NOTE Avian Pathology

Existence of Avian Infectious Bronchitis Virus with a European-Prevalent 4/91 Genotype in Japan

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ABSTRACT. Eight isolates of infectious bronchitis virus (IBV) were obtained from various prefectures in Japan during 2003–2007 and were genetically analyzed by reverse transcriptase-polymerase chain reaction (RT-PCR) coupled with direct sequencing. These IBV isolates were classified into three genetic groups, including two that have already been reported (JP-I and JP-III). The remaining group is related to the 4/91 (also known as 793/B) type, prevalent mainly in European countries, and has not been identified in Japan until now.

KEY WORDS: epidemiology, genotype, infectious bronchitis virus.

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Avian infectious bronchitis virus (IBV), a member of the Coronaviridae family (order Nidovirales, genus Coronavirus), is a highly contagious pathogen of domestic fowls worldwide. It replicates primarily in the respiratory tract but also in the epithelial cells of the gut, kidney and oviduct [4]. Coronaviruses are enveloped and positive-stranded RNA viruses containing an unsegmented genome approximately 27.6 kilobases (kb) in length [1].

The major method of protecting poultry from this disease is the application of live or killed vaccines [4]. In the field, however, the protection afforded by vaccination is not complete, since the high mutation frequency of IBV leads to emergence of new viruses capable of causing disease in chickens [19]. IBV has three major virus-encoded structural proteins: the spike (S) glycoprotein, membrane (M) protein and nucleocapsid (N) protein. The IBV spike is formed by post-translational cleavage of two separate polypeptide components, designated S1 and S2 [3]. Of these, the S1 glycoprotein is associated with virus attachment and is a major target of the neutralizing antibodies in chickens [5, 8], so serotypic evolution in IBV is associated primarily with the sequences of the S1 glycoprotein [10, 18]. Therefore, recent genetic grouping of IBV has been performed mainly on the basis of the nucleotide sequences of the S1 glycoprotein gene [9, 11, 12, 17, 18, 20].

Previously, our phylogenetic analysis of mainly hypervariable (HVR) regions of S1 glycoprotein genes revealed that Japanese IBV strains could be classified into five genetic groups, one of which is indigenous to Japan and can not be placed within the existing groups in other countries [14].

In the present report, to define the epidemiology or relationships among recent IBV isolates in Japan during 2003–2007, we used essentially the same procedure in an attempt to characterize eight Japanese IBV isolates from chickens.

The IBV isolates were obtained from prefecture-based regional animal hygiene service centers in Japan (Table 1). Most of the IBV specimens were isolated by two or three passages using 10-day-old embryonated chicken eggs. The presence of IBV in the inoculated embryos was initially determined by IBV-specific reverse transcriptase-polymerase chain reaction (RT-PCR) as described previously [14] and by observation of characteristic embryonic changes such as dwarfing, stunting or curling. Also, a newly available commercial live attenuated IB vaccine strain (GN), which was developed from an isolate from a chicken with nephritis in Japan, was used in this study. Viral RNA was extracted from infected allantoic fluids using an ISOGENSE kit (Nippon Gene, Tokyo, Japan). Reverse transcription, PCR amplification, sequencing and phylogenetic analysis were performed as described previously [14].

The expected sizes of DNA fragments were successfully amplified by RT-PCR from all the IBV samples in this study. Determination of the nucleotide sequences of the obtained PCR products revealed the diversity in their lengths (677–692 bp).

By phylogenetic analysis, the eight isolates were classified into three genetic groups: JP-I, JP-III and 4/91 (also known as 793/B; Fig. 1). Among them, two groups (JP-I and JP-III) had been identified in a previous study [14]. One isolate (JP/Ehime/2003), as well as the GN vaccine strain, both of which were classified into the JP-I genotype, were clustered only with the Japanese isolates, whereas four isolates (JP/Okayama-5/2004, JP/Chiba/2004, JP/Wakayama-13/2006 and JP/Saitama/2007) classified into the JP-III genotype were clustered with isolates in neighboring countries such as South Korea and P.R.China. Three isolates (JP/Wakayama/2003, JP/Okayama-7/2004 and JP/Saitama/2006) were related to genotype 4/91 (793/B), which is prevalent mainly in European countries. In Japan, the 4/91-type live vaccine has been commercially available since 2002, so we determined the nucleotide sequences of the corresponding regions of this vaccine strain and field isolates for comparison. The results showed that genetically JP/Okayama-7/...
2004 was highly similar to the vaccine strain, with a similarity of over 99%, and so this strain may have been derived from the vaccine strain. However, the remaining two isolates (JP/Wakayama/2003 and JP/Saitama/2006) were most similar to a French isolate (FR-94047–94, Accession Number AJ618987), with a similarity of 96.8% by GenBank searches, and they had similarities of 93.3 and 93.8%, respectively, compared with the vaccine strain; these isolates were not likely to be directly derived from the introduced vaccine strain.

In a previous study, we reported the simple and rapid classification of genotypes of Japanese IBV strains using two restriction endonucleases, Hae II and Eco RI. The strains belonging to the JP-I and JP-III genotypes showed the same profiles as reported previously. However, the newly identified genotype 4/91 (793/B) did not have sites for these 2 enzymes like the Mass type, so differentiation between the 4/91 and Mass types was difficult. Therefore, we selected another endonuclease, Pst I, to differentiate between the 4/91 and Mass types. The strains belonging to the 4/91 type do not have a restriction site for this enzyme, but the strains belonging to the Mass type do. For example, the PCR product of H120 origin, which is a representative Mass-type strain, was digested with 583 and 88 base pairs (data not shown). These results revealed that differentiation of Japanese IBV strains is possible at present by use of three restriction endonucleases.

The present study shows that the 4/91 (793/B) type of IBV has existed in Japan since 2003. Since it was first identified in France in 1985 and subsequently identified in Great Britain in 1990, this genotype has spread to many countries and has become one of the most predominant types in Europe [2, 6, 7]. This IBV genotype has also been detected in Middle Eastern and Asian countries, such as Iran and Thailand [2, 6, 7]. This IBV genotype has been identified in France in 1985 and subsequently identified in Great Britain in 1990, this genotype has spread to many countries and has become one of the most predominant types in Europe [2, 6, 7]. This IBV genotype has been detected in Middle Eastern and Asian countries, such as Iran and Thailand [2, 6, 7].

The introduction route of the 4/91 genotype into Japan is unknown. However, highly virulent infectious bursal disease virus or avian pneumovirus are known to be examples of avian viruses detected in Japan that are genetically similar to those prevalent in Europe [13, 15]. The importation of chicks from European countries, such as the UK, France, the Netherlands and Germany is extensive in Japan; over 500,000 chicks were imported to Japan each year between 2002 and 2006. This may be one of the transfer routes for the 4/91 genotype, and other IBV genotypes may be introduced to Japan in the same way. Detection of the novel 4/91 genotype in this study suggests that continuous isolation and genetic analysis of IBV is required. Furthermore, to understand the epidemiology of IBV in Japan, it will be important to know the prevalence of viruses not only in neighboring countries but also around the world.

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REFERENCES


