Incidence and Molecular Characterization of Infectious Bursal Disease Virus in Commercial Broilers in Pakistan

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Abstract

The study was conducted to determine the prevalence of infectious bursal disease virus (IBDV) at different ages in commercial boilers. From Oct 2003 to Nov 2004, bursa samples collected from both vaccinated as well non-vaccinated commercially reared broiler chickens suspected of having infectious bursal disease were analyzed for the presence of IBDV using reverse transcriptase polymerase chain reaction (RT-PCR). A 743-bp fragment of the VP2 hypervariable region from the nucleotides 701 to 1444 was amplified. Out of the 237 tested samples 103 (43.45%) were found to contain IBDV genome using RT-PCR. The percentage of positive results in all age groups despite of the discrimination between vaccinated and non-vaccinated birds was 26.53 % in 0-3 weeks, 56.30 % in 3-6 weeks and 33.34 % in 6-8 weeks of age, respectively. Results indicated increased incidence of IBDV in non-vaccinated (47.91 %) birds as compared to vaccinated birds (35.05 %).

Key Words: IBDV; Commercial Broilers; RT-PCR; Pakistan

Introduction

Infectious bursal disease is an acute, highly contagious viral disease of young chickens that causes significant losses to the poultry industry worldwide1. After infection, the IBDV multiplies in the developing B-lymphocytes in the bursa of Fabricius (BF) leading to immuno-suppression and susceptibility to the other infections2. Two distinct serotypes of IBDV designated as serotype I and serotype II, have been identified. The serotype I strains are pathogenic to chickens and vary in their virulence, whereas serotype II strains isolated from turkeys are apathogenic to both chickens and turkeys1,2,3.

IBDV is the member of the genus *Avibirnavirus* in the family *Birnaviridae*. The viral genome is composed of two segments of dsRNA, designated as A and B, enclosed within a capsid shell of icosahedral symmetry with a diameter of 55-60 nm1,4. Segment A (± 3400 bp) contains two open reading frames (ORFs) of 3039 bp and 438 bp, which partially overlap at the 5’ end of the genome. The larger ORF encodes the polyprotein (NH2-VP2-VP4-VP3-COOH), which is cleaved into three proteins designated as
VP2 (40-42 KDa), VP3 (32 KDa) and VP4 (28 KDa). The smaller ORF encodes for a 17KDa VP5 protein. The VP2 is the major structural protein of the viral capsid, carries highly conformational epitopes responsible for the induction of protective antibodies. The smaller segment B (±2800 bp) encodes VP1 (90 KDa) protein with polymerase activity.

RT-PCR has been described as a sensitive technique for the molecular detection of IBDV in clinical samples. Studies on the serum antibodies against IBDV exist in commercial broiler birds in Pakistan but merely seropositivity does not correlate with the magnitude of the problem. Further, currently no information is available about the molecular epidemiology of IBDV genome that could indicate the percentage of affected birds at different ages. Therefore, in an attempt to provide the data that would show the current situation of IBDV prevailing in commercially reared broilers using RT-PCR, the present study was designed.

Materials and Methods

1.1. Bursa Samples

A total of 237 bursa samples were collected from vaccinated (144/237; 60.75%) as well as non-vaccinated (93/237; 39.24 %) broiler flocks showing clinical signs suspected of IBD from Oct 2003 to Nov 2004. All broiler birds included in the present study were commercial poultry flocks of at least 3000 broilers. Clinical signs, gross and microscopic lesions were also observed.

1.2. Viral RNA Preparation

One gram from each bursa sample was homogenized in TNE buffer (Tris HCl (pH 8.3) 10mM, NaCl 100mM and EDTA 1mM). To 500 µl homogenate, equal volume of chloroform was added, mixed gently and centrifuged @ 10000 X g at 4°C for 10 minutes. The aqueous phase, 0.5 ml of the TRIzol (LS- Reagent, Life Technologies Inc., Frederick, MD) and 0.1 ml of the chloroform were mixed and centrifuged @ 10000 X g for 10 minutes at 4°C. The aqueous phase was mixed with 0.5 ml of isopropanol and centrifuged @ 10000 X g at 4°C for 10 minutes. The pellet was washed using 70% ethanol, air dried and resuspended in a 100 µl volume of 90% dimethyl sulphoxide (DMSO) (Serva Feinbiochemica, NY).

