Molecular Monitoring of D1466 Genotype of Avian Infectious Bronchitis Virus In Iran: A Retrospective Study (2013-2017)

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Abstract

Infectious bronchitis is one of the most common diseases in the poultry industry in many countries, especially in countries with a dense poultry farming industry. The disease agent is a gammacoronavirus, and chickens and poultries are considered as natural reservoirs of the virus. Various strains of the infectious bronchitis virus have been reported in the poultry around the world, and in terms of pathogenicity, a spectrum of diseases ranges from moderate respiratory tract to kidney diseases and reproductive and do not cause cross-immunity against each other, which makes it difficult to control the disease. Based on the analysis of the highly variable region of glycoprotein S1 gene, isolated strains in Iran were classified into seven different phylogenetic groups including Massachusetts, QX, IS-720, IS-1494, 793/B, IR-1 and IR-2. D1466 genotype has not been reported in the country, but the killed vaccine is used in mother farms. In this study, tissue specimens from 700 farms (broiler farms, egg laying farms and broiler Breeder farms) suspected to infectious bronchitis during 2013 to 2017 were examined using Real-time RT-PCR. D1466 genotype was not detected in any of the studied specimens. Due to the lack of immunity from D1466 serotype against the dominant types in the country, one has to be careful in choosing the right vaccine. Continuous monitoring of the circulation status of the various serotypes of viruses in the country is necessary in order to identify the dominant serotypes and possible new serotypes for the utilization of the appropriate vaccine.

Keywords: D1466 Infectious Bronchitis, Commercial farms, Real-time RT-PCR, Iran
Abstract

Avian Infectious bronchitis (IB) is one of the most common diseases in the poultry industry in many countries, especially in countries with a dense poultry farming industry. Various strains of the IB virus have been reported in the poultry around the world, and regarding pathogenicity, a spectrum of diseases ranges from moderate respiratory tract to kidney diseases. Based on the analysis of the highly variable region of glycoprotein S1 gene, isolated strains in Iran were classified into seven different phylogenetic groups including Massachusetts, QX, IS-720, IS-1494, 793 / B, IR-1, and IR-2. D1466 genotype has not been reported in the country, but the killed vaccine is used in mother farms. In this study, tissue specimens from 700 farms (broiler farms, egg-laying farms and broiler Breeder farms) suspected to IB from 2013 to 2017 were examined using Real-time RT-PCR. D1466 genotype was not detected in any of the studied specimens. Due to the lack of immunity from D1466 serotype against the dominant types in the country, one has to be careful in choosing the right vaccine. Continuous monitoring of the circulation status of the various serotypes of viruses in the country is necessary in order to identify the dominant serotypes and possible new serotypes for the utilization of the appropriate vaccine.

Keywords: D1466, Avian Infectious Bronchitis, Real-time RT-PCR, Iran

Introduction:

Avian Infectious bronchitis (IB) is one of the most common diseases in the poultry industry in many countries, especially in countries with a dense poultry farming industry. Production decline due to pathogenicity and mortality and weight loss in broiler chicken and reduction of egg production and loss of egg quality in egg laying poultry is one of the most important consequences of this disease (Ignjatovic and Sapats, 2000). IB is a highly contagious disease of the upper respiratory tract in chickens and other poultry (Jackwood and de Wit, 2013). The disease agent is a gammacoronavirus, and chickens are considered as natural host of the virus (Ignjatovic and Sapats, 2000). The IB virus (IBV) is a RNA virus with a diameter of approximately 120 nm and single-stranded with a positive sense. The RNA genome is ~ 27.6 kb and has at least 10 open reading frame (ORF) and from 5' to 3', includes: 5'- (1a-1b-S (S1, S2) -3ca, b, (E) -M-5ba , - N - Poly (A))-3'. The genome is coded into four main structural proteins that include: glycoprotein spike (S), small membrane protein (E), membrane glycoprotein (M), and Nucleocapsid protein (N), and some non-structural proteins. The first type of glycoprotein S is composed of three homopolymers, which is a binding factor to the target cell receptor and for the fusion of the virus and cell membranes. Protein S consists of
two or three copies of two subunits that are included in S1 and S2 subunits. Although it has been reported that S1 is the most important protein in the variability of coronavirus (Casais et al., 2003).

