Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2019; 7(1): 1041-1042

Received: 09-11-2018
Accepted: 13-12-2018

Preeti
Department of Veterinary Public Health and Epidemiology, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India

Suresh Kumar
Department of Veterinary Public Health and Epidemiology, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India

Naresh Jindal
Department of Veterinary Public Health and Epidemiology, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India

Ramkaran
Department of Veterinary Physiology and Biochemistry, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India

Correspondence
Preeti
Department of Veterinary Public Health and Epidemiology, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India

Detection of infectious bursal disease virus by using one-step PCR method

Preeti, Suresh Kumar, Naresh Jindal and Ramkaran

Abstract
The present study was conducted to detect the infectious bursal disease virus by using one-step reverse transcription polymerase chain reaction (RT-PCR) from IBD-affected broiler flocks in Haryana, India. Of the 85 pooled bursal samples, 83 (97.64%) bursal samples were found positive for IBDV by one step RT-PCR using VP2 gene specific primers.

Keywords: Infectious bursal disease, reverse transcription polymerase chain reaction, RNA etc.

Introduction
Infectious bursal disease virus (IBDV) causes infectious bursal disease (IBD) which is a highly contagious and immunosuppressive disease in young chicken. It was first discovered by Cosgrove [3] in 1962 from an outbreak in Gumboro area of Southern Delaware, so it was called Gumboro disease. Infectious bursal disease virus (IBDV) belongs to family *Birnaviridae* of the genus *Avibirnavirus*. The virus is double stranded RNA, bi-segmented (segments A and B), non-enveloped and icosahedral (Dobos *et al*., 1979; Murphy *et al*., 1995, Nwagbo *et al.*, 2016) [4, 11, 12]. Two serotypes: serotype-1 and serotype- 2 have been discovered on the basis of virus neutralization test. Serotype-1 IBDVs vary in virulence and pathogenicity, and cause disease and immunosuppression in chickens.
In India the IBD was reported for the first time by Mohanty *et al*. (1971) [8] and it remained in its classical form until 1990's. In an earlier study, Mor *et al*. (2013) [9] collected bursal samples from 30 IBD-affected commercial broiler flocks during 2008-2009 and reported the presence of IBDVs in 28 flocks by reverse transcription-polymerase chain reaction (RT-PCR). Hence, the present study was undertaken to detect the IBDV from IBD affected flocks of Haryana, India.

Material and Methods
Sample collection: Bursal samples were collected aseptically from 85 chicken flocks suspected to be suffering from IBD during the period from 2012- 2016 from different parts of Haryana, India. In almost all the IBD-affected flocks, the symptoms of dullness, depression, anorexia, ruffled feathers and yellowish white or greenish yellow diarrhoea were observed. The post-mortem lesions included oedematous and swollen bursa, the presence of gelatinous exudates around bursa with haemorrhages and haemorrhages on thigh muscles were recorded. The bursal samples of 4-5 birds in a flock were collected and pooled. Such 85 pools were collected. Pooled sample hereinafter will be referred as 'Sample'. The samples immediately after collection were stored at -20^{0}C until further processed for total RNA isolation.

RNA isolation and RT-PCR
Bursal tissue suspension was prepared by homogenizing 5mg of bursa sample in 5ml phosphate buffer saline. The homogenate was clarified by centrifugation at 5000rpm for 4min. Total RNA from bursal tissue homogenate was extracted by TRIzol reagent (Life technologies Inc.). One step reverse transcription-polymerase chain (RT-PCR) was carried out to amplify the hypervariable region of VP2 gene of IBDV using one-step RT-PCR Kit (Qiagen, Germany). A primer pair, VP2 forward 5′-GGCCCAGAGTCTACACCATAAC-3′ and reverse 5′-CCGGATTATGTCTTTGAAGCC-3′ was used which generated PCR product of size 743 bp. The primers were reconstituted in nuclease free water to 100 pmol stock solution. The one step PCR was performed in 20µl reaction mixture containing 4µl of 5 x RT-PCR buffer,

0.8µl of dNTP mix, 1.2µl of forward and reverse primers, 1.5µl of template RNA and 0.8µl of enzyme mix. Amplification steps consisted of RT at 50^{0}C for 30 min, Taq activation at 95^{0}C for 15 min, followed by 35 cycles of denaturation at 94^{0}C for 1 min, annealing at 58^{0}C for 1 min and extension at 72^{0}C for 1 min, and a step of final extension at 72^{0}Cfor 10 min. The PCR products were gel electrophoresed and bands of 743 bp were observed in positive cases. IBD vaccine procured from local market acted as positive control. Bursal tissue sample was also collected from unvaccinated uninfected bird; this sample acted as a negative control. Total RNA from the vaccine strain as well as from bursal tissue sample of unvaccinated uninfected bird was extracted as done for the bursal samples from IBD-affected birds.

Results and Discussion

The Department provides disease investigation services to the livestock and poultry farmers in the state. During the year 2015-16, IBD was detected in 7.11% (195/2741) flocks and in many flocks, the disease was observed even after vaccination at day 12-15 of age in commercial broiler flocks. Though there can be many reasons for the disease to occur in vaccinated flocks; presence of very virulent strains can be one of them. The aim of the present study was to detect IBDV from broiler flocks affected with IBD. One-step RT-PCR technique was used for the amplification of hypervariable region of VP2 gene of IBDVs from bursal samples. The authenticity of these amplicons was verified by their size in agarose gel electrophoresis. A band of 743 bp for VP2 gene was observed in 83 (97.64%) of the 85 samples from IBD-affected flocks and the positive control (Fig. 1).

