Journal of Animal and Veterinary Advances 5 (9): 734-736, 2006

A First Report of Isolation of Infectious Bronchitis Virus from Broiler Chicken in Kerala State of India

[1]A. Arun, [2]M. MINI, [3]Leo Joseph and [4]V. Jayaprakasan
[1]Kerala Agricultural University, Thrissur, Kerala, India
[2,4]Department of Microbiology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, India
[3]University Poultry and Duck Farm, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, India

Abstract: This study of isolation of avian Infectious Bronchitis Virus (IBV) for the first time from Kerala state of India. An IBV isolate was recovered from the diseased broiler bird having hemorrhagic lesions in the respiratory tract. It was isolated from homogenized tissues samples by embryo inoculation. The virus was identified by characteristic dwarfing of embryo, Agar Gel Precipitation Test (AGPT) and reverse transcriptase polymerase chain reaction (RT-PCR).

Key words: Infectious ronchitis virus, egg inoculation, agar gel precipitation test reverse. transcriptase-polymerase chain reaction

INTRODUCTION

Infectious bronchitis virus is a highly contagious disease of poultry replicates in respiratory and urogenital tract of chicken. Young chicken typically develop respiratory disease and adult hens experience decrease in egg production and egg quality problem[1,2]. Infectious bronchitis virus is the prototype strain of the Coronaviridae, which has a single, positive-stranded RNA approximately 27.6 kb in length[3]. This paper describes the first report on the isolation of IB virus from Kerala state, India from an ailing broiler bird suffered from respiratory infections. The isolate was identified by Agar Gel Precipitation Test (AGPT) and confirmed by reverse transcriptase polymerase chain reaction (RT-PCR).

MATERIAL AND METHODS

History: Ailing broiler chickens having the history of continued morality were brought to the department of microbiology, college of veterinary and animal sciences, Thrissur, Kerala, India for disease investigation. The postmortem lesions were nephropathy, congestion of lung and hemorrhages in trachea.

Clinical material: Tissue samples like lung, trachea, liver and kidney were collected, pooled, homogenized and 20% suspension (w/v) was prepared in sterile Phosphate Buffered Saline (PBS). Antibiotic solution was added at the rate of 1000 I.U penicillin and 1 mg of streptomycin/mL to this suspension and the suspension was incubated at 37°C for one h. This was used as inoculum for embryo inoculation.

Virus isolation: Viable and healthy nine to ten days old Embryonated Chicken Eggs (ECE) were selected by candling. Eggs were inoculated with 200 μ L of processed tissue samples through allantoic cavity route and incubated at 37°C. the inoculated eggs were candled twice daily. The embryos that died within 24 h of inoculation were considered non-specific and were discarded. The embryos found viable even after three days of post inoculation were killed by keeping them at 4°C overnight. All the embryos were examined for the presence of characteristic lesions of IB and harvested Amnioallantoic Fluid (AAF) was subjected to AGPT.

Hyper immune serum: Adult male rabbit and six to eight weeks old chicks were used to raise hyperimmune serum against IBV. One ml of the AAF was mixed with one ml of Freund's complete adjuvant and injected intramuscularly at the rate of one ml per rabbit and 0.5 mL per chick. In addition, chicken was also inoculated intranasally. The second and third dose of antigen was given with Freund's incomplete adjuvant at weekly intervals then day after the third injection, the rabbits and chicks were bled and sera were checked for the presence of the antibodies by AGPT. The serum was adsorbed with uninfected AAF to remove nonspecific proteins.

Corresponding Author: A. Arun, Kerala Agricultural University, Thrissur, Kerala, India

Virus identification

Agar Gel Precipitation Test (AGPT): This test was carried out using 0.8% agarose gel was prepared on precoated glass slides. Three mm diameter wells were punched at an interval of 3 mm so as to get one central and five peripheral wells. The central well was filled with 20 μL of hyperimmune serum and 20 μL of positive and negative samples of antigen was loaded in two of the peripheral wells. The remaining wells were filled with 20 μL of each of test sample. The slides were incubated at room temperature in a humid chamber for 48 h and were examined in diffuse light for the presence of precipitin lines. The slides were then stained with amidoblack for clear visualization of precipitin lines.

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

RNA extraction: OnemL of Trizol™ reagent was mixed with 250 μL of allantoic fluid and was shaken well. Chloroform at the rate of 0.2 mL was added, mixed well and the mixture was incubated at room temperature for three minutes. The mixture was centrifuged at 12,000 x g for 15 min at 4°C. The aqueous phase was transferred to fresh tube and 0.5 mL of isopropyl alcohol was added. The mixture was incubated at room temperature and then centrifuged at 12,000 x g for 10 min at 4°C. The RNA pellet was washed with one mL of 75% ethanol at 7500 x g for five minutes at 4°C. The RNA pellet was air dried and finally dissolved in 3 μL of Diethyl Pyrocarbonate (DEPC) water.

Reverse transcription: The RT-PCR kit, which was supplied by M/s MBI Fermentas, USA was used. Primers for a 464 bp nucleotide sequence of S1 gene (Genotype PCR)[4] were obtained from Microsynth, Switzerland.

