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Original Article

Molecular Monitoring of D1466 Genotype of Avian Infectious Bronchitis Virus in Iran: A Retrospective Study in 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 the regions with a dense poultry farming industry. Gammacoronavirus is the etiologic agent of this disease, with the chickens and poultries as the natural reservoirs of the virus. Various strains of infectious bronchitis virus have been reported in poultry around the world. In terms of pathogenicity, this virus can induce a spectrum of diseases ranging from the moderate respiratory tract to kidney and reproductive diseases. The serotypes of this virus do not cause cross-immunity against each other. This issue makes it difficult to control the disease. Based on the analysis of the highly variable region of the glycoprotein S1 gene, the 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. The D1466 genotype has not been reported in the country; however, the killed vaccine is used in broiler breeder farms. In this study, tissue specimens were collected from 700 farms (i.e., broiler, egg-laying, and broiler breeder farms) suspected of infectious bronchitis within 2013-2017. The samples were examined using real-time reverse transcription-polymerase chain reaction. The D1466 genotype was not detected in any of the studied specimens. Due to the lack of immunity of the D1466 serotype against the dominant types in the country, one has to be careful in choosing the right vaccine. It is necessary to perform continuous monitoring of the circulation status of the various serotypes of viruses in the country to identify the dominant and possible new serotypes for the utilization of the appropriate vaccine.

Keywords: D1466 infectious bronchitis, Commercial farms, Real-time RT-PCR, Iran

Surveillance Moléculaire du Génotype D1466 du Virus de la Bronchite Infectieuse Aviaire en Iran: une Étude Rétrospective en 2013-2017

Résumé: La bronchite infectieuse est l'une des maladies les plus courantes dans l'industrie de la volaille dans de nombreux pays, en particulier dans les régions où l'industrie de l'élevage de volaille est dense. Le *gammacoronavirus* est l'agent étiologique de cette maladie, les poulets et les volailles étant les réservoirs naturels du virus. Diverses souches de virus de la bronchite infectieuse ont été signalées chez les volailles du monde entier. En termes de pathogénicité, ce virus peut induire un spectre de maladies allant des voies respiratoires modérées aux maladies rénales et reproductives. Les sérotypes de ce virus ne provoquent pas d'immunité croisée les uns contre les autres. Ce problème rend difficile le contrôle de la maladie. Sur la base de l'analyse de la région hautement variable du gène de la glycoprotéine S1, les souches isolées en Iran ont été

classées en sept groupes phylogénétiques différents, dont le Massachusetts, QX, IS-720, IS-1494, 793/B, IR-1, et IR-2. Le génotype D1466 n'a pas été signalé dans le pays; cependant, le virus inactivé est utilisé dans les fermes d'élevage de poulets de chair. Dans cette étude, des échantillons de tissus ont été prélevés de 700 fermes (c.-à-d. les Fermes d'élevage de poulets de chair, de poules pondeuses, et de poulets reproducteurs) soupçonnées de bronchite infectieuse en 2013-2017. Les échantillons ont été examinés en utilisant une transcription inverse - réaction en chaîne par polymérase (TI-RCP) en temps réel. Le génotype D1466 n'a été détecté dans aucun des échantillons étudiés. En raison du manque d'immunité du sérotype D1466 contre les types dominants dans le pays, il faut être prudent dans le choix du bon vaccin. Il est nécessaire d'effectuer une surveillance continue de l'état de circulation des différents sérotypes de virus dans le pays pour identifier les nouveaux sérotypes dominants et possibles pour l'utilisation du vaccin approprié.

