

Detection and characterization of group A and D avian rotaviruses in India

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The present study was undertaken to determine the association of rotaviruses with diarrhea in poultry. Avian rotaviruses were detected on the basis of migration pattern of discrete 11 segments of dsRNA in the RNA-polyacrylamide gel electrophoresis (RNA-PAGE). The prevalence of rotavirus infection in diarrhoeic (n=46) and environmental (n=31) samples was 17.39% (8/46) and 3.22% (1/31), respectively. Electrophoretic migration pattern characteristic of group A (5:1:3:2) and group D (5:2:2:2) avian rotaviruses was observed. Overall the prevalence of group D avian rotavirus was higher (77.8%; 7/9) than group A rotavirus (22.22%; 2/9) in central India. Occurrence of different electrophoretic migration patterns suggested existence and circulation of genetically diverse strains of avian rotaviruses. This study establishes the presence of group D avian rotavirus in India and need for expansion of such work to cover more geographical areas.

Keywords: Avian rotavirus, diarrhea, diversity, genomic pattern, group A and D, RNA-PAGE

Rotaviruses are the major cause of acute viral gastroenteritis in infants and young animals of many mammalian and avian species¹. Rotaviruses belong to the family *Reoviridae*, their genome consists of 11 segments of double-stranded RNA (dsRNA). Electrophoretic patterns of rotaviruses are distinct and, hence, used to classify rotaviruses into seven groups² and also to differentiate between avian and mammalian rotaviruses. The genomic RNA segments cluster into four regions—I to IV. Mammalian group A rotaviruses have a 4:2:3:2 pattern, while avian group A rotaviruses have a 5:1:3:2 pattern and group D have 5:2:2:2 pattern⁴. Avian rotaviruses have been

isolated from diarrhoeic chicken, turkey poult and other avian species in various parts of the world⁵⁻⁷. However, very little is known about the circulation of avian rotaviruses in India. Recently, in India, mammalian like Group A rotavirus⁸ and avian group A⁹ have been reported in chicken, but to date there is no report on the occurrence of avian group D rotavirus. Present investigation reports the genomic characterization of avian rotaviruses isolated from 46 diarrhoeic and 31 samples collected from poultry shed, litter, and water troughs (environmental samples).

A total of 46 diarrhoeic fecal samples and 31 environmental samples (moist swabs from poultry cages, water, feed, litter, etc) were collected from Poultry Farm, College of Veterinary Science & A H, JN Krishi Vishwa Vidyalaya, Adhartal, Jabalpur, as well as from CPDL-Phoenix and other poultry farms located in and around Jabalpur city during September 2005 to February 2006. Samples were collected from both broilers and layers. Samples were kept on ice and transported to the laboratory for immediate processing for rotavirus detection.

The diarrheic fecal and environmental samples (feed and litter) were suspended in phosphate buffer saline (PBS), pH 7.2 to make 10% suspension. From this suspension, 1 mL was used for viral nucleic acid extraction and the rest was archived. Water samples from poultry sheds were centrifuged at 5,000 rpm for 10 min and the supernatant was used for viral nucleic acid extraction. Swabs collected from poultry cages were vortexed in 2 mL of PBS and 1 mL was used for viral nucleic acid extraction.

The viral nucleic acid was extracted using phenol:chloroform method as described by Herring *et al*¹⁰. RNA-PAGE was carried out for initial screening of all the samples for detection of avian rotaviruses obtained from different sources on the basis of presence of 11 RNA segments and their typical migration pattern in PAGE after silver staining¹¹. Stained gels were documented using gel doc system (Alpha Innotech, USA).

Of total 77 diarrheic (n=46) and environmental (n=31) samples, 9 (11.69%) were found positive for avian rotavirus by RNA-PAGE. Electrophoretic

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characteristic of group A (5:1:3:2) and group D avian rotaviruses (5:2:2:2) was observed (Fig. 1). Of the total 9 rotavirus positive samples, 8 (17.39%) were from diarrheic cases ($n=46$), while one (3.22%) was from litter ($n=31$). Of the 9 positive samples, 2 samples including 1 from litter belonged to group A avian rotavirus with a typical migration pattern of 5:1:3:2 in RNA-PAGE (Fig. 2), while remaining 7 diarrheic fecal samples exhibited a migration pattern of 5:2:2:2 in RNA-PAGE indicative of group D avian rotavirus. Thus majority of the samples revealed presence of avian group D rotavirus (77.8%; 7/9).

Comparison of migration pattern of RNA segments of groups A avian rotaviruses showed that the group A avian rotaviruses were of short electropherotypes as genome segment 10 and 11 migrated closely, while they showed differences in other segments. Segments 4 and 5 migrated closely in the environmental rotavirus positive sample, while were distant in another group A rotavirus sample. Genome segments

7, 8 and 9 migrated closely in one isolate, whereas segment 9 migrated separately from 7 and 8 in another sample. Thus, genomic analyses showed presence of two different electropherotypes within group A avian rotaviruses.

