Isolation, identification and molecular characterization of IBV variant from out break of visceral gout in commercial broilers

Amit Gaba, Hanish Dave, J.K.Pal and K.S.Prajapati*[1]

Hester Biosciences Limited
16/10 Devendra Society, Naranpura, Ahmedabad-380013, India
1. Professor and Head, Department of Pathology, Veterinary College, Anand-388001, India.
* Corresponding author e-mail: kanti_prajapati@yahoo.com, Cell phone: 919825079191

Absrtact

Kidney tissue samples of field outbreak of visceral gout in commercial broilers from Anand District of Gujarat, India were processed for virus isolation, identification and molecular characterization. Isolates produced typical embryo lesions of curling and stunting suggestive of infectious bronchitis after three passages. RT-PCR and sequence analysis was carried out from infected allantoic fluid. The nucleotide sequence was found to have 99% nucleotide sequence identity with avian infectious bronchitis virus strain 4/91(pathogenic). Phylogenetic analysis using Clustal W program, the virus was found to group with 793/B and 4/91 strain of viruses.

Keywords: Molecular, Virus, Commercial Broiler, Visceral Gout, Outbreak, Mortality.

Introduction

Infectious Bronchitis (IB) is currently one of the major diseases in poultry flocks all over the world caused by infectious bronchitis virus (IBV). IBV is the prototype species of the Coronaviridae family. More than 20 serotypes of IBV have been identified worldwide (Xu et al., 2007). The S glycoprotein comprises of two glycopolypeptides S1 and S2. Neutralizing and serotype specific antibodies are mainly directed against the S1 glycoprotein (Cavanagh et al., 1992). IBV is highly mutating virus and the small differences in S1 contribute poor cross protection and vaccine failure. There for it is necessary to identify various serotypes prevailing in major poultry growing areas of the country. Until recently Indian subcontinent was free of variant forms of IBV (Bayry, et al., 2005).The most prevalent form of IBV was only respiratory form related to Massachusetts strain. Outbreaks of visceral gout and nephrosis in commercial young broiler chicken are widely prevalent through out the country and causes severe economic losses. The condition is caused by wide number of etiology involving nutritional, metabolic, managemental, infectious and toxicity. Infectious causes are gaining importance particularly the emergence of nephropathic variants of IBV. Hence an attempt was made to identify the involvement of IBV variant in cases of visceral gout in Anand District which is a major poultry belt of Gujarat.

Material and Methods

An out break with rising mortality at 7 days age in Cobb broiler flock near Anand, Gujarat was attended to investigate into the reason of mortality. Clinical signs were recorded and post mortem examinations were carried out.

Isolation: Kidney tissue samples were collected during post mortem showing lesions of visceral gout. Clarified suspensions (10% w/v) of homogenate were prepared and inoculated into allantoic cavity of 11 day old embryonated SPF eggs. After 3 passages the embryos were examined for any lesions and the allantoic fluid was harvested and used for RNA extraction.

RT-PCR and sequence analysis: Total RNA was extracted from 200µl infected allantoic fluid with RNeasy kit (QIAGEN) using manufacturer's protocol. For cDNA synthesis, Enhanced Avian First Strand Synthesis Kit (SIGMA) was used as described by the manufacturer. Briefly 8µl of RNA was mixed with 1µl of random nanomer and 1µl of dNTPs. The mixture was incubated at 950C for 5min and then immediately put on ice. To this mixture 2µl 10xRT buffer, 1µl RNAse inhibitor and 1µl eMVRT was added. Final volume of reaction mixture was made up to 20µl by addition of nuclease free water. The cDNA synthesis was then carried out in thermocycler following the cycle of 25°C for 15min, 45oC for 50min and 900C for 5min.