Primer 1 (5'- CAC TGG TAA TTT TTC AGA TGG-3')
Primer 2 (5'-CTC TAT AAA CAC CCT TAC A-3')

The extracted RNA was used to synthesize cDNA using random hexamors. The cDNA was synthesized using Revert Aid MmLV Reverse Transcriptase (200 U μL). The reaction mix for cDNA synthesis was made up as follows.

RNA -11 μL
Random hexamer (0.4 μg μL) - 1 μL.

This was heated at 70°C for five minutes, snap cooled in ice for one minute and the following were added. 5X reaction buffer-4 μL, dNTP mix (10mM each)- 2 μL, Rnase inhibitor (20U μL)-1 μL. This was kept at 25°C for five min. Then 200 units of MmLV reverse transcriptase (1 μL) were added. This reaction mix was kept in a thermal cycler with following cyclic conditions. 25°C for 10 min, 42°C for 60 min and 70°C for 10 min. The cDNA synthesized was stored at -20°C until further use.

Polymerase Chain Reaction (PCR): The composition of master mix for a reaction volume of 50 μL used for PCR was as follows. 10X PCR buffer-5 μL, dNTP mix(10mM)-2 μL, Sense primer (20 pmol)-1 μL, Antisnse primer (20 pmol)- 1 μL, Taq DNA polymerase (25U μL)-1 μL, cDNA template -5 μL, Dnase Rnase free distilled water-35 μL. This was kept in a thermal cycler (Eppendorf, Germany), with the following cycling conditions: Initial denaturation at 94°C for three min, 35 cycles of 94°C for 30 seconds; 55°C for one min; 72°C for 1.5 min, followed by a final extension of 72°C for seven min.

Electrophoresis: The amplified PCR product were detected by running a 1% agarose gel electrophoresis in 1X Tris-borate EDTA (TBE) buffer at 60V for 1.5 h. The pUC 19 DNA/Msp 1 digest (Genei, Bangalore, India) was used as _DNA molecular size marker. The gel was stained with ethidium bromide and was visualized under UV transilluminator and the results were documented in a gel documentation system.

RESULTS AND DISCUSSION

The IBV isolate produced characteristic dwarfing of embryo at sixth passage level. Similar embryopathies were also observed by Verma and Malik[5] and Sukumar and Prabhakar[4]. In the AGPT, the IBV isolate showed identical precipitation line with positive control. Negative control in the form of uninfected allantoic fluid did not show any precipitation line. These results were in agreement with the findings of Verma and Malik[5].

In this study the amplification of S1 gene of IBV isolate yielded an expected size of 464 bp product as obtained by Adzhar[6] and confirmed the identity of the IB virus. The S1 gene of IBV codes for S1 subunit of spike protein induces protective immunity and is responsible for serotype specificity[7]. Few changes in amino acids result in distinct serotype and farther the genotypic relationship between the viruses less the protection between them[8]. Serotyping of the isolate, analysis of the sequence of S1 gene and comparison with vaccine strains will probably reveal the type of virus.

Previously there were complaints about IB like infections from some of the poultry farms in Kerala. Isolation of IBV isolate from ailing broiler chicken has thrown some light on the status of IBV infection in Kerala. It warrants a systematic study, sero-monitoring and proper investigation of disease outbreaks, which will describe the status of the IB virus explicitly.

ACKNOWLEDGEMENT

We are grateful to Dr. Kumanan, Associate Professor, Department of Animal Biotechnology, Madras Veterinary College, Chennai, India for his generous help for providing facilities for identification of IBV by RT-PCR.

REFERENCES

1. Crinion, R.A.P. and M.S. Hofstad, 1972. Pathogenicity of four serotypes of avian infectious bronchitis virus for the oviduct of young chickens of various ages. Avian Dis., 16: 351-363.
2. Winterfield, R.W. and S.B. Hitchner, 1962. Etiology of an infectious nephritis-nephrosis syndrome of chickens. Am. J. Vet. Res., 23: 1273-1279.
3. Boursnell, M.E.G., T.D.K. Brown, I.J. Foulds, P.F. Green, F.M. Tomely and M.M. Binns, 1987. Completion of the sequence of the genome of the coronavirus avian infectious bronchitis virus. J. Gen. Virol., 68: 57-77.
4. Sukumar, S. and T.G. Prabhakar, 1993. An outbreak of infectious bronchitis among poultry in Tamil Nadu. Ind. J. Anim. Sci., 63: 820-822.
5. Verma, K.C. and B.S. Malik, 1971. Isolation of Infectious Bronchitis Virus (IBV) of poultry in India. Ind. Vet. J., 48: 887-892.
6. Adzhar, A., R.A. Gough, D. Haydon, K. Shaw, P. Britton and D. Cavanagh, 1997. Molecular analysis of the 793/B serotype of infections bronchitis in Great Britain. Avian Pathol., 26: 625-640.
7. Cavanagh, D., P.J. Davis, J.K.A. Cook, D.L.A. Kant and G. Koch, 1992. Location of aminoacid differences in the S1 spike glycoprotein subunit of closely related serotypes of infectious bronchitis virus. Avian Pathol., 21: 33-43.
8. Cavanagh, D., M.M. Ellis and J.K.A. Cook, 1997. Relationship between sequence variation in the S1 spike protein of infectious bronchitis virus and the extent of cross - protection *in vivo*. Avain Pathol., 26: 63-74.