MOLECULAR CHARACTERIZATION OF INFECTIOUS BURSAL DISEASE VIRUSES FROM THE RECENT OUTBREAKS IN POULTRY IN HARYANA, INDIA

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ABSTRACT

The present study was conducted to characterize the infectious bursal disease virus (IBDV) by reverse transcription- polymerase chain reaction (RT-PCR). Detection of IBDV by RT-PCR was carried out in bursal samples from 30 commercial broiler chicken flocks and four vaccine strains by amplifying the hypervariable region of VP2 gene. The IBDV was detected in bursal samples from 28 out of 30 flocks and in all the four vaccine strains. Phylogenetic analysis based on the nucleotide sequencing of VP2 gene of 10 IBDVs revealed that nine were of very virulent nature due to the presence of 795G, 827T, 833C, 857C, 897A, 905T, 908T and 1011A specific to vvIBDV strains. The amino acids isoleucine at positions 242, 256 and 294, glutamine at position 253 and serine at position 299 were also observed in these nine IBDVs. Amino acid changes from isoleucine to threonine at position 272 in one IBDV (HR/9097/07), asparagine to aspartic acid at position 279 in four IBDVs (HR/8664/07, HR/10277/08, HR/10612/08 and HR/8044/07), glutamic acid to alanine at position 300 in two IBDVs (HR/8664/07 and HR/10277/08) and valine to serine at position 357 in one IBDV (HR/8044/07) were also observed. The remaining one strain had valine, leucine and asparagine at positions 242, 294 and 299, respectively; all of which are features of a classical strain. Nucleotide and deduced amino acid sequencing revealed the presence of vvIBDV strains in commercial broiler flocks and also suggested the continued genetic drift.

Key words: Infectious bursal disease virus, VP2 gene, molecular characterization, broiler chickens

Infectious bursal disease virus (IBDV) is a single shelled, non-enveloped, RNA virus belonging to the genus Avibirnavirus under the Birnaviridae family. On the basis of virus neutralization test, two serotypes of IBDV have been recognized. Serotype 1 includes strains that are pathogenic to chickens but vary in their virulence and antigenicity. On the basis of virulence, these strains have been classified as attenuated, classical virulent, intermediate virulent and very virulent strains. Serotype 2 viruses were initially isolated from turkeys and are non-pathogenic for both turkeys and chickens (van den Berg, 2000). Very virulent (vv) strains of IBDV (vvIBDVs) emerged in the late 1980s in different parts of Europe (Chettle et al., 1989) and later, these strains were reported from Asia and Africa where they caused high mortality (Chettle et al., 1989; Nunoya et al., 1992). The vvIBDVs are capable of establishing themselves even in the presence of high levels of maternal antibodies that normally are protective against classical virulent IBDVs (Kim et al., 2010; Jackwood and Sommer-Wagner, 2011). Infectious bursal disease (IBD) caused by the IBDV has been reported from many parts of the world including India (Kataria et al., 2001; Jeon et al., 2008; Fernandes et al., 2009; Mor et al., 2010).

The IBDV has a double stranded, bi-segmented RNA genome. The major structural protein VP2 displays the greatest amount of nucleotide and amino acid sequence variation among different strains of IBDV (Bayliss et al., 1990). The variable region of VP2 gene has been used for the detection and differentiation of IBDVs (Yamaguchi et al., 2007; Yuwen et al., 2008; Jackwood and Sommer-Wagner, 2010; Li et al., 2011). In this study, we characterized IBDVs from IBD outbreaks in commercial broiler chickens in Haryana, a northwestern state of India. Molecular characterization of the IBDVs would generate data that may help understand the epidemiology better.

MATERIALS AND METHODS

Source of Samples: Bursal samples were collected from 30 IBD-affected commercial broiler flocks from different parts of Haryana during July 2008 to June 2009. Bursae collected from 4 to 5 birds in a flock were pooled to
represent a single sample; thus a total of 30 samples were collected in 50% buffered glycerin. Bursal samples were also collected from a normal, unvaccinated broiler flock to be used as a negative control. Four commonly used vaccine strains (V1,V2,V3 and V4) used in this region were used as positive controls.

RNA Extraction: Total RNA from bursal pools was extracted using TRIzol reagent as per the manufacturer’s protocol (Life Technologies Inc.). Briefly, bursal tissue suspension (10% w/v) was prepared in 1 ml TNE buffer (50mMTris, 150 mMNaCl and 10 mM EDTA, pH 8.0). To 0.25 ml of tissue suspension, 1.0 ml TRIzol reagent was added and mixed. Further processing for total RNA extraction was done following the manufacturer’s protocol. The RNA pellet was resuspended in 20 μl nuclease free water and was stored at -20°C till further use.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR): RT-PCR was carried out to amplify the hypervariable region of VP2 gene of IBDV using the primer pair, F: 5’ACAGGCCCAGAGTCTACACCATAA3’ and R: 5’ATCCTGTGTGCCACTCTTTCGTAGG3’ as reported earlier (Cao et al., 1998). This primer pair generated a product of 479 bp.