For PCR 10µl of cDNA was amplified using

15pmol of each primer (Forward, 5'- CAC TGG TAA TTT TTC AGA TGG nucleotide positions 728-749; Reverse, 5'- CTC TAT AAA CAC CCT TAC A -3' nucleotide positions 1168-1193). The S1 region of S protein gene was chosen for the selection of primers. The amplification was carried out in thermocycler by initial denaturation at 940C for 5min and 35 cycles of 94°C for 1min, 48°C for 1min 30 s and 72°C for 2min, followed by final elongation at 720C for 7 min. The amplified product was electrophoretically separated in a submerged two percent agarose gel and visualized under ultraviolet light. The amplified product was sent to Lab India Instruments Private Limited, Gurgaon for sequencing. For further comparison nucleotide sequences of IBV isolates with different pathogenecity were retrieved from the Genbank database (Table: 1). All the nucleotide sequences were aligned for phylogenic analysis using the Clustal W program.

Table: 1. IBV viruses used for comparison

Strain	Serotype	Origin	Accession No.
4-91-Attenuated	793/B	UK	AF093793
4-91-Pathogenic	793/B	UK	AF093794
793B	793/B	UK	Z83979
Ark99	Arkansas	USA	M99482
Beaudette	Mass	USA	Af151954
Connecticut	Connecticut	USA	L18990
Florida	Florida	USA	AF027512
Georgia	Georgia	USA	U16157
Gray	Gray	USA	L14069
H120	Mass	Holland	M21970
Holte	Holte	USA	L18988
Ma5	Mass	Europe	AY561713
SDW	Unknown	China	DQ070840
T	Australia-T	Australia	AY775779

Results and Discussion

Clinical signs and post mortem lesion: Affected chicks showed mild respiratory rales and difficulty in breathing. The carcasses were dehydrated and showed white urate deposits on various serosal and mucosal surfaces particularly on pericardium, liver capsule and kidneys (Fig.1). Kidney lobs were swollen with prominent and dilated tubules.

Isolation: After 3rd passage the embryo showed lesions of stunting, curling, legs compressed upon head and urate deposition in the kidney and ureter (Fig.2). These lesions are specific for infectious bronchitis.

RT-PCR and sequence analysis: Sample produced an amplicon of approximately 464 bp as expected with this primer pair by RT-PCR (Fig.3). Using the blast programme of NCBI the obtained nucleotide sequence was found to have 99% nucleotide sequence identity with avian infectious bronchitis virus strain 4/91 (pathogenic). On phylogenetic analysis using Clustal W program, the virus was found to group with 793/B and 4/91strain of virus (Fig.4).

Visceral gout in baby broiler chicks is a major disease in commercial broiler farming through out India causing major economic losses to the farmer. Disease usually appears at the end of first week or in the second week and runs for 4-5 days causing mortality up to 30%. Earlier the managemental and nutritional causes were given more emphasis without much benefit. Of late the infectious causes, particularly the IBV variant as a cause of these out breaks were under scrutiny. The present work proves the involvement of nephropathic IBV variant in the out breaks of visceral gout. The out breaks were also seen in flocks vaccinated with regular Mass type vaccine suggesting poor cross protection. There fore, it is imperative to know the prevalent status of IBVs and the major circulating strains in a region or country, in order to select the appropriate vaccines to prevent the disease.

In the present study the matching of nucleotide identities revealed close identity with 793/B and 4/91 strains of IBV. There is very poor cross protection by Mass strains of IBV with these variants and hence the increased frequencies of vaccine failures. Elankumaran et al. (1999) also showed the serological evidence for a 793/B related avian infectious bronchitis virus in India. In India very limited work has been done on identifying the variant IBVs from the field cases both in layers and broilers. Bayry et al. (2005) reported the

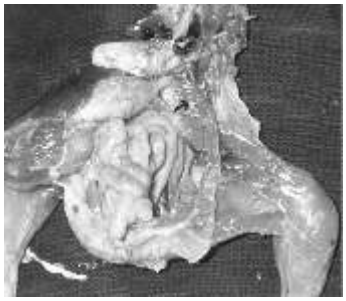

Figure 1. Visceral gout in broiler chick. Note the wide spread deposits of uric acid on serosal surfaces and kidneys.

