Iranian Journal of Virology 2018;12(2): 1-5

Original Article

Molecular Characterization of a very Virulent Infectious Bursal Disease Virus from Iran Demonstrates its Similarity with Recent Isolates from the Middle East

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Abstract

Background and Aims: Infectious bursal disease (IBD) is an acute, very contagious disease of juvenile chickens. High mortality following its acute clinical form on the one hand, and immunosuppressive effects resulting from subclinical infections, on the other hand, made IBD an economically important disease. In Iran, despite regular vaccination, cases of IBD are still diagnosed clinically, with limited information on their molecular epidemiology. The present study was conducted to characterize IBD viruses responsible for a recent outbreak.
Materials and Methods: Samples of the bursa of Fabricius were collected from IBD suspected pullets with up to 40% mortality. The viral RNA was isolated, and an RT-PCR targeting hypervariable region within the VP2 gene was carried out. One positive sample was sequenced and phylogenetically analyzed.
Results: The virus detected in this study had the highest homology to a very virulent IBD virus (vvIBDV) identified in 2018 in Iran. It also shared a high level of homologies to vvIBDVs isolated from Kuwait, Iraq, and Turkey.
Conclusions: Despite using vaccines, very virulent IBD viruses are circulating in Iran. The close relationships of the detected virus with vvIBD viruses circulating in neighboring countries is an alarming issue announcing the necessity of imposing strict rules on importation and exportation of birds.
Keywords: Infectious bursal disease, Molecular characterization, Iran, Pullets.

Introduction

Infectious bursal disease (IBD) is an acute, very contagious disease of juvenile chickens. High mortality following its acute clinical form on the one hand, and immunosuppressive effects resulting from subclinical infections, on the other hand, made IBD an economically important disease. A non-enveloped virus from Avibirnavirus genus belongs to the Birnaviridae family is the causative agent (1, 2).Infectious bursal disease virus (IBDV) is a double-stranded RNA virus, comprises two segments termed A and B. Segment B with the size of 2.8 kbp encodes the VP1, a 90 kDa protein representing the RNA dependent RNA polymerase. Segment A, approximately 3.2 kbp in size is made up of two ORFs; the smaller ORF encrypts for a 17 kDa non-structural polypeptide named VP5. The larger ORF of segment A encrypts for a

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115 kDa polyprotein which is self-cleaved to generate the two capsid proteins pVP2 and VP3 plus the VP4 (2, 3). Either VP4 or the puromycin-sensitive aminopeptidase cut the pVP2 at the carboxyl terminus to produce the intermediate pVP2, which is again refined by VP2 itself eventually creating the mature VP2. The hypervariable region within the VP2 induces a protective immune reaction, thus responsible for antigenic variations. Apart from mutations in the hypervariable region of VP2, reassortment events, and homologous recombination within segments also lead to IBDV variation (4).

Two serotypes of IBDV have been characterized. While serotype 1 viruses cause diseases in chickens, serotype 2 viruses are not pathogenic in any avian species (5). Strains of serotype 1 are further divided into classical virulent IBDV (cvIBDV), very virulent (vvIBDV), antigenic variant IBDV (avIBDV) and attenuated IBDV (at IBD) (6). The phylogenetic analysis of IBDV is based on the sequence of a hypervariable region within the VP2 (3).

vvIBDV emerged in 1991 in Iran, and disease outbreaks have been occurring even in flocks vaccinated with IBDV intermediate or intermediate plus vaccines. This study was conducted to genetically identification of IBDVs responsible for a new outbreak in a vaccinated pullet flock. The information provided by this work is basically needed to implement effective preventive strategies.

Methods

Samples. An IBD suspected outbreak causing up to 40% mortality occurred in a pullet flock (30 days old) located in Semnan province, Iran in May 2019. Birds were vaccinated twice with IBDV 2 intermediate vaccines. Samples of the bursa of Fabricius were aseptically collected from birds, transported under the cold chain to the Faculty of Veterinary Medicine Laboratory, Tehran. Iran. Ten ml of phosphate-buffered saline was added to 100 mg of each bursal tissue and homogenized by a pestle and mortar. Suspensions were clarified by centrifu-gation and supernatant was collected and stored at -70 °C.

IBDV detection. The viral RNA was isolated by SinaPure ONE kit (SinaClon Co., Iran) from the supernatants as recommended by the manufacturer. The cDNA was synthesized using the Thermo Scientific reverse transcriptase PCR (RT-PCR) kit using random hexamer (Thermo Scientific, USA). A PCR method described by Lin et al. (7) was used to amplify a 474 bp product within the variable region of VP2 gene.

