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MOLECULAR EPIDEMIOLOGIC STUDY ON AVIAN ROTAVIRUS PREVAILING IN BANGLADESH

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ABSTRACT

The present study on molecular epidemiologic study on avian rotavirus prevailing in Bangladesh was attempted to know the present status of avian rotavirus in Bangladesh. A total of 210 faecal samples of chickens were collected from August 2006 to March 2007 and examined by polyacrylamide gel electrophoresis and silver staining (PAGE-ss) technique for the presence of rotavirus dsRNA. The prevalence of avian rotavirus infection was 13.81% in chickens. The prevalence of avian rotavirus infection in Chittagong, Mymensingh, Gazipur and Barisal were 2.86%, 21.25%, 5% and 22.5%, respectively. The highest prevalence was recorded in Barisal (22.5%) and the lowest in Chittagong (2.86%). The highest prevalence (40%) was observed in 12 day-old birds and the lowest (5.71%) in 22 days old birds. The highest percentage (18%) of avian rotavirus was detected in the summer season. The migration patterns of all detected positive strains were similar in gel electrophoresis and their migration speed was same as previously designated avian rotavirus group D.

Key words: Epidemiology, PAGE, rotavirus, chickens

INTRODUCTION

Rotavirus gastroenteritis is a worldwide disease affecting primarily infants, young children and a wide variety of young mammalian and avian species (Estes *et al.,* 1983 and McNulty *et al.,* 1984). Rotavirus infection in avian species was first reported by Bergeland *et al.* (1977) who found particles morphologically indistinguishable from rotavirus in intestinal contents of poults with watery droppings and increased mortality. Since then it has become apparent that rotaviruses infect many species of domestic birds. As in mammals, rotavirus infection in avian species is frequently associated with outbreaks of diarrhoea. The economic significance of rotaviral enteritis to the poultry industry has not yet been defined, but by analogy with the situation in mammals it is likely to be significant. The rotaviruses belonging to the family Reoviridae contain a genome of 11 segments of double stranded RNA (dsRNA), which can be separated into distinct bands by electrophoresis. The migration pattern of the 11 genome segments following electrophoresis of the viral RNA in polyacrylamide gel is called the RNA electropherotype (Estes *et al.,* 1984). Rotavirus in birds belongs to groups A, D, F and G. (Saif *et al.,* 1985).

Detail studies on the epidemiology of rotavirus associated diarrhoea in poultry has been performed in advanced countries. Recently avian rotavirus like virus was detected in Bangladesh. The number of detection was very low approximately 0.86% in 232 broiler chicks (Ahmed and Ahmed, 2006). This result raised a question whether avian rotaviruses do exist at all in Bangladesh. Since sample size was small and detection was too low, we felt necessity to substantiate this finding whether avian rotavirus is responsible for enteritis in chicken in Bangladesh. Moreover, Electrophoretic identification of rotavirus strains in different regions of Bangladesh has not yet been performed. In order to accomplish this we undertook the present study covering wide range of areas and collected faecal samples from different age group of chickens. The present paper describes the prevalence of avian rotavirus infection and rotavirus RNA electropherotypes in chicken in Bangladesh.

MATERIALS AND METHODS

Collection and preparation of samples

Faecal samples were collected from 210 diarrhoeic and nondiarrhoeic poultry birds. The samples were collected from a) Chittagong: Pahartoli Zonal Poultry Farm (PZPF), Nazma Poultry Farm; b) Barisal: Ahmed Ali Poultry Farm, Kader Poultry Farm; c) Mymensingh: Tania Poultry Farm, Soma Poultry Farm and d) Gazipur: Shaikat Poultry Farm during the period from August 2006 to March 2007. The faecal samples were collected from the cloaca of the birds and from the litter (bedding) immediately after voiding by the poultry birds (layer and broiler). For each bird, 5-10 g sample was collected. Precautions were taken to avoid contamination of one sample with other. The date of collection, age, clinical signs and environmental history were recorded for each case. After collection, the samples were transported to the laboratory of the Department of Medicine, BAU, Mymensingh with ice pack and stored at -20^0C until used for electrophoresis. After thawing the stored samples, 1 g of each sample was taken in a test tube and 9 ml of PBS of P^H 7.4 was added. Faeces and PBS were thoroughly suspended by using a spinmix.

