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Molecular and antigenic characteristics of Massachusetts genotype infectious bronchitis coronavirus in China

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ABSTRACT

In this study, 418 IBVs were isolated in samples from 1717 chicken flocks. Twenty-nine of the isolates were classified as the Massachusetts genotype. These 29 isolates, as well as two previously isolated Massachusetts genotype IBV strains, were studied further. Of the 31 strains, 24 were H120-like and two were M41-like isolates as determined by complete genomic sequence analysis, indicating that most of the IBV isolates were likely the reisolated vaccine virus. The remaining five IBV isolates, ck/CH/LHB/111172, ck/CH/LSD/111219, ck/CH/LHB/130598, ck/CH/LDL/110931, and ck/CH/LHB/130573, were shown to have originated from natural recombination events between an H120-like vaccine strain and other types of viruses. The virus cross-neutralization test found that the antigenicity of ck/CH/LHB/111172, ck/CH/LSD/111219, and ck/CH/LHB/130598 was similar to that of H120. Vaccination with the H120 vaccine offered complete protection against challenge with these isolates. However, isolates ck/CH/LDL/110931 and ck/CH/LHB/130573 were serotypically different from their parental viruses and from other serotypes in this study. Furthermore, vaccination with the H120 vaccine did not provide protection against challenge with these two isolates. The results of this study demonstrated that recombination is the mechanism that is responsible for the emergence of new serotype strains, and it has the ability to alter virus serotypes. Therefore, IBV surveillance of chicken flocks vaccinated with IBV live vaccines, as well as the consideration of new strategies to effectively control IBV infection using inactivated or/and genetically engineered vaccines, is of great importance.

1. Introduction

Avian infectious bronchitis virus (IBV) is a ubiquitous, highly contagious respiratory pathogen of chickens that inflicts serious economic losses to the commercial poultry industry worldwide. IBV belongs to the genus *Gammacoronavirus* in the subfamily *Coronavirinae*, family *Coronaviridae*, order *Nidovirales* (de Groot et al., 2012). It has a single-stranded, positive-sense RNA genome of approximately 27.6 kilobases in length that encodes four structural proteins: the nucleocapsid (N), membrane (M), envelope (E), and spike (S). Serotype and genotype classifications, which are usually based on features of the S1 part of the S protein gene, are used to classify IBV strains (de Wit, 2000). Furthermore, recombination may be involved in the evolution of IBV, and it probably occurs at many positions within a given genome during mixed infections (Lai and Cavanagh, 1997).

Historically, the Massachusetts (Mass)-type viruses were believed to be the first and only serotype found in the USA and other regions of the world. However, many IBV serotypes have arisen and disappeared in the poultry industry since then (Cook et al., 2012). The control of IB is mostly accomplished through the use of live attenuated vaccines. The use of commercially produced Mass-type modified live virus vaccines began in the 1950s and has continued to this day. This type of vaccines, such as the Mass strains M41 and H120, are the most commonly used around the world because such vaccines have proven to be capable of protecting against a wide range of IBV strains (Cavanagh and Gelb, 2008). However, the Mass-type of IBVs is constantly isolated from chicken flocks with respiratory clinical signs.

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In this study, the surveillance of IBVs was conducted on 1717 chicken flocks suspected to be infected with IBV between 2009 and 2013 in China. Twenty-nine Mass genotype IBVs were isolated and, together with our previously isolated two strains, the genomic and antigenic characteristics of these IBV isolates were investigated to provide a better understanding of the emergence, circulation, evolution, and antigenicity of Mass-type IBVs in chicken flocks in China.

2. Material and methods

2.1. Eggs and chicks

Fertile White Leghorn specific pathogen-free (SPF) chicks were obtained from the Harbin Veterinary Research Institute, as were White Leghorn SPF chicken eggs. The birds were maintained in isolators with negative pressure, and food and water were provided *ad libitum*.

2.2. Samples, testing, and virus isolation

Samples of tissues and organs were obtained from 1717 chicken flocks with suspected IB clinical signs in China between 2011 and 2013 that were vaccinated with the H120 vaccine (Table 1). These samples were first screened for IBV by reverse transcription-polymerase chain reaction (RT-PCR) as previously described (Liu et al., 2009). Then the IBV-positive samples were used for virus isolation using 10-day-old embryonated SPF chicken eggs as previously described (Liu et al., 2009). Subsequently, a Mass genotype-specific RT-PCR assay targeting the IBV S1 gene (Cavanagh et al., 1999) was conducted on the infected allantoic fluids, and only those viruses of the Mass genotype were used in this study.