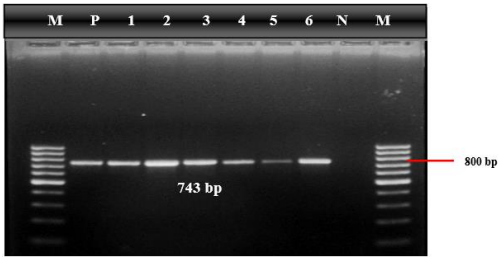

Fig 1: Gel picture of 743bp PCR products of field samples using VP2 gene specific primers of IBD virus on 1.5% agarose gel Lane M: 100bp DNA molecular size marker; Lane N: Negative control; Lane P: Positive control, Lanes 1-6: Field samples.

The specificity of RT-PCR was further confirmed by the absence of amplification in the negative control. Many workers have verified the authenticity of PCR products by the size and nucleotide sequencing of the amplicons using hypervariable region of VP2 gene of IBDV (Rojs *et al.*, 2008; Adamu *et al*., 2013; Jenberie *et al.*, 2013; Amin and Jackwood, 2014; Mawgod *et al*., 2014; Vukea *et al*., 2014; Owolodun *et al.*, 2015; Xu *et al.*, 2015; Morla *et al*., 2016) [14, 1, 6, 2, 7, 16, 13, 17, 10]. Jackwood and Sommer (2010) [5] examined 47 pooled bursa samples; of which 12 (25.5%) samples were positive for IBDV by RT-PCR. Mawgod *et al.* (2014) [7] reported 20 of the 52 bursal samples to be positive for IBDV by RT-PCR.

References

1. Adamu J, Owoade AA, Abdu PA, Kazeem HM, Fatihu MY. Characterization of field and vaccine infectious bursal disease viruses from Nigeria revealing possible virulence and regional markers in the VP2 minor hydrophilic peaks. Avian Pathol. 2013; 42(5):420-433.
2. Amin OG, Jackwood DJ. Identification and molecular analysis of infectious bursal disease in broiler farms in the Kurdistan Regional Government of Iraq. Trop. Anim. Hlth. Prod. 2014; 46:1297-1301.
3. Cosgrove AS. An apparently new disease of chickens avian nephrosis. Avian Dis. 1962; 9:385-389.
4. Dobos P, Hill BJ, Hallet R, Kells DTC, Becht H, Teninges D. Biophysical and biochemical characterization of five animal viruses with bisegmented double-stranded RNA genomes. J Virol. 1979; 32:593-605.
5. Jackwood DJ, Sommer SE. Detection and characterization of infectious bursal disease viruses in broilers at processing. Prev. Vet. Med. 2010; 97:45-50.
6. Jenberie S, Lynch SE, Kebede F, Christley RM, Gelaye E, Negussie H *et al*. Genetic characterisation of infectious bursal disease virus isolates in Ethiopia. Acta Trop. 2013; 130(100):39-43.
7. Mawgod SA, Arafa AS, Hussein HA. Molecular genotyping of the infectious bursal disease virus (IBDV) isolated from broiler flocks in Egypt. Int. J Vet. Sci. & Med. 2014; 2:46-52.
8. Mohanty GC, Pandey AP, Rajya BS. Infectious bursal disease in chicken. Curr. Sci. 1971; 40:181-184.
9. Mor SK, Narang G, Mittal D, Lather A, Rakha NK. Molecular characterization of infectious bursal disease virus from the recent outbreaks in poultry in Haryana, India. Haryana Vet. 2013; 52:12-16.
10. Morla S, Deka P, Kumar S. Isolation of novel variants of infectious bursal disease virus from different outbreaks in Northeast India. Microbiol. Pathogenesis. 2016; 93:131-136.
11. Murphy FA, Faugnet CM, Bishop DHL, Ghabrial SA, Jarvis AW, Martelli GP *et al*. Virus taxonomy, classification and nomenclature of viruses. Sixth Report of the International Committee on Taxonomy of Viruses. Springer, Wein, New York. Arch. Virol. Suppl. 1995; 10:240-244.
12. Nwagbo IO, Shittu I, Nwosuh CI, Ezeifeka GO, Odibo FJC, Michel LO *et al*. Molecular characterization of field infectious bursal disease virus isolates from Nigeria. Vet. World. 2016; 9(12):1420-1428.
13. Owolodun OA, Yakubu B, Jambol AR, Audu BJ, Dogonyaro BB, Luka PD. Further evidence for very virulent infectious bursal disease virus in vaccinated chickens in Nigeria. Trop. Anim. Hlth. Prod. 2015; 47:1437-1441.
14. Rojs OZ, Krapez U, Slavec B, Mankoc S, Juriric-Cizerl R, Barlic-Maganja D. Molecular characterization of infectious bursal disease viruses isolated in recent acute outbreaks in Slovenia. Acta. Vet. Hung. 2008; 56:255-64.
15. Vera F, Craig M, Olivera V, Rojas F, Konig G, Pereda A *et al*. Molecular characterization of infectious bursal disease virus (IBDV) isolated in Argentina indicates a regional lineage. Arch. Virol. 2015; 160:1909-1921.
16. Vukea PR, Willows-Munro S, Horner RF, Coetzer TH. Phylogenetic analysis of the polyprotein coding region of an infectious South African bursal disease virus (IBDV) strain. Infect. Genetics Evol. 2014; 21:279-286.
17. Xu M, Lin S, Zhao Y, Jin J, Tang N, Zhang G. Characteristics of very virulent infectious bursal disease viruses isolated from Chinese broiler chickens (2012-2013). Acta Tropica. 2015; 141:128-134.