Short Communication

Conventional and Molecular Detection of Infectious Bursal Disease Virus in Broiler Chicken

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A B S T R A C T

The present study was conducted to compare the different diagnostic methods for detection of Infectious Bursal disease virus (IBDV) in broiler chickens. For this purpose a total of 100 samples of Bursa of Fabricius were collected from poultry flocks of Punjab Pakistan during the period (from December 2012 to May 2013). IBDV was isolated from field samples by inoculating the suspected bursa samples in embryonated chicken eggs through chorioallantoic membrane. Virus was confirmed through conventional serological method Agar Gel precipitation test and molecular method Reverse transcriptase-polymerase chain reaction (RT-PCR). The 743-bp region of VP2 gene of IBDV was amplified by using specific primers. Agar gel precipitation test revealed the presence of IBDV in 25 (71.4%) field infected bursal samples and RT-PCR revealed the IBDV in 28 (80%) samples. RT-PCR is more accurate, sensitive and specific method for rapid detection of infectious bursal disease virus from field samples.

Infectious bursal disease (IBD) is a highly contagious acute viral disease of young chickens of 3-6 weeks old that causes a fatality or immunosuppression by damaging bursa of Fabricius and impaired growth of young chickens which results significant economic losses in the poultry industry (Islam, 2005). The causal agent of IBD is infectious bursal disease virus (IBDV), a non-enveloped double stranded RNA (dsRNA) virus belonging to the family *Birnaviridae* (Jackwood *et al.,* 1984). IBDV strains have been classified into two distinct serotypes 1, pathogenic and 2, non-pathogenic (Van den Berg *et al.*, 2000).

The disease is manifested by debilitation, dehydration and development of depression with watery diarrhea, swollen and blood stained vent (Islam and Samad, 2004). Severity of the signs depends on the virus strain and the age and breed of the chickens (Van den Berg *et al*., 1991). Infection with less virulent strains may not show obvious clinical signs but the birds may have fibrotic or cystic bursa of Fabricius that become atrophied prematurely (before six months of age) and may die of infections by agents that would not usually cause disease in immunocompetent birds. Infectious bursal disease (IBD) is commonly encountered lymphocytolytic disease that adversely affects the defene mechanism of birds and results in immunosuppression and failure to develop

satisfactory immunity (Beenish *et al*., 2013). The postmortem findings were haemorrhages in the thigh/pectoral muscles, enlarged, edematous and hyperemic bursa or atrophic in chronic cases and hemorrhage in the junction between gizzard and proventriculus (Chettele *et al.,* 1989). Though gross lesions of IBD affected poultry are considered sufficient for diagnosis but are sometimes confused with other diseases (Banda, 2001).

Various diagnostic methods like indirect haemagglutination (IHA) test, virus neutralization test (VNT), enzyme linked immunosorbent assay (ELISA), fluorescent antibody technique (FAT) and agar gel immunodiffusion test (AGIDT) are used limitedly to detect IBDV and molecular techniques like reverse transcriptase polymerase chain reaction (RT-PCR) have frequently used to detect viruses from the field samples (Gohm *et al.,* 2000; Mathivanan *et al.*, 2004). The VP2 gene encodes major protective epitopes, contains determinants for pathogenicity, and is highly variable among IBDV strains (Abdel-Alim and Saif, 2001) and have been used for detection of IBDV.

In Pakistan, IBD continues to be a serious problem. Severe outbreaks of the disease occurred in commercial broiler flocks, causing up to 60% mortality despite vaccination (Lone *et al*., 2009). The diagnosis of the disease until now has depended mainly on clinical signs, gross pathology, and serological tests, and there has been a lack of information about the molecular detection of IBDV strains in Pakistan. The aim of present study was

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to isolate and identify the IBD virus from field samples by using different techniques, serological method agar gel precipitation test and molecular method using RT-PCR to diagnose IBDV from bursal field samples.