RNA extraction and electrophoresis of rotaviral RNA

This was done according to the method of Laemmli (1970) with some modification. After thawing, the faecal suspension was thoroughly mixed and vortexed and centrifuged at 15000 rpm for 15 minutes and then at 10000 rpm for 10 minutes. 300 µl of supernatant was collected separately in eppendorf tube and 60 µl of disrupting solution (6% Sodium Dodecyl Sulphate, 0.6% 2-Mercaptoethanol and 0.036 M EDTA) was added in each eppendorf tube and vortexed for 30 seconds. Then the eppendorf tubes were incubated at 37^0C for 30 minutes. After incubation, 500 µl of saturated phenol was added to each eppendorf tube and vortexed for 30 seconds. The eppendorf tubes were then centrifuged at 10000 rpm for 1 minute in eppendorf microcentrifuge. The upper clear aqueous phase was collected separately in another eppendorf tube. A volume of 1:10 of 5 M sodium chloride and 3 volume of chilled ethanol were added to each eppendorf tube. RNA was precipitated at -20^0C for overnight (Steele and Alexander, 1987 and Dimitrov *et al.*, 1984). After thawing, RNA was pelleted by centrifugation the eppendorf tube at 10000 rpm for 3 minutes. The liquid from the eppendorf tube was discarded carefully. The RNA pellet was dried at 37^0C. The dried RNA was suspended in 10 µl of sample buffer (0.12 M Tris-hydrochloride, 15% glycerol and 0.001% bromophenol blue).

Polyacrylamide gel electrophoresis

Electrophoresis of the viral RNA was carried out in 10% polyacrylamide slab gels. After preparing the gels in slabs, the two gel slabs were set on the electrophoresis chamber. Electrode buffer P^H 8.3 (0.25 M Tris-hydrochloride, 0.192 M glycine, 0.001 M EDTA) was pored on the chamber and in between the two slabs. Thereafter, 10 µl of diluted RNA was loaded separately on the wells of the gels and the corresponding well was recorded. The electrophoresis was performed at 40 mAmp (for double gels) for 16 hours.

Silver staining, developing and viewing

After electrophoresis, the gels were stained with staining solution (0.011 M silver nitrate) for 2 hours with continuous shaking. Then, the gels were washed three times in distilled water for 5 minutes. Finally the reduction step was performed by adding developing solution (0.75 M sodium hydroxide, 0.1 M formaldehyde) in gel trays and with continuous shaking. The RNA bands appeared at this stage and reduction was continued until the bands were clearly visible. The reduction reaction was stopped by replacing developing solution with 5% acetic acid. The discrete bands of 11 segmented double stranded RNA appeared in positive cases.

Electropherotyping

The double-stranded 11 RNA segments of avian rotavirus were divided into 4 groups for better classification. RNA segment bands 1-5 are denoted as group I, bands 6 and 7 as group II, bands 8 and 9 as group III and bands 10 and 11 as group IV. Within each group the individual segments from different strains of rotavirus may show variations in the distance migrated relative to each other. In some cases migration of segments is very closely co-migrated which may appear to be single band rather than several segments but this pattern is characteristic for each strain of avian rotavirus.

RESULTS AND DISCUSSION

A total of 210 faecal samples of day-old layer chicks and broiler birds (4-22 days) were screened by PAGE, of which 29 broiler birds (13.81%) had rotavirus infection (Table 1), but lower rate of rotavirus infection have been documented by Ahmed and Ahmed (2006) who found only 0.86% rotavirus like virus infection in diarrhoeic broiler chicks in Bangladesh (Mymensingh and Gazipur). This result indicates that there is an increasing trend of rotavirus infection in chickens in Bangladesh.

Table 1. Prevalence of avian rotavirus infection in chickens on the basis of different regions of Bangladesh

Name of the districts	Total no. of faecal samples tested	No. of positive sample	Prevalence of avian rotavirus (%)
Chittagong	70	2	2.86
Mymensingh	80	17	21.25
Gazipur	20	1	5
Barisal	40	9	22.5
Total	210	29	13.81

Although detection rate of avian rotavirus is comparatively low in clinical samples in Bangladesh, but higher rate of rotavirus infection have been documented by Villareal *et al.* (2006) where 45.3% of chickens in Brazil were avian rotavirus positive. Decaesstecker *et al.* (1988) reported that electron microscopic technique showed a positive frequency of 25% for avian rotavirus in 102 diarrhoeic faecal samples from broiler chicken up to a month old in Belgium. McNulty *et al.* (1981) reported that 40% of chicken farms and 59% of bird on those farms in USA were rotavirus seropositive. Saif *et al.* (1985) also reported that rotavirus detection rate in 50% of farms samples and 58% of the flocks positive on these farms. McNulty *et al.* (1984) further reported that 70% of serum samples from broiler breeder from 14 farms in Ireland were seropositive for rotavirus like virus. This result indicates that there is a possibility of causing higher rate of rotavirus infection in broiler birds of Bangladesh in near future.

The prevalence of avian rotavirus infection in Chittagong, Mymensingh, Gazipur and Barisal were found 2.86%, 21.25%, 5%, and 22.5% respectively (Table 1). It has been observed that the prevalence of avian rotavirus infection in chicken showed significant variation in different regions of the country. The highest prevalence (22.5%) was found in Barisal and the lowest (2.86%) in Chittagong. These differences may be due to several factors such as geoclimatic situation, passive immunity level, infecting dose, strain virulence, simultaneous infection with different avian rotavirus serotypes or even with others enteropathogens, stress, management errors and biosecurity failures.