1.3. cDNA synthesis

A 5 µl RNA (approximately containing 5µg of RNA) was denatured at 95°C for 5 minutes and then used for the synthesis of cDNA using RevertAid™ First Strand cDNA synthesis kit (MBI Fermentas, Graiciunau 8, Vilnius 2028, Lithuania) according to the manufacturer’s instructions. Briefly, to 5 µl of RNA, 1 µl of random hexamer primer (0.2 µg/ µl) and 12 µl deionized DEPC treated water (nuclease free) were added and incubated at 70°C in water bath for five minutes. Then 5X reaction buffer, 20 U of Ribonuclease inhibitor and 2 µl of dNTPs (10mM) were added and incubated at 25°C for five minutes. Finally, 200 U of M-MuLV RT was added and incubated at 25°C for ten minutes and then at 42°C for one hr. The reaction was stopped by heating at 70°C for ten minutes, chilled on ice and stored at -20°C until used for PCR.

1.4. Polymerase Chain Reaction (PCR)

A primer pair that amplified a 743-bp fragment of the VP2 gene hypervariable region was used as described by Jackwood and Jackwood. Each reaction tube contained 5 ul of 10X PCR buffer (Sigma) [200
mM Tris-HCl (pH8.3), 500 mM KCl and 15 mM MgCl₂, 1ul of 10 mM dNTPs each, 2 U of Taq polymerase (Eppendorf) and 35 ul of DEPC nuclease free water. MgCl₂ concentration was kept at 2.5 mM in a total reaction volume of 50 µl. The reaction conditions consisted of denaturattion at 95°C for 30 sec, annealing at 56°C for 30 sec and extension at 72°C for 1 min. A final extension was given at 72°C for 7 min. The reaction steps were repeated for 35 cycles. A 8 µl f the PCR product was electrophoresed (80 V for 40 minutes) on 1.5% agarose gel. Amplified bands were visualized under UV light at a wavelength of 254 nm with Eagle Eye Gel Documentation System (Stratagene, USA) after ethidium bromide staining @ 0.5µg/ml. The size of the bands was confirmed with the help of a 100-bp DNA ladder as a molecular size marker (Invitrogen Life Technologies).

Results and Discussion

IBD was first recognized as a disease entity in “Gumboro” district of Delaware State in USA in 1957 by A. S. Cosgrove. In Our neighboring country India, the disease was first seen in 1971 in Uttar Pradesh. Sporadic outbreaks of IBD were seen in Karachi and Faisalabad areas in 1983. It has now become an endemic disease in poultry birds in all areas of Pakistan and causes great economic losses to the industry due to its increased mortality directly and immuno-suppression indirectly. In order to assist the re-evaluation of the disease control policy the present study was designed with the aim to determine the prevalence of the virus among different age groups of commercial broiler birds.

Table 1: Prevalence of IBDV at different age groups in Vaccinated and Non-Vaccinated Commercial Broiler Birds

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Age (Weeks)</th>
<th>Non-Vaccinated</th>
<th>Vaccinated</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. Tested</td>
<td>No. Positive (%)</td>
<td>No. Tested</td>
</tr>
<tr>
<td>1</td>
<td>0-3</td>
<td>32</td>
<td>9 (28.12 %)</td>
<td>17</td>
</tr>
<tr>
<td>2</td>
<td>3-6</td>
<td>75</td>
<td>42 (56.0 %)</td>
<td>44</td>
</tr>
<tr>
<td>3</td>
<td>6-8</td>
<td>37</td>
<td>18 (48.64 %)</td>
<td>32</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>144</td>
<td>69 (47.91 %)</td>
<td>93</td>
</tr>
</tbody>
</table>

Different Superscript values differ significantly in that column (p<0.05)

Of the clinical signs noted, the birds appeared sick, lethargic with chalky white diarrhea first and later anorexic with severe depression and prostration leading to death. In some birds soiling of vent feathers with pecking their own vent was observed. Grossly the carcass was dehydrated and dark in color with severe hemorrhages on breast and thigh muscles. Bursa showed characteristic changes i.e. edematous, enlarged and severely hemorrhagic. In some birds the bursa contained the clotted blood. Spleen and liver were slightly enlarged and contained yellowish foci on the surface. In Some birds, hemorrhages in the
mucosa at the juncture of proventriculus and gizzard were also observed. Microscopically the bursa revealed degeneration of lymphocytes in the medullary area of bursal follicles.