Phylogenetic Analysis: PCR products of ten randomly selected IBDV-positive bursal samples (HR/6646/06, HR/6893/06, HR/8044/07, HR/8492/07, HR/8664/07, HR/9097/07, HR/9269/07, HR/9307/08, HR/10277/08 and HR/10612/08) were purified using a QIAquick PCR purification kit (Qiagen, Valencia, CA). The purified PCR products were sequenced using both forward and reverse primers. Phylogenetic differences based on the partial nucleotide sequences of VP2 gene of IBDV were studied using previously published sequences of VP2 gene of IBDV (Table 1). All the ten viral sequences of this study were found to belong to serotype 1 IBDVs. Nine of the ten IBDVs of this study clustered with previously reported vv strains of IBDVs from India and abroad thereby suggesting them to be of vv nature. Of these nine IBDVs, three IBDVs (HR/8492/07, HR/8664/07 and HR/10277/08) were phylogenetically more close to a vvIBDV from Tamil Nadu, India (EF066494/TN-04). The remaining six (HR/6646/06, HR/8044/07, HR/9097/07, HR/9269/07, HR/9307/08 and HR/10612/08) were more close to previously reported vvIBDVs from Haryana state and also from other parts of India. However, the latter vvIBDVs were in different subgroups. The remaining one IBDV (HR/6893/06) was found close to the classical strain in VP2 gene phylogeny and clustered with STC strain (Fig. 1). The OH strain

### Table 1

<table>
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<th>Accession No.</th>
<th>Strain name</th>
<th>Geographic origin</th>
<th>Virulence</th>
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<td>Very virulent</td>
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RESULTS AND DISCUSSION

RT-PCR: The IBDV RNA was detected in 28 bursal samples and in all the four vaccines (V1, V2, V3 and V4). The PCR amplicons yielded a specific band of 479 bp on gel electrophoresis. The specific amplification was not observed on gel electrophoresis in the negative control.

Sequencing and Phylogeny: The sequence data from ten IBDV positive samples were aligned and analyzed for nucleotide and deduced amino acid sequences by comparing them with 16 previously reported partial VP2 gene sequences of IBDV strains (Table 1). All the ten viral sequences of this study were found to belong to serotype 1 IBDVs. Nine of the ten IBDVs of this study clustered with previously reported vv strains of IBDVs from India and abroad thereby suggesting them to be of vv nature. Of these nine IBDVs, three IBDVs (HR/8492/07, HR/8664/07 and HR/10277/08) were phylogenetically more close to a vvIBDV from Tamil Nadu, India (EF066494/TN-04). The remaining six (HR/6646/06, HR/8044/07, HR/9097/07, HR/9269/07, HR/9307/08 and HR/10612/08) were more close to previously reported vvIBDVs from Haryana state and also from other parts of India. However, the latter vvIBDVs were in different subgroups. The remaining one IBDV (HR/6893/06) was found close to the classical strain in VP2 gene phylogeny and clustered with STC strain (Fig. 1). The OH strain
phylogeny. The amino acids isoleucine at positions 242, 256 and 294, glutamine at position 253 and serine at position 299 were also observed in these nine IBDVs. The serine rich heptapeptide sequence (SWSASGS) was observed in all nine IBDVs. Amino acid changes from isoleucine to threonine at position 272 in one IBDV (HR/9097/07), asparagine to aspartic acid at position 279 in four IBDVs (HR/8664/07, HR/10277/08, HR/10612/08 and HR/8044/07), glutamic acid to alanine at position 300 in two IBDVs (HR/8664/07 and HR/10277/08) and valine to serine at position 357 in one IBDV (HR/8044/07) were also observed (Fig. 2). The amino acids threonine and valine were observed at positions 270 and 329, respectively in strain HR/6893/06 of this study. This strain had valine, leucine and asparagine at positions 242, 294 and 299, respectively.