Mots-clés: Bronchite infectieuse D1466, Fermes commerciales, TI-RCP en temps réel, Iran

INTRODUCTION

Avian infectious bronchitis (IB) is one of the most common diseases in the poultry industry in many countries, especially in regions with a dense poultry farming industry. A decline in production due to pathogenicity, mortality, and weight loss in broiler chickens, as well as the reduction of egg production and quality in egg-laying poultry, is one of the most important consequences of this disease (Ignjatovic and Sapats, 2000). The IB is a highly contagious disease of the upper respiratory tract in chickens and other poultry (Jackwood and de Wit, 2013). Gammacoronavirus is the etiologic agent of this disease, with chickens being considered the natural host of the virus (Ignjatovic and Sapats, 2000). The IB virus (IBV) is a positive-sense single-stranded RNA virus with a diameter of approximately 120 nm. The RNA genome is ~ 27.6 kb and has at least 10 open reading frames (ORFs), from 5' to 3', including 5'-1a-1b-S(S1, S2)-3c,a,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 facilitates 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. It has been reported that S1 is the most important protein in the variability of coronavirus (Casais et al., 2003). Various genotypes of IBV have been reported in the poultry around the world. In terms of pathogenicity, this virus can induce a spectrum of diseases ranging from the moderate respiratory tract to kidney and reproductive diseases (Tan et al., 2016). The various serotypes of IBVs do not cause cross-immunity against each other, thereby making it difficult to control the disease. On the other hand, these viruses reproduce rapidly and have high mutational power. This explains the wide genetic variability of this virus (Jackwood, 2012). One of the most important ways of controlling the disease is vaccination. Genetic diversity of this virus and lack of safety coverage by different serotypes necessitates the development of several vaccines (Ignjatovic and Sapats, 2000). More disease control is achieved through the use of live attenuated vaccines; however, 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-based vaccines to the vaccination

program. Protective immunity against common dominant strains in the field is possible using the dominant strains of homologous vaccines (Jackwood and de Wit, 2013). The real-time reverse transcript polymerase chain reaction (RT-PCR) is an effective method for the identification of an IBV. Even though the region encoding protein S1 is exposed to mutations, diagnostic methods are designed to identify this gene and 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 IBV was first identified in Iran in poultry flocks in 1994 (Aghakhan et al., 1994; Modiri Hamadan et al., 2017). Based on the analysis of the highly variable regions of glycoprotein *S1* gene, the 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; Modiri Hamadan et al., 2017). In our country, a variety of live and killed vaccines are used to control the disease. So far, the D1466 genotype has not been reported in the country, but the killed vaccine is used in layer and breeder farms. With this background in mind, the present study was conducted to investigate the likely prevalence of the D1466 genotype of IBV in Iranian commercial poultry farms during 2013-2017.

MATERIAL AND METHODS

Statistical Population and Specimens. This study was performed on IBV-positive tissue specimens of the trachea, lung, and kidney, collected from broiler, layer, and broiler breeder farms by the Department of Microbiology, Faculty of Veterinary Medicine, the University of Tehran from 2013 to 2017. The identification of the IBV-positive specimens was accomplished using real-time PCR with 5 UTR as a primer (data are not shown).

Molecular Diagnosis. The tissue specimens were provided separately for each poultry. Suspensions were prepared and used for molecular testing after low-speed centrifugation. The 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 (TTACAGCCTGGCAATGTCTT) and reverse (CAACATCCTCMRTAAAGTTAGAAC) primers, as well as FAM-CYAGTGTGTTTCTAAAYGGCAACCTT-BHQ1 probe, were used to trace the D1466 genotype (Domanska-Blicharz et al., 2017). This reaction was performed using the QuantiTect Probe RT-PCR Kits (Qiagen Co.). The reaction conditions included 50 °C for 30 min, 95 °C for 15 min, as well as 40 cycles of 94 °C for 10 sec and 60 °C for 60 sec. This reaction was placed on the specimens. Also, the 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, IBV-positive tissue specimens were collected from 700 farms, including 610 broiler farms, 70 layer farms, and 20 broiler breeder farms, located in the different provinces of Iran. The D1466 genotype was not detected in any of the studied specimens during 2013-2017. The use of positive (D1466; antigen GD Animal Health) and negative controls in the reaction indicated that the reaction was set correctly.

