

Frequency Distribution of Avian group-D Rotavirus in Southern and Northern Parts of India

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Enteric infections foist massive losses in commercial and backyard poultry sector. Rotavirus, the leading cause of enteric infections, is of great concern among researchers and industry, as the epidemiological reports confirm that new rotavirus groups are evolving in poultry and might inflict the growing poultry sector in the coming years. The diversity and epidemiology in avian group A rotaviruses throughout the world have been studied by several researchers, but epidemiological studies are barely accessible for other groups of rotaviruses like D, F and G. A timely identification of rotavirus is, therefore, critical for control of disease and to minimize losses to the poultry sector. Therefore, the frequency distribution of avian RVD infection in northern and southern states of India was ascertained by using RNA-PAGE and a RT-PCR assay. RVD was detected in 6.04% (13/215) of the intestinal contents examined during January 2012 to January 2013. The results confirm the existence of RVD in chicken of temperate northern India. Sequence based inquiry confirmed higher homology with existing type D avian rotavirus isolates from rest of the world. Thus, an understanding of the viruses that cause gastrointestinal tract disease in poultry, along with an understanding of their epidemiology is necessary for the development of be-fitting control measures.

Key words: Avian group-D rotavirus, RT-PCR, Epidemiology, India.

India ranks 2nd in the world's largest poultry market with an annual 6% growth in egg and 10% growth in meat production and contributes to nearly 1% of national GDP. However, diseases like rotavirus (RV), causing runting and stunting syndrome (RSS) and linked with severe gastroenteritis, are known to levy colossal economic loss to the growing poultry sector world over¹. Rotavirus belongs to the family *Reoviridae* and is a non-enveloped, 11 segmented, double stranded RNA genome virus². Based on the constitution of major inner capsid protein (VP6) virus has been classified in to seven groups or

types, designated as rotavirus type A (RVA), type B (RVB), to RVG³. Amid these, RVA strains predominate and affect both mammals and birds, while RVD, RVF and RVG are reported only in birds. Accurate epidemiological data on occurrence of RVD in developing countries, in particular from India, is almost derisory. Existence of avian rotaviruses has been recognized from India only on few occasions^{4,5,6,7,8}. For diagnosis, ribonucleic acid-polyacrylamide gel electrophoresis (RNA-PAGE) test is the one which is more commonly employed for avian rotavirus infection confirmation^{1,9}. Recently, a reverse transcription-polymerase chain reaction (RT-PCR) based diagnostic assay was developed and in-house validated in our laboratory for the specific detection of RVD¹⁰ (Kattor *et al.*, 2014). Using this

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molecular assay, an epidemiological survey was planned to detect the distribution of RVD in poultry from southern and northern parts of India.

MATERIALS AND METHODS

Sample collection and processing

A total of 215 faecal samples were collected during January 2012 to January 2013 from diarrhoeic and post mortem enteritis cases of chicken (205) and quails (10) between 1 to 15 weeks age from Northern [Uttarakhand (142), Uttar Pradesh (23) and Haryana (10)] and Southern parts [Tamil Nadu (15) and Kerala (25)] of India. Suspensions (10% w/v) of faecal samples in phosphate buffered saline (PBS) were prepared as per our previous reports¹¹.

Molecular detection of group D rotavirus

The procedures for extraction of total RNA from 10% faecal suspension, reverse-transcription for cDNA synthesis from viral RNA and PCR, as optimized and reported earlier from our laboratory were implemented¹⁰. RNA-PAGE was performed for all the samples according to the methods described in our previous studies^{6,11}. For RT-PCR, self-designed primers¹⁰ were used with sense primer (5'-GCTATACATTTTCGCTGCATTTG-3') and anti-sense primer (5'-TGGCCAATAGTGTGTGGCAGCT-3'). All the primers were custom synthesized (Integrated DNA Technologies Inc., India). The PCR amplified products were resolved in 1.5% agarose gel and visualized in Transilluminator-UV[®]300 (UVP Inc., Upland, USA).

Sequence and phylogenetic analysis

Selected RVD positive PCR products were sequenced and evolutionary history was inferred using the Neighbor-Joining method and evolutionary distances were computed using the Maximum Composite Likelihood method. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA6¹².

RESULTS AND DISCUSSION

In this study, RNA-PAGE analysis of all the 215 faecal samples collected from diarrhoeic and post mortem enteritis cases of chicken and quails from Northern and Southern parts of India

revealed specific genomic migration banding pattern representative of RVD (5:2:2:2) in two of the samples (0.93%) collected from Uttarakhand (temperate region). Based on PAGE results, lower prevalence of RVD in the samples tested (0.93%) was observed as compared to previous reports from Madhya Pradesh, 9.09% (Savita *et al.*, 2008) and Maharashtra, 7.84%¹³. The higher occurrence of RVD in central India (9.09%) and Maharashtra (7.84%) state of India reported earlier might be due to the smaller sample size in comparison to the present study, where 215 diarrhoeic cases were screened. None of the sample from southern and other northern parts exhibited positive banding representative of rotavirus.

