; 28: 593-605. https://doi.org/10.1080/03079459994399

14. Seyfi Abad Shapouri M, Mayahi M, Charkhkar S, Shahriari AR. Serotype identification of recent Iranian isolates of infectious bronchitis virus by type-specific multiplex RT-PCR. Archives of Razi Institute 2002; 53: 79-85.

15. 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 Veterinaria Hungarica 2004; 52: 163-166. https://doi.org/10.1556/AVet.52.2004.2.4

16. Shoushtari AH, Toroghi R, Momayez R, Pourbakhsh SA. 793/B type, the predominant circulating type of avian infectious bronchitis viruses 1999-2004 in Iran: a retrospective study. Archives of Razi Institute 2008; 63 (1): 1-5.

17. Seifi S, Asasi K, Mohammadi A. Natural co-infection caused by avian influenza H9 subtype and infectious bronchitis viruses in broiler chicken farms. Veterinarski Arhiv 2010; 80 (2): 269-281.

18. Ghalyanchilangeroudi A, Hosseini H, Mehrabadi MHF, Ghafouri SA, Hamdan AM et al. Genotyping of avian infectious bronchitis virus in Iran: detection of D274 and changing in the genotypes rate. Comparative Immunology, Microbiology and Infectious Diseases 2019; 65: 110-115. https://doi.org/10.1016/j.cimid.2019.05.011

19. Cook JK, Orbell SJ, Woods MA, Huggins, MB. Breadth of protection of the respiratory tract provided by different live-attenuated infectious bronchitis vaccines against challenge with infectious bronchitis viruses of heterologous serotypes. Avian Pathology 1999; 28 (5): 477-485. https://doi.org/10.1080/03079459994506

20. Habibi M, Karimi V, Langeroudi A, Ghafouri S, Hashemzadeh M et al. Combination of H120 and 1/96 avian infectious bronchitis virus vaccine strains protect chickens against challenge with IS/1494/06 (variant 2)-like infectious bronchitis virus. Acta Virologica 2017; 61: 150-160. https://doi.org/10.4149/av_2017_02_04

21. Adzhar A, Shaw K, Britton P, Cavanagh D. Universal oligonucleotides for the detection of infectious bronchitis virus by the polymerase chain reaction. Avian Pathology 1996; 25: 817-836. https://doi.org/10.1080/03079459608419184

22. Jones R, Ellis R, Cox W, Errington J, Fuller C et al. Development and validation of RT-PCR tests for the detection and S1 genotyping of infectious bronchitis virus and other closely related gammacoronaviruses within clinical samples. Transboundary and Emerging Diseases 2011; 58: 411-420. https://doi.org/10.1111/j.1865-1682.2011.01222.x

23. Montassier MFS, Brentano L, Montassier HJ, Richtzenhain LJ. Genetic grouping of avian infectious bronchitis virus isolated in Brazil based on RT-PCR/RFLP analysis of the S1 gene. Pesquisa Veterinária Brasileira 2008; 28: 190-194. https://doi.org/10.1590/S0100-736X2008000300011

24. Lin Z, Kato A, Kudou Y, Umeda K, Ueda S. Typing of recent infectious bronchitis virus isolates causing nephritis in chicken. Archives of Virology 1991; 120: 145-149. https://doi.org/10.107/BF01310957

25. Keeler Jr CL, Reed KL, Nix WA, Gelb Jr J. Serotype identification of avian infectious bronchitis virus by RT-PCR of the peplomer (S-1) gene. Avian Diseases 1998; 42: 275-284.

26. Mohammadi P, Karimi V, Hashemzadeh M, Ghlaynachi A, Ghafouri S et al. Combination of H120 and 793/B types of infectious bronchitis virus vaccine protects chickens against challenge with QX like strain of the virus. Iranian Journal of Virology 2014; 8: 20-24.

27. De Wit J, Cazaban C, Dijkman R, Ramon G, Gardin Y. Detection of different genotypes of infectious bronchitis virus and of infectious bursal disease virus in European broilers during an epidemiological study in 2013 and the consequences for the diagnostic approach. Avian Pathology 2018; 47: 140-151. https://doi.org/10.1080/03079457.2017.1387231

28. Reddy VR, Trus I, Desmarets L, Li Y, Theuns S. Productive replication of nephropathogenic infectious bronchitis virus in peripheral blood monocytic cells, a strategy for viral dissemination and kidney infection in chickens. Veterinary Research 2016; 47 (1): 1-19. https://doi.org/10.1186/s13567-016-0354-9

29. De Wit J, Nieuwenhuisen-van Wilgen J, Hoogkamer A, Van De Sande H, Zuidam G et al. Induction of cystic oviducts and protection against early challenge with infectious bronchitis virus serotype D388 (genotype QX) by maternally derived antibodies and by early vaccination. Avian Pathology 2011; 40: 463-471. https://doi.org/10.1080/03079457.2011.599060

30. Stenzel T, Dziewulska D, Śmiałek M, Tykałowski B, Kowalczyk J, Koncicki A. Differentiation of infectious bronchitis virus vaccine strains Ma5 and 4/91 by TaqMan real-time PCR. Polish Journal of Veterinary Sciences 2017; 20: 599-601. https://doi.org/10.1515/pjvs-2017-0073