Various genotypes of the IBV have been reported in the poultry around the world, and regarding pathogenicity, a spectrum of diseases ranges from moderate respiratory tract to kidney diseases and reproductive system (Tan et al., 2016). The various serotypes of the IBVs do not cause cross-immunity against each other, which makes it difficult to control the disease. On the other hand, these viruses reproduce rapidly and have high mutational power, which is why a wide genetic variety of virus is created (Jackwood, 2012). One of the most important ways of controlling the disease is vaccination, and due to genetic diversity and lack of safety coverage by different serotypes, several vaccines are necessary (Ignjatovic and Sapats, 2000). More disease control is achieved through the use of live attenuated vaccines, but vaccination can lead to the emergence of antigenic variants. (Moore et al., 1998; Ignjatovic et al., 2002; Meir et al., 2004). Vaccination with a single serotype does not provide sufficient protection against the dominant virus in the field, and it is necessary to add other serotype vaccines to the vaccination program. Protective immunity against common dominant strains in the field is possible using dominant strains of homologous vaccines (Jackwood and de Wit, 2013). The Real-Time RT-PCR is an effective method for identifying an IBV. In spite of the fact that the region encoding protein S1 is exposed to mutations, diagnostic methods are designed to identify this gene, and to identify other pedigrees such as GI-1 (Massachusetts, Connecticut), GI-9 (Arkansas), (SAI) GI-11, (SAI) GI-16 and (DE072) GIV-1 (Acevedo et al., 2013; Roh et al., 2014; Marandino et al., 2016). This disease in our country is also one of the most critical challenges in the poultry industry, especially in broiler farming. The first isolation of the IBV in Iran was first identified in poultry flocks in 1994 (Aghakhan et al., 1994; Hamadan et al., 2017). Based on the analysis of the highly variable region of glycoprotein S1 gene, isolated strains in Iran were classified into seven different phylogenetic groups including Massachusetts, QX, IS-720, IS-1494, 793 / B, IR-1 and IR-2 (Najafi et al., 2016; Hamadan et al., 2017). In our country, a variety of live and killed vaccines are used to control the disease. So far D1466 genotype has not been reported in the country, but the killed vaccine is used in layer and breeder farms. This study aimed to investigate the probable prevalence of D1466 genotype of IBV in Iranian commercial poultry farms during 2013-2017.

Materials and Methods:
Statistical Population and Specimen

The population in this study was IB positive tissue (based on 5 UTR real-time PCR; DATA has not been shown) specimens (Trachea, lung, and kidney), broiler flocks, layer, and broiler breeder farms that collected at Department of Microbiology, Faculty of Veterinary Medicine, University of Tehran; 2013 to 2017.

Molecular Diagnosis:

The tissue specimens were provided separately for each poultry; suspensions were prepared and used for molecular testing after low-speed centrifugation. RNA isolation of the virus from the suspension was performed using the RNA easy Mini-kit (Qiagen Co.) according to the manufacturer’s instructions. Forward primer (TTACAGCCTGGCAATGTCTT) and reverse (CAACATCCTCMSRTAAAGTTAGAAC) and FAM-CYAGTGTGTTTCTAAAYGGCAACCTT-BHQ1-) probe were used to trace the genotype D1466 (Domanska- Blicharz et al., 2017). This reaction was performed using QuantiTect Probe RT-PCR Kits (Qiagen Co.). The reaction conditions included 50 °C, 30 minutes, 95 °C, 15 minutes and 40 cycles of 94 °C, 10 seconds and 60 °C for 60 seconds. This reaction was placed on them. Rotor Q device (Qiagen Co., D1466 antigen GD Animal Health (Cat No: 1232)) was used to ensure the correctness of the various stages of the test.

Results

In this study, IB positive tissue specimens from 700 farms (including 610 Broiler farm, 70 layer farms and 20 Broiler breeder farms) from the different provinces of the country were investigated. D1466 genotype was not detected in any of the studied specimens during 2013-2017. Positive (D1466; antigen GD Animal Health) and negative controls performed precisely in the reaction, which indicated that the reaction was set correctly.

Discussion

IBV strains in a region are often unique and distinct, although viruses in some countries are common regarding antigenicity (Ignjatovic and Sapats, 2000). Since the multiplicity of IBV genotypes that do not have cross-immunity, continuous monitoring and identification of IBVs worldwide are important (Jackwood, 2012). The high rate of IBV replication and non-proof reading lead to genetic mutation and the creation of a recombinant genome and the emergence of new virus serotypes, which, due to the lack of cross-immunity, complicates the control of
the disease by using vaccination (Jackwood and de Wit, 2013). In studies conducted in the country, various genotypes of the IBV have been circulating in the country during multiple periods of time. During the years 1994-2004, serotype 793B was the dominant serotype in the country (Hosseini et al., 2015). In the study of Pourbakhsh et al. (2008) genotype, 793/B was identified in 83 farms with IB. In addition to genotype 793/B, the genotype of Massachusetts was also identified (Pourbakhsh et al., 2008). In a study by Seifi et al. (2010) genotype 4/91 in 11 farms and Massachusetts in one farm out of 30 studied farms were identified (Seifi et al., 2010). In a study conducted in 2010 to 2014 on 250 farms with bronchitis, seven different genotypes including Massachusetts, IS720, 793/B, Variants 2, QX, IR-I and R-II in the country were identified (Hosseini et al., 2015). In other studies, the dominant genotypes in Iran during the years 2015 to 2017 were variants 2 and 793/B (Ghalyanchi-Langeroudi et al., 2015; Modiri Hamadan et al., 2017).