DISCUSSION

The IBV strains in a region are often unique and distinct, although these viruses are common in some countries in terms of antigenicity (Ignjatovic and Sapats, 2000). The multiplicity of IBV genotypes and their lack of cross-immunity highlight the importance of continuous monitoring and identification of IBVs worldwide (Jackwood, 2012). The high rate of IBV replication and non-proofreading lead to genetic mutation, the creation of a recombinant genome, and the emergence of new virus serotypes, which

complicates the control of the disease by vaccination due to the lack of cross-immunity (Jackwood and de Wit, 2013). In the studies conducted in Iran, various genotypes of IBV have been reported to circulate in the country during multiple periods. During 1994-2004, 793B was the dominant serotype in this country (Hosseini et al., 2015). In a study conducted by Pourbakhsh et al. (2008), 793/B genotype was identified in 83 farms with IB. In addition to this genotype, the genotype of Massachusetts was also identified. In a study carried out by Seifi et al. (2010), investigating 30 farms, Massachusetts and *4/91* genotypes were identified in 1 and 11 farms, respectively. In a study conducted within 2010-2014 on 250 farms with bronchitis, seven different genotypes, including Massachusetts, IS720, 793/B, Variants 2, QX, IR-I, and R-II, were identified in Iran (Hosseini et al., 2015). In other studies, the dominant genotypes identified in this country during 2015-2017 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 Iran. In the current study, all samples suspected of 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. (2007) examined the prevalence of IBV strains in commercial flocks. They concluded that 88% of the flocks were positive for M41 antibodies, while 40%, 52%, and 8% of them were positive for D274, D1466, and 4/91, respectively. The variant of D1466 (GII-1 lineage, also called D212) belonging to IBV was first recognized in the Netherlands in 1970 as an agent for the drop of egg production (Davelaar et al., 1984; Sjaak de Wit et al., 2011). Further studies showed that the antigenic and molecular properties of this variant differed significantly from those of other IBV strains (Kusters et al., 1987; Kusters et al., 1989). 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, 2001a, b). Differences between the D1466 variant and the rest of European strains with safety studies also revealed that there is low 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 cause severe disease in poultry (Sjaak de Wit et al., 2011). The results of molecular analysis in 2005-2006 showed that the issues of the D1466 genotype in Western European countries are escalating (Worthington et al., 2008). In the UK and France, only a few diseases are identified by D1466 variant; however, in other countries, the dynamics of the disease is escalating. In 2005, the prevalence of Pesuedo-D1466 variants in Belgium was moderate; nonetheless, in 2006, the incidence rates of this variant were obtained as 7%, 10%, and 16% among other IBV genotypes reported that year. So far, there has been no study on the presence 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, the TaqMan real-time RT-PCR was applied for the precise and fast detection of D1466 (GII-1 pedigree) belonging to the IBV. Until now, the nested RT-PCR method has been used to identify the genotypes of IBV (Cavanagh et al., 1999). Both methods involve the use of the same portion of the spike protein gene to identify IBV genotypes. However, the nested RT-PCR method requires a lot of time, a specialist workforce, and the use of genetic material, which can lead to contamination and false-positive results among the various stages of work. Meanwhile, the implementation of real-time RT-PCR can prevent these issues (Domanska-Blicharz et al., 2017). Domanska-Blicharz et al. (2017) validated the real-time RT-PCR method by evaluating the spike protein-encoding region in the proprietary *S* gene of the GII-1 pedigree (Pseudo-D1466 variant), belonging to IBV. This genotype is different from the other IBVs in Europe that belong 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 the real-time RT-PCR method were very high, and its variable coefficient was less than 4%. In this test, 100 positive specimens were investigated for IBV using this method, and GII-1 strains were identified in four 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 technique for monitoring the virus, which is used to check the progression of the disease (Domanska-Blicharz et al., 2017). In the present study, this method was used due to its high sensitivity and specificity for monitoring, which indicates the high precision of this research.