Sequencing and phylogenetic analysis. From RT-PCR products, one was sent to Bioneer Company (Korea) for sequencing. The alignment was carried out using Clustal W. Sequences of reference strains and other related IBDVs retrieved from the GenBank database. The distance-based neighbor-joining tree was constructed using MEGA version 7 with up to 1000 bootstrapping replicates.

Results

In this work, we employed RT-PCR followed by nucleotide sequencing of hypervariable VP2 to assess phylogenetic relations between Iranian IBDVs and other global strains (Fig. 1).

Table 1. The similarity matrix calculated using Mega 7 for Iranian IBDVs and other selected IBD viruses based on the partial VP2 gene sequences.

		1	2	3	4	5	6	7	8	9	10
1	UT-PCR_Keivanfar_2019										
2	IRAN_IBD_AVAK_2018	98.56									
3	Isolate_422_Iraq_(MF142534.1)	97.81	99.29								
4	Isolate_101_Kuwait(MF142502.1)	97.81	99.29	99.53							
5	wIBDV_TR_B607/102/2017(MH137952.1)	97.04	98.57	99.29	98.81						
6	IBDV-w-Iran07(EU697938_)	95.19	96.81	97.07	97.07	96.30					
7	IR599(EU091537)	95.19	96.81	97.07	97.07	96.30	99.06				
8	IR399(EU091535)	95.19	96.81	97.07	97.07	96.30	99.06	100.00			
9	IR197(EU091532)	94.65	96.30	96.56	96.56	95.78	98.57	99.53	99.53		
10	ShirazIBD_(JX983160.1)	94.62	96.27	96.54	96.54	95.75	97.82	98.32	98.32	97.83	

As shown in table 1, UT-PCR-Keivanfar-2019 had the highest homology of 98.56% to a vvIBDV identified in 2018 in Iran. The virus of this study also shared homologies ranging between 97-98% to IBDVs isolated from Kuwait (2014), Iraq (2015) and Turkey (2017). More distantly with the sequence homologies of 94.62% and 95.19%, respectively, UT-PCR-Keivanfar-2019 was related to the Iranian vvIBDVs isolated in 2012 (JX 983160) and 2007.