The RT-PCR assay, being much more sensitive, detected 6.04% samples (13/215) positive for RVD. The specific PCR amplicons (185 bp) in all the positive samples were visualized in 1.5% ethidium bromide stained agarose gel (Fig. 1). A number of isolates which were detected positive in RT-PCR did not show any presence of virus RNA in PAGE, which could be due to low viral load and thus its detection failed due to lower sensitivity limit of RNA-PAGE. Comparative evaluation results of RT-PCR and RNA-PAGE assay exhibited unexpected correlation over the number of samples tested, as in RT-PCR based screening, 6.04% (13/215) samples were found positive in comparison to 0.93% (2/215) by RNA-PAGE for RVD, i.e. RT-PCR when showed 100% sensitivity, RNA-PAGE displayed only 15.4% sensitivity. Bezerra *et al.*¹⁴ also indicated the sensitivity of

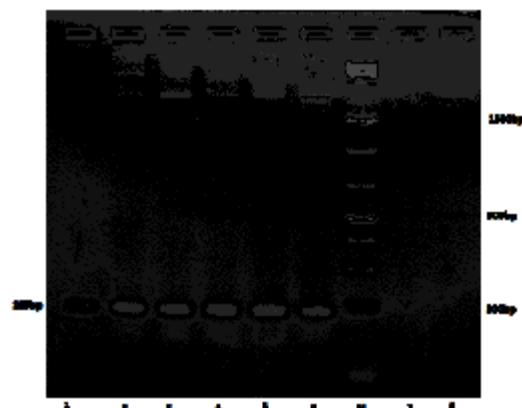


Fig. 1. Agarose gel electrophoresis of RT-PCR products of avian group D rotavirus (RVD). Lanes, M: 1 Kb Plus DNA Ladder (Thermo scientific); 1-6: Field samples from Uttarakhand; 7-8: NTC (Non template control)

RNA-PAGE as 56.3% in comparison to 100% sensitivity of RT-PCR. The higher sensitivity of RT-PCR over RNA-PAGE and 28.2% detection of avian RVD were also shown in a study by Otto *et al.*¹⁵.

The results also disclosed the regional localization of avian RVD in Uttarakhand state which need further studies to understand the evolving epidemiology of rotaviruses in avian species in temperate zones of the country. None of the sample from Uttar Pradesh, Tamil Nadu and Kerala yielded positive result, but before

concluding absence of avian RVD in these regions, extensive epidemiological studies on more number of enteritis/diarrhoeic samples needs to be assessed. Selected RVD positive clinical samples were further confirmed by sequencing and NCBI blast analysis with other strains of RVD showed high sequence correspondence (>95%) with existing RVD strains from Bangladesh, Germany and Brazil (Fig. 2). The evolutionary history was inferred using the Neighbor-Joining method and evolutionary distances were computed using the Maximum Composite Likelihood method. The

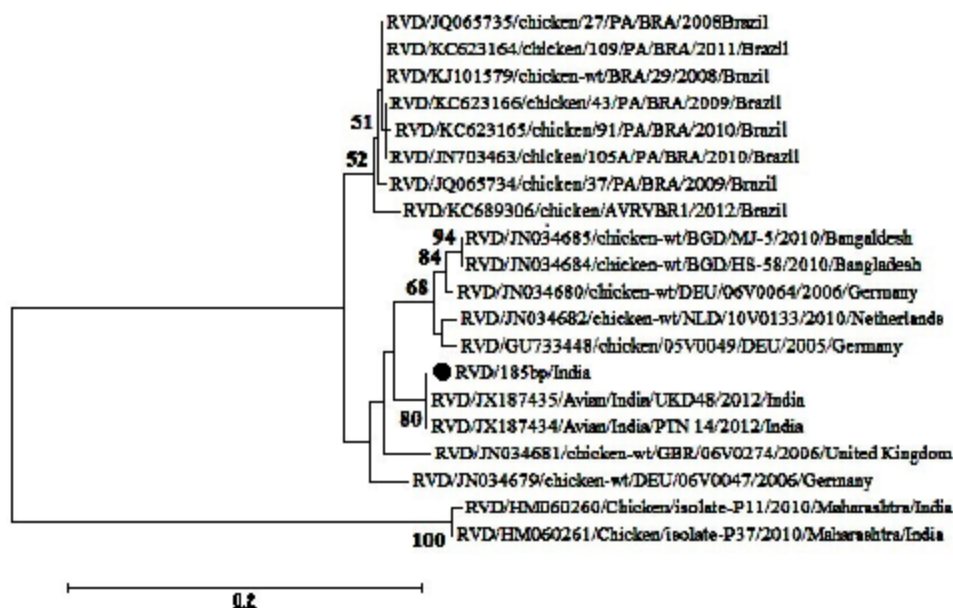


Fig. 2. Phylogram based on nucleotide sequences of avian group D rotavirus (RVD) partial inner capsid protein gene (VP6) indicated as (●). Sequences of different RVD strains were retrieved from the NCBI GenBank database (<http://www.ncbi.nlm.nih.gov/>). Construction of the tree is based on 185 nucleotide segment of the VP6 gene region by neighbor-joining (NJ) method implemented in MEGA6 (<http://megasoftware.net/>). Numbers on branches indicate percentages of bootstrap support from 2,500 replicates. The scale bar indicates the estimated 0.2 nucleotide substitutions per site

sequence and phylogenetic analysis showed that the two RVD isolates from Maharashtra, India¹³ clustered separately from rest of the world's RVD isolates, stressing the need to study the origin of these isolates and confirmation of their RVD genome nature. Overall, the results confirmed the presence of RVD infection in chicken in northern India.

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