At different time intervals, various types of IBVs have been circulating in Iran. To control the disease in the country, live, and killed vaccines are used in broiler breeders, commercial egg layers, and broiler flocks. Based on the evidence, 2 and 4/91 variant serotypes are currently dominant in the country. This study was the initial study of the D1466 genotype in the country. However, the results of the real-time RT-PCR test showed that this genotype does not exist in the country. These results are reliable due to the sensitivity of the method applied in this study. Even though there is no report on the presence 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 the D1466 serotype against the dominant types in Iran, one has to be careful in choosing the right vaccine. Accordingly, it is necessary to perform continuous monitoring of the circulation status of various IBV genotypes in this country to identify the dominant and possible new serotypes for the utilization of an appropriate vaccine.

Ethics

We hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

The authors declare that they have no conflict of interest.

Authors' Contribution

Study concept and design: Ghalyanchilangeroudi, A.
Acquisition of data: Khaltabadi Farahani, R.
Analysis and interpretation of data: Maghsoudloo, H.
Drafting of the manuscript: Ghalyanchilangeroudi, A.
Critical revision of the manuscript for important intellectual content: Ghafouri, S. A.
Statistical analysis: Fallah Mehrabadi, M. H.
Administrative, technical, and material support: Khaltabadi Farahani, R.; Ghalyanchilangeroudi, A.

References

Acevedo, A.M., Perera, C.L., Vega, A., Rios, L., Coronado, L., Relova, D.*, et al.*, 2013. A duplex SYBR Green I-based real-time RT-PCR assay for the simultaneous detection and differentiation of Massachusetts and non-Massachusetts serotypes of infectious bronchitis virus. Mol Cell Probes 27, 184-192.

Aghakhan, S., Abshar, N., Fereidouni, S.R.N., Marunesi, C., Khodashenas, M., 1994. Studies on Avian Viral Infections in Iran. Arch Razi Inst 44.45, 1-10.

Ahmed, Z., Naeem, K., Hameed, A., 2007. Detection and seroprevalence of infectious bronchitis virus strains in commercial poultry in Pakistan. Poult Sci 86, 1329-1335.

Casais, R., Dove, B., Cavanagh, D., Britton, P., 2003. Recombinant avian infectious bronchitis virus expressing a heterologous spike gene demonstrates that the spike protein is a determinant of cell tropism. J Virol 77, 9084-9089.

Cavanagh, D., Mawditt, K., Britton, P., Naylor, C.J., 1999. Longitudinal field studies of infectious bronchitis virus and avian pneumovirus in broilers using type-specific polymerase chain reactions. Avian Pathol 28, 593-605.

Cook, J.K., Orbell, S.J., Woods, M.A., Huggins, M.B., 1999. 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 Pathol 28, 477-485.

Davelaar, F.G., Kouwenhoven, B., Burger, A.G., 1984. Occurrence and significance of infectious bronchitis virus variant strains in egg and broiler production in the Netherlands. Vet Q 6, 114-120.

Domanska-Blicharz, K., Lisowska, A., Pikula, A., Sajewicz-Krukowska, J., 2017. Specific detection of GII-1 lineage of infectious bronchitis virus. Lett Appl Microbiol 65, 141-146.

Ghalyanchi-Langeroudi, A., Karimi, V., Jannat, A., Hashemzadeh, M., Fallah, M., Gholami, F., *et al.*, 2015. Genotyping of infectious bronchitis viruses in the east of Iran, 2015. Iran J Virol 9, 31-35.

Hosseini, H., Fard, M.H., Charkhkar, S., Morshed, R., 2015. Epidemiology of Avian Infectious Bronchitis Virus Genotypes in Iran (2010-2014). Avian Dis 59, 431-435.

Ignjatovic, J., Ashton, D.F., Reece, R., Scott, P., Hooper, P., 2002. Pathogenicity of Australian strains of avian infectious bronchitis virus. J Comp Pathol 126, 115-123.