Materials and methods

A total of 100 samples of Bursa of Fabricius were collected from poultry flocks on reported outbreaks by field veterinarians and from the farmers visiting Postmortem Section of Pathology Department and University Diagnostic Lab of University of Veterinary and Animal Sciences, Lahore, Pakistan (during the period from the Dec 2012 to May 2013), for the purpose of postmortem and lab-based disease diagnostics. During the samples collection detailed information regarding age, breed, and history of previous disease outbreak, vaccination and treatments were recorded. The samples collected for diagnosis labeled and preserved under refrigeration. The tissues samples were divided in three parts in which thirty field bursal samples were processed for virus isolation, thirty five were processed for IBD virus identification through agar gel precipitation test and thirty five were processed for molecular detection.

For isolation of IBD virus, 10 day-old embryonated chicken eggs were inoculated through chorio-allantoic membrane (CAM) at dose rate of 0.2ml of IBDV inoculum having titer of (EID50 $10^{5.50}$/100ul) (0.1 ml virus suspension + 0.1 ml antibiotic mixture). For IBDV samples of CAM were collected with PBS (phosphate buffered saline) to prepare 50% suspension and stored at -80°C for further use (Majed *et al.,* 2013).

The triturated bursal field samples (50% inocula) were used for agar gel precipitation (AGPT) test (Wood *et al.*, 1979). Briefly, the central well of a glass slide coated with melted agarose gel was loaded with known hyperimmune sera against IBDV and peripheral wells with reference antigen (taken from Veterinary Research Institute, Lahore) of IBDVs and bursal suspensions. Slides were kept in moist chamber at 40°C and observed at 24, 48 and 72 h interval for antigen antibody reaction in the form of appearance of precipitation lines in between the central and peripheral wells.

For molecular detection viral RNA of the IBD virus was extracted from 150 µl of suspected bursal field sample and laboratory isolated virus using FavorPrep™ Viral Nucleic Acid Extraction Kit (Favorgen, Fisher Biotech, Australia) according to the manufacturer's procedure. The RNA was extracted in 50 µl of elution buffer and used as template directly for RT-PCR assay or stored at -80°C until further use.

A commercial cDNA synthesis kit (Fermantas, USA) was used to make cDNA. The procedure adopted was taken from instruction manual of manufacturer. To amplify a 743 bp fragment of VP2 hypervariable region (Bayliss *et al.*, 1990), we used Forward primer 5'- GCCCAGAGTCTACACCAT -3 and Reverse primer 5'- CCCGGATTATGTCTTTGA -3' (Jackwood and Nielsen, 1997). The amplification products were detected by gel electrophoresis in 1.5% agarose gel in TAE buffer. Gels were run for 1.5 h at 80 V, stained with ethidium bromide (0.5 µg/ml), exposed to ultraviolet light and photographed (Visi-Doc-It system, UVP, UK). A commercial 100-bp DNA ladder (Fermentas) was used as molecular-weight marker in each gel running.

Results and discussion

A total of 100 bursal field samples from poultry flocks on reported outbreaks were collected after postmortem findings. Out of the 30 bursal field samples, 5 (16.6%) were positive for isolation of virus. In positive cases the embryos died within 24 to 96 h post-inoculation. The CAM of infected embryonated egg was thickened, dead embryos were congested and hemorrhagic similar to the findings of Hitchner (1970) and Takase et al. (1996). The reduced rate of virus isolation may be due to absence or low concentration of virus in the inoculums or due to the presence of maternal antibody in the embryonated eggs. By AGPT, out of 35 field samples, 25 (71.4%) samples were positive and prominent white line of precipitation was noticed between known positive anti-IBDV hyper-immune serum of the central well and bursal homogenates of the peripheral wells due to antigen and antibody reaction within 24 and 48 h. The results are in agreement with the findings of Muhammad et al. (1996) and Gupta et al. (2001). The nucleic acid based detection tests like RT-PCR have been used for the detection of viruses (Kataria et al., 2000). IBD viral RNA was extracted from both 35 field samples and direct IBD isolated virus using specific primers conducted on a 743-bp fragment of the VP2 gene. Out of 35 field samples 28 (80%) were positive for IBDV and all virus isolates were positive for RT-PCR.

To conclude RT-PCR allows rapid diagnosis of IBDV from bursal tissue samples as has been earlier reported by Jackwood and Sommer (1997, 1998) and Majed *et al.* (2013).

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