MOLECULAR DETECTION OF INFECTIOUS BURSAL DISEASE VIRUS FROM POULTRY IN THRISSUR DISTRICT

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Abstract

Three ailing broilers of age 3 weeks, 5 weeks and 9 weeks respectively were presented to the Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy from three different private farms in Thrissur district for disease investigation. Owners reported a high mortality rate among the flocks and the birds were showing symptoms such as anorexia, depression, ruffled feathers, white watery diarrhoea and death. On post mortem examination, enlargement of bursa suggestive of infectious bursal disease (IBD) was noticed. The further confirmation of the cases were done with a reverse transcriptase polymerase chain reaction (RT-PCR) targeting the VP2 gene of Infectious bursal disease virus (IBDV), which revealed an amplicon size of 480 bp, specific for VP2 gene.

Key words: *Infectious bursal disease; RT-PCR; Thrissur district*

Infectious bursal disease is a contagious immunosuppressive disease of chicken, caused by infectious bursal disease virus (IBDV). This disease is associated with high economic losses to the broiler industry. The causative virus is a member of the family *Birna viridae*, belonging to the genus *Avibirna virus*. Two known serotypes of IBDV are present viz., serotype 1 and 2. Serotype 1 is pathogenic to chicken, while serotype 2 is found to be non pathogenic. In infected birds, there will be immunosuppression, inflammation and atrophy of bursa of Fabricius, nephritis and haemorrhages in thigh muscles. The first report of a specific disease affecting the bursa of Fabricius in chickens was documented by Cosgrove (1962) from Gumboro, Delaware, United States of America. In India, the disease is highly prevalent among the broiler flocks causing severe economic losses. In Kerala, there is high incidence of the disease among poultry and no routine vaccination is carried out in our state apart from a very few organized farms.

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The tentative diagnosis of IBD can be done based on the clinical signs and post mortem findings. Isolation and identification of the causative agent, which is the gold standard test, is laborious and time consuming. The diagnostic methods, includes detection of viral antigens in bursa by using agar gel immunodiffusion (AGID), Immunofluorescence test (IFT), antigen capture enzyme linked immunosorbent assay (ELISA) and reverse transcriptase –polymerase chain reaction (RT-PCR). Among these tests, RT-PCR offers a rapid, highly sensitive and specific test for the confirmative diagnosis of the disease which would help in controlling the disease, thereby reducing the economic losses significantly.

The present study deals with the identification of infectious bursal disease virus from infected birds of 3, 5 and 9 week age groups from three different private farms in Thrissur district employing an RT-PCR.

Materials and Methods

Post mortem examination was conducted on the three sacrificed birds under aseptic conditions and samples from liver, spleen and heart blood were collected. The isolation of the causative agent was done using standard bacteriological methods with special emphasis on *Salmonella* (OIE, 2012). The organ samples collected during post mortem examination were streaked onto Blood agar (BA), brain heart infusion agar (BHIA) and Mac Conkey agar (MCA) for primary isolation. Intestinal contents were inoculated into 10 mL of buffered peptone water as pre-enrichment for *Salmonella*. After incubation for 12 h at 37 °C, about 0.1 mL of the pre-enrichment broth was transferred to 10 mL of selective broth *viz.*, Rappaport-Vassiliadis broth and then incubated at 42°C for 48 h. Following the incubation period, a loopful of inoculum was transferred to MCA and incubated for 24 h under aerobic condition in a bacteriological incubator. The samples were inoculated in duplicate in Sabouraud's dextrose agar (SDA) and kept at 37°C and room temperature.

For conducting RT-PCR, bursal samples were collected aseptically in RNA later solution and RNA was extracted from the samples employing Trizol method. The RNA extracted from the IBD vaccine (Nobilis, Intervet) was kept as the positive control.

The primers used in the study for the detection of infectious bursal disease were selected as per Singh *et al*. (2014) and RT-PCR (Biorad) was carried out with minor modifications. The first strand cDNA was synthesized using RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific). The PCR conditions were standardized as per the details given in the Table 1. The PCR product was detected by electrophoresis in one per cent agarose gel in 1 X TAE buffer (Thermoscientific). The gel was visualized and the results were documented in a gel documentation system (Biorad).

Results and Discussion

On post-mortem examination, inflamed, edematous bursa with caseous exudates inside was observed, suggestive of infectious bursal disease. The liver, spleen and heart appeared to be little congested. On BA, BHIA and MCA, no bacterial organisms of pathogenic significance could be isolated. No growth could be detected on SDA at 37°C and even after incubation at room temperature for up to seven days. An RT-PCR was conducted, targeting the VP2 gene of infectious bursal disease virus which revealed an amplicon size of 480 bp according to Singh *et al*. (2014) which confirmed the cases as IBD (Fig 1).

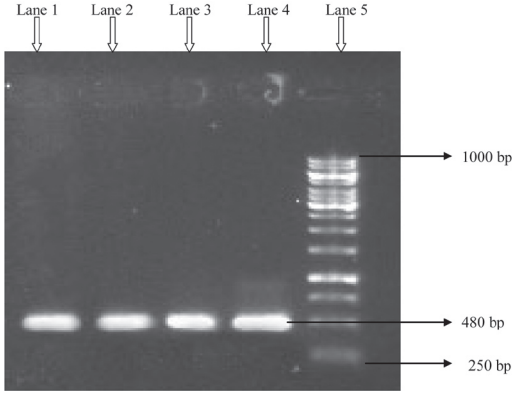

Fig1. PCR targeting the VP2 gene of Infectious Bursal Disease

Lane 1, 2, 3: 480 bp; Lane 4: positive; Lane 5: 1000 bp Ladder

Table 1. PCR conditions for amplification of infectious bursal disease virus

Steps		Temperature	Time
Initial denaturation		95ºC	5 min.
34 cycles	Denaturation	95ºC	30 sec.
	Annealing	59.5ºC	60 sec.
	Extension	72ºC	30 sec.
Final extension		72ºC	6 min.
Hold		4ºC	10 min.

Chickens infected with IBDV between three and six weeks of age group usually develops clinical signs of IBD, which may result in death, but those infected at less than three weeks of age may have few or no clinical signs (Okoye and Uzoukwu, 1981) but experience permanent and severe immunosuppression. The reason for young chicken exhibiting no clinical signs of the disease is not known. However, immunosuppression occurs due to damage of bursa of Fabricius. The affected birds have poor body weight and feed conversions. The poor performance of chicken is due to the immunosuppression caused by subclinical IBD. The clinical forms of IBD usually occur in chicken from three to six weeks of age. The disease has also been observed in chickens older than six weeks, even in up to 20-week-old chickens. In the present study, clinical disease has a sudden onset and mortality rate in the flock increased rapidly and the signs exhibited by the birds include dehydration, trembling, ruffled feathers, vent picking, whitish watery diarrhoea and depression (Tehsome and Admassu, 2015). On post-mortem examination, the bursa of Fabricius was enlarged and the kidney tubules were well distended. Histopathological sections of the bursa were characterized by edema, destruction of lymphocytes, and heterophilic infiltration (Okoye *et al*., 1981).

Various techniques like virus isolation, IFA, Antigen capture ELISA and AGID were employed earlier for the detection of infectious bursal disease. But now a day, these techniques are replaced by molecular techniques which are more sensitive, rapid and enable a rapid diagnosis. Among the various diagnostic techniques currently available, RT-PCR was found to be the appropriate technique being highly sensitive for the detection of IBDV (Yousif, 2005).

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