Ignjatovic, J., Sapats, S., 2000. Avian infectious bronchitis virus. Rev Sci Tech 19, 493-508.

Jackwood, M., de Wit, S., 2013. Infectious bronchitis. In: Swayne, D. (Ed.), Diseases of poultry, Wily Blackwell, New Jersey, pp. 139-160.

Jackwood, M.W., 2012. Review of infectious bronchitis virus around the world. Avian Dis 56, 634-641.

Kusters, J.G., Niesters, H.G., Bleumink-Pluym, N.M., Davelaar, F.G., Horzinek, M.C., Van der Zeijst, B.A., 1987. Molecular epidemiology of infectious bronchitis virus in The Netherlands. J Gen Virol 68, 343-352.

Kusters, J.G., Niesters, H.G., Lenstra, J.A., Horzinek, M.C., van der Zeijst, B.A., 1989. Phylogeny of antigenic variants of avian coronavirus IBV. Virology 169, 217-221.

Lee, C.W., Jackwood, M.W., 2000. Evidence of genetic diversity generated by recombination among avian coronavirus IBV. Arch Virol 145, 2135-2148.

Lee, C.W., Jackwood, M.W., 2001a. Origin and evolution of Georgia 98 (GA98), a new serotype of avian infectious bronchitis virus. Virus Res 80, 33-39.

Lee, C.W., Jackwood, M.W., 2001b. Spike gene analysis of the DE072 strain of infectious bronchitis virus: origin and evolution. Virus Genes 22, 85-91.

Marandino, A., Tomas, G., Hernandez, M., Panzera, Y., Craig, M.I., Vagnozzi, A., *et al.*, 2016. Development of RT-qPCR assays for the specific identification of two major genotypes of avian infectious bronchitis virus. J Virol Methods 235, 21-25.

Meir, R., Rosenblut, E., Perl, S., Kass, N., Ayali, G., Perk, S., *et al.*, 2004. Identification of a novel nephropathogenic infectious bronchitis virus in Israel. Avian Dis 48, 635-641.

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

Moore, K.M., Bennett, J.D., Seal, B.S., Jackwood, M.W., 1998. Sequence comparison of avian infectious bronchitis virus S1 glycoproteins of the Florida serotype and five variant isolates from Georgia and California. Virus Genes 17, 63-83.

Najafi, H., Langeroudi, A.G., Hashemzadeh, M., Karimi, V., Madadgar, O., Ghafouri, S.A., *et al.*, 2016. Molecular characterization of infectious bronchitis viruses isolated from broiler chicken farms in Iran, 2014-2015. Arch Virol 161, 53-62.

Pourbakhsh, S., Momayez, R., Toroghi, R., Shoushtari, A., 2008. Ninetythree B type, the Predominant Circulating Type of Avian Infectious Bronchitis Viruses 1999-2004 in Iran: a retrospective study. Arch Razi Inst 63, 1-5.

Roh, H.J., Hilt, D.A., Jackwood, M.W., 2014. Detection of infectious bronchitis virus with the use of real-time quantitative reverse transcriptase-PCR and correlation with virus detection in embryonated eggs. Avian Dis 58, 398-403.

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

Sjaak de Wit, J.J., Cook, J.K., van der Heijden, H.M., 2011. Infectious bronchitis virus variants: a review of the history, current situation and control measures. Avian Pathol 40, 223-235.

Tan, L., Liao, Y., Fan, J., Zhang, Y., Mao, X., Sun, Y., *et al.*, 2016. Prediction and identification of novel IBV S1 protein derived CTL epitopes in chicken. Vaccine 34, 380-386.

Worthington, K.J., Currie, R.J., Jones, R.C., 2008. A reverse transcriptase-polymerase chain reaction survey of infectious bronchitis virus genotypes in Western Europe from 2002 to 2006. Avian Pathol 37, 247-257.