The D1466 variant has not yet been identified in the country. In this study, all samples, all of which were suspected to IB, were negative for the virus. The virus has not been reported in any of the neighboring countries of Iran and the Middle East except Pakistan. In Pakistan, Ahmed et al. Examined the prevalence of IBV strains in commercial flocks in Pakistan, concluding that 88% of the flocks were positive for M41 antibodies while 40%, 52% and 8% of flocks respectively, were positive relative to D274, D1466 and 4/91 (Ahmed et al., 2007).

The variant of D1466 (GII-1 lineage, also called D212) belonging to the IBV was first recognized in the Netherlands in 1970 as an agent for the drop of egg production (Davelaar et al., 1984; De Wit et al., 2011). Further studies showed that the antigenic and molecular properties of this variant differed significantly from other strains of the IBV (Kusters et al., 1987; Kusters et al., 1989). Interestingly, the only strain in the phylogenetic tree with the D1466 variant which is in a branch is the Dutch strain V1397, and the isolates of this strain are similar to those of North America DE072 and GA98 (Lee and Jackwood, 2000; Lee and Jackwood, 2001a, b). Differences between the D1466 variant and the rest of European strains with safety studies also revealed that there is less cross-immunity with the use of heterologous vaccines against this strain (Cook et al., 1999).

The D1466 variant is commonly found in Europe, which according to these findings has low pathogenicity compared to other types of IBV such as 793/B, 624/I or QX, which causes severe disease in poultry (De Wit et al., 2011). The results of molecular care in 2005-2006 showed that the issues of the D1466 genotype in Western European countries are escalating
In the UK and France, only a few diseases are identified by the D1466 variant, but in other countries, the dynamics of the disease is escalating. In 2005, the prevalence of Pesuedo-D1466 variants in Belgium was modest, but in 2006 the occurrence of this variant was 7, 10 and 16 percent among other genotypes of the IBV that year. So far, there has been no study on the existence of this genotype in the country. One of the reasons for this could be the detection method of this virus in comparison with another IBV. In the present study, Taq Man-probe method based on RT-PCR real-time method was used for precise and fast detection of D1466 (GII-1 pedigree) belonging to the IBV. Until now, the nested RT-PCR method was used to identify the genotypes of the IBV (Cavanagh et al., 1999). Both methods use the same portion of the spike protein gene to identify IBV genotypes.

However, the nested RT-PCR method requires a lot of time, specialist workforce, and the use of genetic material, which can lead to contamination and false positive results among the various stages of work, while implementing Real-Time RT-PCR can prevent these issues (Domanska- Blicharz et al., 2017). Duminska et al. validated the Real-Time RT-PCR method by evaluating the Spike protein encoding region in the proprietary S gene of GII-1 pedigree (Pseudo-D1466 variant) belonging to the IBV. This genotype is different from the other IBV in Europe that belongs to the genotype GI. The method used in this test is 30 times more sensitive than the nested RT-PCR method, which has the detection limits of 56 copies of the RNA in each reaction. The repeatability and regeneration of this method were very high, and its variable coefficient was less than 4%. In this test, 100 positive specimens for IBV were investigated using this method, and GII-1 strains were identified in four specimens out of all specimens (31%), indicating a decrease in the GII-1 outbreak in the Netherlands. This method is validated to identify the GII-1 pedigree among IBV strains and is also a reliable method for monitoring the virus, which is used to check the progression of the disease (Domanska- Blicharz et al., 2017). In this study, this method was used for monitoring which, given the high sensitivity and specificity, indicates the high precision of this research.

At different time intervals, different types of IBV have been circulating in the country. To control the disease in the country, live, and killed vaccines are used in mother flocks, commercial egg layers, and broilers. Studies show that currently variants 2 and 4/91 serotypes are dominant in the country. This study was the initial study of D1466 genotype in the country. However, according to the RT-PCR Real Time test, it turned out that this genotype does not exist in the country and is reliable due to the sensitivity of the method of the results.

Even though there is no report of the D1466 variant in the country, the D1466 variant vaccine
is used domestically. On the one hand, due to the lack of immunity from D1466 serotype against the dominant types in the country, one has to be careful in choosing the right vaccine. On the other hand, continuous monitoring of the circulation status of the various IBV genotypes in the country is necessary to identify the dominant serotypes and possible new serotypes for the utilization of the appropriate vaccine.

References:


