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Short Communication

MOLECULAR DETECTION OF AVIAN INFECTIOUS BRONCHITIS VIRUS IN HARYANA STATE

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SUMMARY

The present study was aimed to screen Respiratory Disease Complex (RDC) suspected samples for avian infectious bronchitis virus (IBV) in the state of Haryana. A total of 50 tissue samples (trachea and kidney) suspected for IBV with variable clinical signs were used for PCR amplification using primers targeting 5' and 3' UTR by reverse transcriptase-polymerase chain reaction (RT-PCR). Out of 50 suspected tissue samples, 33 samples are found to be positive on 2% agarose gel electrophoresis. The study provides information of high prevalence of IBV in the state.

Keywords: Haryana, Infectious bronchitis virus, Primers, RT-PCR

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The poultry segment is the fastest-growing sector in India. Infectious bronchitis (IB) is an acute highly contagious viral disease of respiratory and urogenital tracts of poultry affecting all age groups. The disease causes considerable economical loss to poultry industry which is mainly attributed to production losses like poor weight gain and reduced egg production up to 50 percent (Cavanagh, 1997; Cavanagh, 2005). Avian infectious bronchitis virus (IBV) is a prototype member of avian species coronavirus belonging to genus Gammacorona virus in the family Coronaviridae. Present study was envisaged with the objective to screen the tissue samples of poultry for the presence of avian infectious bronchitis virus by reverse transcriptase-polymerase chain reaction (RT-PCR) in the state of Haryana.

A total of 50 tissue samples (trachea and kidney) of chicken from both backyard and organized farms of western Haryana were taken in the present study. Each sample is a pooled sample of 3-5 birds which reflects one flock. The tracheal samples were collected from birds suspected of suffering from Respiratory Disease Complex (RDC) and shows signs like respiratory sounds and tracheal congestion. The kidney samples were taken from birds affected with gout. The samples were stored at -20ºC without any media till further processing.

A small piece of sample weighing approximately 300 mg was cut with the help of a sterile scissor and transferred to a sterile mortar containing liquid nitrogen. The tissue samples were grinded using a sterile pestle and then liquefied in 3 ml of guanidine isothiocyanate (GIT) lysis buffer. The resultant suspension (10% w/v) was centrifuged at 5000 x g for 5 min to settle down the debris. The aliquots of resultant supernatant were stored at -20°C and later used for genomic RNA extraction. Commercially available Massachusetts H120 strain IP vaccine strain was used as positive control in this study.

The viral RNA was isolated by GIT lysis method (Chomczynski and Sacchi, 1987). The viral genomic RNAs of the suspected samples were used as template for one step RT-PCR amplification using one-step RT-PCR kit (QIAGEN) and in-house designed IBV specific primer pair specific targeting the 3'UTR (untranslated region) of viral genome. The sequence of forward and reverse primer is 5'-CTATCGCCAGGGAAATGTCTAATYTG-3' and 5'-TCCTAGTGCTGTACCCTCGRTC-3', respectively.

RT-PCR was carried out in a 12.5 µl reaction mixture containing 1.5 µl viral RNA, 0.6 µl forward and reverse primer (10 µM each), 0.5 µl one step RT-PCR enzyme mix (Qiagen), 0.5 µl dNTP mix (10 mM), 2.5 µl of 5x buffer. All the components were completely thawed; vortexed, spin down and kept on ice before use. The thermal conditions included a reverse transcription step at 50°C for 30min; and amplification steps with initial denaturation at 95°C for 15 min and 35 cycles of 95°C for 30 sec., 51°C for 30 sec., 72°C for 20 sec and final extension at 72°C for 5min.

The PCR product was resolved by AGE using 2% agarose gel containing ethidium bromide (0.5 µg/ml) in 1X Tris-acetate EDTA (TAE) buffer. The standard DNA ladder of 100 bp (CS Cleaver Scientific Ltd) was used with the amplified products. The 12.5 µl PCR product was mixed with 3 µl of 6X loading dye and was loaded into the well. The electrophoresis was carried out at 8V/cm of gel in 1X TAE running buffer in horizontal electrophoresis unit (BIO-RAD, USA) till the tracking dye reached upto two third of gel. After that, gel was visualized under UV transilluminator gel documentation. The expected product size was observed on the basis of standard DNA markers.

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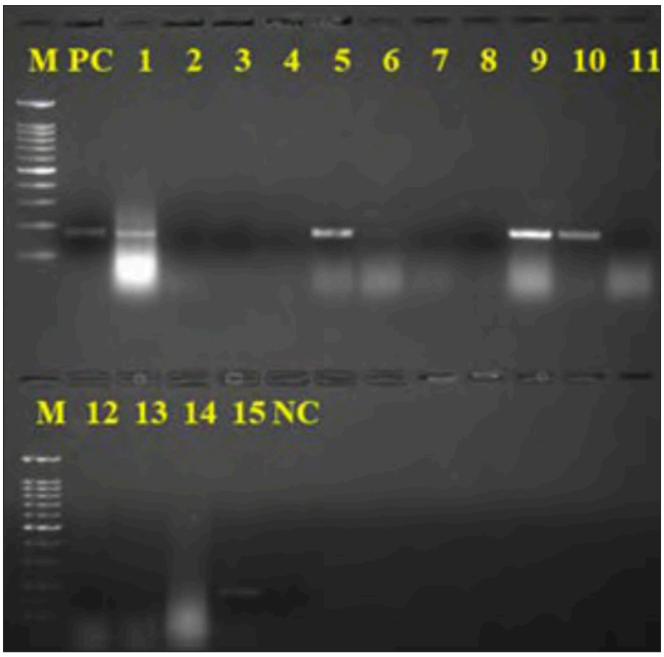

Fig. 1. Screening of IBV suspected samples by one-step RT-PCR using in-house designed primers targeting 3'-UTR region. M: 100bp (CS Cleaver Scientific Ltd); PC: Positive control; NC: no template control; UTR: untranslated region; Lanes: 1: 7491; 2: 6717; 3: 7356; 4: 7372; 5:7340; 6:7060; 7: 74/1; 8:7268; 9: 7381 K; 10: 7090; 11: 7321; 12: desi RDC 8/1/21; 13:7489; 14: 7055 K; 15:7381T

The amplified amplicons were expected to yield a product size of 171 bp.

The positive control yielded a PCR product of 171 bp while no amplification was observed in no-template control (NTC). Out of 50 suspected samples for IB, 33 samples (66%) (two kidney samples and 31 tracheal samples) were found positive (Fig. 1).

Previously, in Haryana, Beaudette and the Mass 41 (genotype I lineage 1) strains of the virus were identified (Jakhesara *et al.*, 2018). Verma (2018) developed indirect peptide ELISA for diagnosis of IB that showed appreciable sensitivity and specificity when compared to two commercial ELISA kit viz. IDEXX and BIOCHECK. He concluded that peptide based diagnostic assay is easy, sensitive, robust and cost-effective making it ideal diagnostics for IB. Rajalakshmi *et al.* (2019) reported that out of 87 tissue samples suspected for IB, 37 samples (42.5 percent) were found positive by RT-PCR targeting 5' UTR and concluded that nucleotide sequencing and phylogenetic analysis are required to identify the prevalent strains so as to develop suitable vaccination protocol for effective control and prevention of disease in the state. The first strain 4/91 type in India was isolated by Sumi *et al.* (2012), which may be a new vaccine strain variant based on its genomic sequence.

The RT-PCR results confirmed that IBV is circulating poultry flocks in Haryana state with very high occurrence rate. The epidemiological studies are indispensable for genetic characterization of viral strains and there is requisite of regular monitoring and surveillance programme in the region which could help in designing of a control strategy and strengthening of the vaccine licensing system against this disease.

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