This study was carried out to characterize the IBDVs detected from IBD-affected commercial broiler chicken flocks at molecular level. Such types of studies are helpful in generating epidemiological data which may help to develop effective vaccine. Continuous monitoring of the genetic variability (long-term tracking of viral evolution) is essential in order to provide optimal protection against IBDV (He et al., 2012). In this study, the nucleotide and amino acid substitutions that are specific to vvIBDVs were observed in nine of the ten IBDVs. The amino acids A222, I256 and I294 had been reported to be unique to vvIBDV strains (Hoque et al., 2001; Banda and Villegas, 2004). In addition, the amino acids I242, I256, I294 and S299 have been reported to be highly conserved among vvIBDV strains (Rudd et al., 2002). All nine vvIBDVs of this study had amino acids I242, I256, I294 and S299 as also reported by Jackwood and Sommer-Wagner (2007). Similarly, the IBDVs in China were characterized as vvIBDVs on the basis of conserved amino acid residues: P222A, V256I, L294I, and N299S (Yuwen et al., 2008; Li et al., 2011).

The amino acid glutamine at position 253 was observed in all nine vvIBDVs of this study. The impact of this mutation on the antigenicity or pathogenicity of the virus was not studied. Mutation at this position was considered important for virulence (Li et al., 2011). It has been reported that a single change in amino acid at position H253Q markedly increased the virulence of IBDV (Jackwood et al., 2008). Thus it can be inferred upon that the IBDV with glutamine at position 253 may cause severe disease in commercial broiler chickens. This observation which is a serotype 2 IBDV was altogether in a different clade from all other IBDVs.

Nucleotide sequence analysis revealed that the IBDVs of this study had 92.0-98.9% similarity among themselves. The homology with previously published Indian strains was 91.2-98.9% and with strains from other countries was 91.2-97.2%. Whereas the IBDVs of this study showed only 71.5-73.4% homology with Ohio, USA strain at the nucleotide level.

The nucleotide substitutions at 795G, 827T, 833C, 857C, 897A, 905T, 908T and 1011A were present in all nine IBDVs that clustered with vvIBDVs in VP2 gene phylogeny. The amino acids isoleucine at positions 242, 256 and 294, glutamine at position 253 and serine at position 299 were also observed in these nine IBDVs. The serine rich heptapeptide sequence (SWSASGS) was observed in all nine IBDVs. Amino acid changes from isoleucine to threonine at position 272 in one IBDV (HR/9097/07), asparagine to aspartic acid at position 279 in four IBDVs (HR/8664/07, HR/10277/08, HR/10612/08 and HR/8044/07), glutamic acid to alanine at position 300 in two IBDVs (HR/8664/07 and HR/10277/08) and valine to serine at position 357 in one IBDV (HR/8044/07) were also observed (Fig. 2). The amino acids threonine and valine were observed at positions 270 and 329, respectively in strain HR/6893/06 of this study. This strain had valine, leucine and asparagine at positions 242, 294 and 299, respectively.

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needs further confirmation. Similarly, the impact of amino acid changes at one or two positions observed in vvIBDVs of this study needs to be examined further. The serine rich heptapeptide sequence (326SWSASGS332), observed in 9 of the 10 IBDVs of this study indicated the circulation of very virulent strains of IBDV in commercial broiler chicken flocks. Divergence of vvIBDVs (1.1-93.3%) of this study from previously reported vvIBDVs indicated the continuation of genetic changes in vvIBDVs.

The change of amino acid from alanine to threonine at position 270 as observed in one strain (HR/6893/06) of this study had been reported in classical and attenuated IBDV isolates (Domanska et al., 2004). This strain (HR/6893/06) had V 242, L 294 and N 299, a characteristic of classical strains. Classical strains of IBDVs have also been detected earlier (Jackwood and Sommer-Wagner, 2007; Yamaguchi et al., 2007; Yuwen et al., 2008; Kim et al., 2010). Thus the IBDV from sample HR/6893/06 based on nucleotide and amino acid substitutions and clustering in VP2 phylogeny may be a classical strain. Percent homology of the IBDVs of this study with previously published sequences of VP2 gene at the nucleotide level is in accordance with earlier reports (Lin et al., 1993; To et al., 1999; Mittal et al., 2006).

Molecular epidemiological studies have indicated that continuous mutation in amino acids of hypervariable region of IBDV leads to antigenic drift in the virus. These specific point mutations may allow virus to escape from immunity induced by vaccines (Jackwood and Sommer-Wagner, 2011). As mentioned earlier, the IBDVs of this study also had point mutations; the impact of such mutations on causation of disease in vaccinated flocks and severity of the disease induced in such flocks needs to be explored further. In conclusion, this study indicates the presence of vvIBDVs in commercial broiler chicken flocks in Haryana and that continuous surveillance involving different types of birds (broilers, breeders and layers) is essential to better understand the epidemiology of IBD.

REFERENCES


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