An amplified product of approximately 743 bp was obtained in all positive cases. Primers used in the present study amplified a fragment fro 701 to 1444 of VP2 gene that has been considered to be the hypervariable region and conserved among all field strains. These primers are licensed by IDEXX laboratories Inc. (Westbrook, ME USA) for commercial use and have been successfully used.

IBDV isolates from vaccinated and non-vaccinated flocks were analyzed through RT-PCR. Majority of the samples were from non-vaccinated chickens of more than 3 weeks. Birds less than three weeks of age showing impaired growth suspected to be suffering from subclinical IBD were also included in the study. In vaccinated birds of 0-3 weeks of age, 4 out of 17 (23.52 %) while in non-vaccinated birds 9 out of 31 (29.03 %) were found positive. We speculated that IBDV-positivity in vaccinated birds might be due to vaccinal virus but non-vaccinated birds of the same group might be suffering from subclinical form of IBD without showing any clinical signs. The subclinical immuno-suppressive form might be due to the inadequate level/ waning of maternally derived antibodies (MDAs) or due to the existence of different strains of environmental IBDV which can even escape through the neutralizing antibodies as in Pakistan day old progeny chicks are being sold by many hatcheries with unknown status of parental immunity. Similar results have been described and they further reported that the best time for the detection of IBDV using RT-PCR is between 3 and 4 weeks of age. The present study showed similar findings and indicated that the virus can be diagnosed in less than 3 weeks of age but the percentage of observing positive samples would be more in birds older than 3 weeks.

In the non-vaccinated 3-6 week age group, 42 out of 75 (56.00 %) whereas in vaccinated birds 25 samples out 44 (56.81 %) were positive. It was noted that many of the infected birds from the age groups 3-6 weeks in both vaccinated as well as non-vaccinated showed some signs of adenovirus hepatitis and clostridial enteritis. Similar results have been described. Anjum et al., reported that non-vaccinated flocks (56.58 %) suffered more than vaccinated flocks (43.42 %) but the present study indicated that both groups are equally susceptible to the field virus challenge and difference only lied in the severity of the infection i.e. disease was found more severe in case of non-vaccinated birds. One possible reason might be the presence of antigenic variants as there are reports of IBDV outbreaks in many farms even after vaccination or perhaps might be due to the injudicious use of IBDV vaccines that cause neutralization of antibodies thus making the birds susceptible to the infection. Our previous studies also supported the argument about the presence of field variants of IBDV in Pakistan. Furthermore, this group showed maximum clinical signs. In the birds of 6-8 weeks age group, out of 37 samples 18 (48.64 %) in non-vaccinated and 5 out of 32 (15.62 %) were found positive. This age group has been considered as least susceptible but the present study indicated that non-vaccinated birds still are more prone to the infection as might be due to the antigenic variants.

Due to the intensive farming, the poultry sector has now become the biggest industry in Pakistan which has lead to the increased incidence of IBD from 26.86% in 1992 to 43.45 % described in the
present report. In India, the increased incidence of IBD in broiler chickens of 3-4 weeks (49.81 %) and 33.58 % in 4-7 weeks has been recorded\textsuperscript{21}. The present study also reported similar findings but with higher percentage in non-vaccinated birds i.e. 47.91% in non-vaccinated birds and 35.05 % in vaccinated birds. Overall percentage of positive results (103/236=43.45%) in all age groups despite of the discrimination between vaccinated and non-vaccinated birds was 26.53 % in 0-3 weeks, 56.30 % in 3-6 weeks and 33.34 % in 6-8 weeks. In conclusion, due to the increased incidence of IBDV as demonstrated by the present study, it is suggested that management and bio-security practices need to be improved. Further, the possible way to control the IBD infections, MDAs must be increased by hyperimmunising or by improving the vaccination schedules in parent flocks.

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**References**