2.3. Total RNA isolation and complete genome sequencing and analysis

Total viral RNA was isolated from 200 μl of infected allantoic fluids using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The complete genomes of 31 Mass genotype of IBV strains were amplified and sequenced as previously described (Liu et al., 2013). The obtained nucleotide sequences were manually edited, assembled, and analyzed using the Clustal W method available in the BioEdit software package (version 7.0.3.0., available at: http://www.mbio.ncsu.edu/bioEdit/bioedit) to produce the final sequences of the viral genomes. The S1 gene and genomes of our 31 IBV strains were compared to those of the M41 and H120 strains and other 19 reference strains available in GenBank. Genetic distances were calculated using a maximum composite likelihood model with 1000 bootstrap replicates as implemented in the MEGA 5.0 program (Tamura et al., 2011).

To further identify the recombinant events, the BLASTN program (Liu et al., 2013) was used to search GenBank for IBV sequences that were homologous to those of the five IBV isolates, ck/CH/LHB/111172, ck/CH/LSD/111219, ck/CH/LHB/130598, ck/CH/LDL/110931, and ck/CH/LHB/130573. The Similarity Plot of the complete genomic sequences (Lole et al., 1999), the nucleotide similarities and phylogenetic trees for each deduced recombinant fragment were analyzed with the MEGA 5.0 program (Schierup and Hein, 2000).

All of the 31 complete genomic sequences reported herein have been deposited in the National Center for Biotechnology Information's GenBank database, and the accession numbers are list in Fig. 1A.

Table 1
Characteristics of the IBVs included in the present study[a].

IBV strain	Year	Province	Day	Vaccine used for immunization	Organ used for virus isolation	Type of chicken
ck/CH/LHLJ/091205	2009	Heilongjiang	20	H120	Trachea	Broiler
ck/CH/LHN/090909	2009	Henan	18	H120	Trachea	Broiler
ck/CH/LDL/110931	2011	Dalian	30	H120	Proventriculus	Layer
ck/CH/LHB/110526	2011	Hebei	20	H120	Kidney	Layer
ck/CH/LHB/110825	2011	Hebei	25	H120	Kidney	Layer
ck/CH/LHB/111172	2011	Hebei	24	H120	Kidney	Layer
ck/CH/LHB/111232	2011	Hebei	18	H120	Proventriculus	Layer
ck/CH/LHB/111268	2011	Hebei	25	H120	Proventriculus	Broiler
ck/CH/LHLJ/110310	2011	Heilongjiang	6	H120	Proventriculus + Kidney	Broiler
ck/CH/LHLJ/111050	2011	Heilongjiang	46	H120	Proventriculus	Layer
ck/CH/LSD/110505	2011	Shandong	21	H120	Kidney	Broiler
ck/CH/LSD/110529	2011	Shandong	15	H120	Proventriculus	Broiler
ck/CH/LSD/110726	2011	Shandong	13	H120	Kidney	Broiler
ck/CH/LSD/111219	2011	Shandong	34	H120	Kidney	Layer
ck/CH/LSD/111241	2011	Shandong	26	H120	Proventriculus	Layer
ck/CH/LSD/1112150	2011	Shandong	23	H120	Proventriculus	Broiler
ck/CH/LDL/120557	2012	Dalian	14	H120	Proventriculus	Layer
ck/CH/LHB/120403	2012	Hebei	45	H120	Proventriculus	Broiler
ck/CH/LHB/121024	2012	Hebei	25	H120	Trachea	Layer
ck/CH/LHB/121040	2012	Hebei	25	H120	Kidney	Broiler
ck/CH/LHB/120749	2012	Hebei	23	H120	Proventriculus	Broiler
ck/CH/LJL/121059	2012	Jilin	35	H120	Proventriculus	Broiler
ck/CH/LSD/121228	2012	Shandong	20	H120	Kidney	Layer
ck/CH/LHB/130573	2013	Hebei	24	H120	Proventriculus + Trachea	Broiler
ck/CH/LHB/130598	2013	Hebei	18	H120	Proventriculus	Broiler
ck/CH/LHB/130642	2013	Hebei	25	H120	Proventriculus	Broiler
ck/CH/LHB/131118	2013	Hebei	20	H120	Proventriculus	Broiler
ck/CH/LHB/131132	2013	Hebei	15	H120	Proventriculus	Broiler
ck/CH/LHB/131142	2013	Hebei	23	H120	Proventriculus	Broiler
ck/CH/LHB/131143	2013	Hebei	15	H120	Proventriculus	Broiler
ck/CH/LHLJ/131216	2013	Heilongjiang	7	H120	Proventriculus + Kidney	Broiler

[a] IBV strains ck/CH/LHLJ/091205 and ck/CH/LDL/090909 were isolated in 2009 (Sun et al., 2011).