A similar observation was seen within group D avian rotaviruses. The 7 isolates of group D avian rotavirus were grouped in 5 electropherotypes depending on their migration patterns. Though group A avian rotaviruses were of short electropherotypes, group D avian rotaviruses showed the presence of both long electropherotypes (4 samples with distant migration of segments 10 and 11) as well as short electropherotypes (3 samples). The differences observed in migration pattern within Group D avian rotaviruses were mainly in segments 3, 4, 5, 7, 8 and 9 (Fig. 2). Detection of various electrophoretic migration patterns of avian rotaviruses in central India

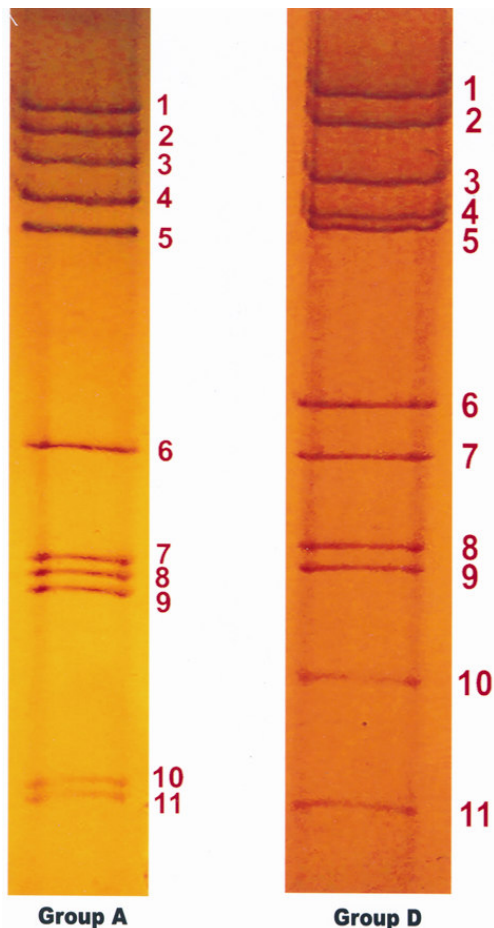


Fig. 1—Genomic migration patterns of group A and group D avian rotavirus in RNA-PAGE.

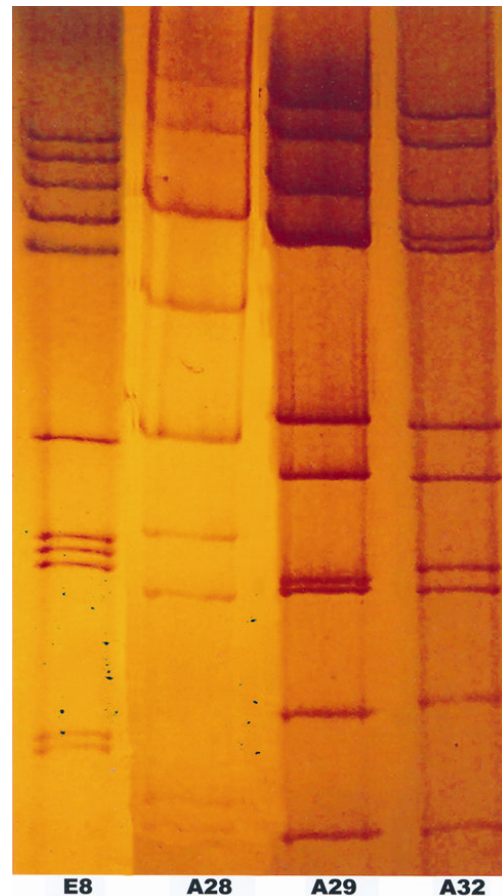


Fig. 2—Genome migration patterns of different field strains of avian rotaviruses. Lane E8: Group A avian rotavirus with migration pattern of 5:1:3:2, lanes A28, A29 and A32: Group D avian rotavirus with migration pattern of 5:2:2:2. Lanes E8 and A28: Short electropherotype, lanes A29 and A32: Long electropherotype

suggested the prevalence of different genomic strains of avian rotaviruses in India. Avian rotaviruses with a migration pattern of 5:2:2:2 had been isolated from chicken, turkey and pheasant¹²⁻¹⁴ in other parts of world, which were earlier named as rotavirus-like virus or atypical rotavirus. However, in India, this is probably the only report for detecting rotaviruses in chicken exhibiting 5:2:2:2 migration patterns. Earlier, Wani *et al*⁸ reported mammalian-like group A rotavirus from diarrheic chickens in India with a migration pattern of 4:2:3:2 and Minakshi *et al*⁹ isolated avian group A rotavirus showing 5:1:3:2 migration pattern.

The detection of rotaviruses has been reported from water, sewage and inanimate objects¹⁵⁻¹⁷ and, during present study, it was detected in poultry sheds. Overall, present study clearly shows the higher prevalence of avian group D rotaviruses than group A rotaviruses in central India and persistence of genetically diverse strains within each group A and D avian rotaviruses. Further studies in this direction are needed to work out the actual prevalence of avian rotavirus in poultry.

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