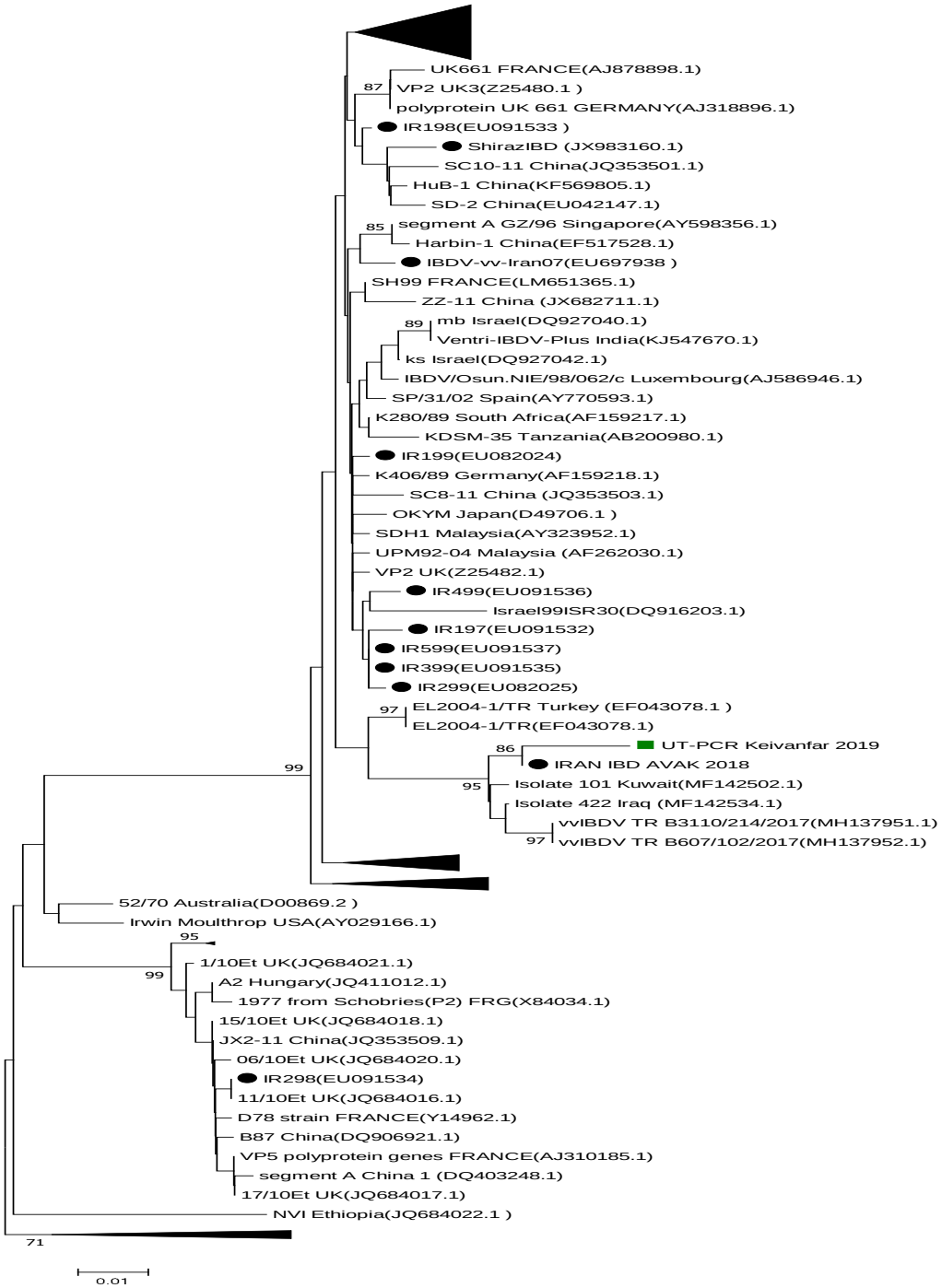

Fig. 1. Neighbor-joining tree showing the relationships between Iranian IBDVs and other selected IBD viruses based on the partial VP2 gene. The UT-PCR-Keivanfar-2019 sequence obtained in this study is marked with a green square, previously known Iranian IBD viruses are marked with black circles. Accession numbers of the sequences from GenBank are shown in parenthesis.

Discussion

IBD is one of the most prominent contagious diseases of chickens imposing a considerable economic burden on the global poultry industry since its first recognition in the USA in 1962. Chicks affected with vvIBDV show prostration, ruffled features, diarrhea and dehydration with moderate mortality (5).

IBDV mainly affects actively dividing B lymphocytes, destructs lymphoid cells in the bursa of Fabricius and result in severe immunosuppression (8). The permanence of suppression of the primary immune responses leads to concurrent bacterial and viral infections (9). Avoiding IBDV consequences, vaccination is essential as a prevention tool (5). Until the 1980s, vaccination was successful in controlling the disease, but vaccine failures were reported after the emergence of very virulent IBDVs by the end of the 1980s. Very virulent IBDV was primarily characterized in Europe, then reported from Japan in the 1990s and finally spread over Asia and extensively around the world (1).

In Iran, IBDV was first isolated from a broiler chicken in 1981. Up to 10 years, losses in infected chicks were mainly due to poor growth and immunosuppression, and the mortality never exceeded 5%. In 1991 new cases of IBD causing up to 75% mortality had happened in the areas with a high density of poultry farms. In this year, the first highly virulent IBDV termed G 2212/91 was identified (10). While IBD intermediate or intermediate plus vaccines are routinely applied in broilers and commercial pullets, but outbreaks are reported periodically. During 2005-2006, IBDV isolates closely related to European and Asian very virulent IBDVs were identified (11, 12). Bahmaninejad et al. isolated three vvIBDVs from IBD outbreaks in layer flocks (13). Razmyar et al. in 2009 reported the presence of vvIBDV in turkeys (14).

Several reports demonstrated high levels of similarity among IBDVs circulating throughout the Middle Eastern countries. While some Iranian IBDVs isolated in 2007 showed homologies to the European and Chinese vvIBDVs, but the majority of them clustered with an Israeli isolate. Amin et al. showed that Iraqi IBDVs detected in 2012, were placed in the same cluster with an Iranian vvIBDV(JX 983160) isolated in the same year. They also reported the close relationships of the Iraqi isolates to Turkish, Israeli, European, and Malaysian vvIBDVs (15).

Such a high similarity of viruses circulating among the Middle Eastern countries is also highlighted in our work, in which the UT-PCR-Keivanfar-2019 is highly related to vvIBDVs from Iraq, Kuwait, and Turkey. It highlights the role of uncontrolled animal movements between Iran and neighboring countries. Extensive trade of live birds and poultry products between Iran and neighboring countries is a critical point should be noted in the control and prevention of IBD. Since the IBD is not monitored at veterinary quarantine services, both legal and illegal importation of either poultry or their by-products can lead to spreading the infection. From neighboring countries, more attention should be paid to those in which IBD is endemic, like Iraq.

Conclusions

Despite using vaccines, very virulent IBD viruses are circulating in Iran. The close relationships of UT-PCR-Keivanfar-2019 with vvIBD viruses circulating in neighboring countries is an alarming issue announcing the necessity of imposing strict rules on importation and exportation of birds.

Conflict of interest

The authors state no conflict of interest.

Acknowledgments

The authors gratefully acknowledge Mr. Asadi for his extensive technical support. demic, like Iraq.

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