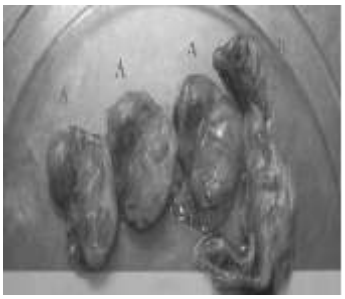

Figure 2. Dwarfing and curling of embryos (A) after 3rd passage. (B) Normal Embryo

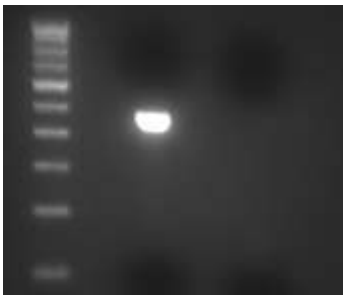

Figure 3. Agarose gel electrophoresis pattern of RT-PCR product (464bp) run along with 100bp marker

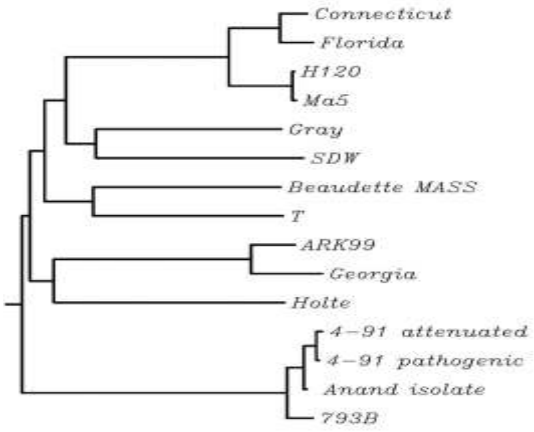

Figure 4. Phylogenetic tree based on nucleotide sequences of the S1 gene of Anand isolate and sequences from Gen Bank.

emergence of a nephropathogenic avian infectious bronchitis virus with novel genotype in India. The Indian isolate PDRC/Pune/Ind/1/00 exhibited < 40% similarity in S1 protein sequence to strains D1466, Mex/1765/99 and DE/072/92 but shared 68% relatedness with strain 6/82. Eight out of 9 Polish isolates had the characteristic band for 793/B and five simultaneously to D275 type (Domanska-Blicharz et al., 2007). Capua et al. (1999) also reported four types of IBV variants viz. 793/B, 624/I, B1648 and Massachusetts in Italy.

The present study further prove the prevalence of IBV variants in Indian poultry and it demands the wide spread survey and policy making decisions on use of variant strains in vaccine production to get maximum advantage of cross protection against prevailing variants in country. This is necessary to minimize the economic losses to the poultry farmers.

References

1. Bayry, J., et.al. (2005): Emergence of a nephropathogenic avian infectious bronchitis virus with a novel genotype in *India. J Clin Microbiol.*, 43: 916-918.
2. Capua, I., Z. Minta, E. Karpinska, K. Mawditt, P. Britton, D. Cavanagh and R.E. Gough.(1999): Co-circulation of four types of infectious bronchitis virus (793/B, 624/I, B1648 and Massachusetts). *Avian Pathology*, 28: 587-592.
3. Cavanagh, D., P.J. Davis, J.K.A. Cook, D. Li, A. Kant and G. Koch (1992): Location of the amino acid differences in the spike glycoprotein subunit of closely related serotypes of infectious bronchitis virus. *Avian Pathology*, 21: 33-43.
4. Domanska-Blicharz, K., K. Smietanka and Z. Minta. (2007): Molecxular studies on infectious bronchitis virus isolated in Poland. *Bull Vet Inst Pulawy*, 51: 449-452.
5. Elankumaran, S., C. et.al.(1999): Serological evidence for a 793/B related avian infectious bronchitis virus in India. *Vet.Rec.*, 144: 299-300.
6. Xu C., J. Zhao, X. Hu and G. Zhang (2007): Isolation and identification of four infectious bronchitis virus strains in China and analyses of their S1 glycoprotein gene. *Veterinary Microbiology*, 122: 61-71.

* * * * * * * *