Table 2. Prevalence of avian rotavirus infection detected from different ages and type of poultry in Bangladesh

Type of chickens	Age	No. of faecal samples tested	No. of positive faecal samples	Prevalence (%)
Layer	Day old {C}	60	0	0
Broiler	4 days {M}	25	6	24
	8 days {B}	20	5	25
	10 days {C}	10	2	20
	12 days {M}	20	8	40
	14 days {B}	20	4	20
	15 days {M}	20	2	10
	22 days {G+M}	{20+15} = 35	{1+1} = 2	5.71
Grand total		210	29	13.81

C = Chittagong, M = Mymensingh, B = Barisal, G = Gazipur.

The association of avian rotavirus infection in relation to age of chicken has been shown in Table 2. During the study period, 60 random (diarrhoeic and nondiarrhoeic) faecal samples were tested from day old chicks of Chittagong and all of which were found negative for avian rotavirus. In Mymensingh the collected faecal samples from 4 days, 12 days and 15 days age group of chickens were found 24% (6/25), 40% (8/20) and 10% (2/20) avian rotavirus positive respectively. The collected faecal samples of 8 days and 14 days age group of chickens from Barisal were found 25% (5/20) and 20% (4/20) avian rotavirus positive respectively. Out of 10 faecal samples 2 (20%) samples were avian rotavirus positive which were collected from 10 days old chickens of Chittagong. Faecal samples collected from Gazipur and Mymensingh of 22 days old chickens were found 5.71% (2/35) avian rotavirus positive. The highest prevalence was recorded as 40% in 12 days old birds whether the lowest was 5.71% in 22 days old birds. From the available literature and the present result it is noted that the incidence of rotavirus infection is remarkably high in young age and the incidence gradually becomes low in older age of birds. The present result revealed that the highest prevalence of avian rotavirus infection occurred in 12 days old chicks. The most susceptible age found in this study is almost similar to the findings of McNulty *et al.* (1981); Yason and Schat (1984) and Theil and Saif (1987) who reported the peak prevalence of avian rotavirus infection between 1 and 2 weeks of age group. Examination of 60 faecal samples collected from day old layer chicks was done and all were found negative for rotavirus. The probable reasons of this result may be the presence of very low amount of virus in samples or absence of rotavirus particle in the faecal samples.

The association of avian rotavirus with different seasons (Table 3) revealed that in rainy season (July-October) 0% (0/30), in winter season (November-February) 13.75% (11/80) and in summer season (March-June) 18% (18/100) faecal samples showed a characteristic electropherotypic mobility of dsRNA of avian rotavirus on PAGE. The present study detected the highest percentage (18%) of rotavirus positive in chicken faecal samples in the summer season. This result does not correlate with the finding made by Ansari *et al.* (1991) who described higher prevalence of rotavirus gastroenteritis in cool dry season in tropical and temperate zones. Although the reasons for the seasonality of rotavirus infection have not been well clarified, it has been suggested that the birds might constantly shedding the virus and disseminate the virus to susceptible chicken in summer season. Moreover, there might have co-infection with other diarrhea causing enteropathogens, which shed in the faeces and thereby facilitating the transmission of avian rotavirus and ultimately resulted in higher prevalence in summer seasons.

Table 3. The prevalence of avian rotavirus in chickens in relation to seasons

Seasons	No. of samples tested	No. of positive samples	Prevalence (%)
Rainy (July-October)	30	0	0
Winter (November-February)	80	11	13.75
Summer (March-June)	100	18	18
Total	210	29	13.81

In this study, 29 samples were found positive for avian rotavirus strain collected from the faeces of broiler birds showed distinct electropherotyping pattern of viral ds-RNA. All the positive samples produced genome electropherotypes characteristic of avian rotavirus. The migration patterns of all detected positive strains were similar in gel electrophoresis and their migration speed was same as previously designated avian rotavirus group D. In the present study, the migration pattern of avian rotavirus genome electropherotype in the first size class segments 1 to 5, segments 6 and 7 migrates in the second size class, segments 8 and 9 migrates in the third size class and segments 10 and 11 migrates in the fourth size class. This findings correlate with the observation made by Theil *et al.* (1986). The rotavirus that was detected in this study from faecal materials of chickens recognized as rotavirus belonging to group D avian rotavirus. The genome electropherotypic pattern revealed two segment migration pattern in first size class, segment 1 migrated separately; segments 2 and 3 and segments 4 and 5 migrated closely. The segments 6 and 7 migrated closely in the second size class. In the third size class, the segments 8 and 9 migrated as close spaced couplet. The segments 10 and 11 migrated separately in fourth size class (Fig. 1). This findings correlate with the findings made by Pedley *et al.* (1986) who designated an antigenically distinct rotavirus isolated from chicken in North Ireland by McNulty *et al.* (1981) as the prototype member for the group D rotaviruses.

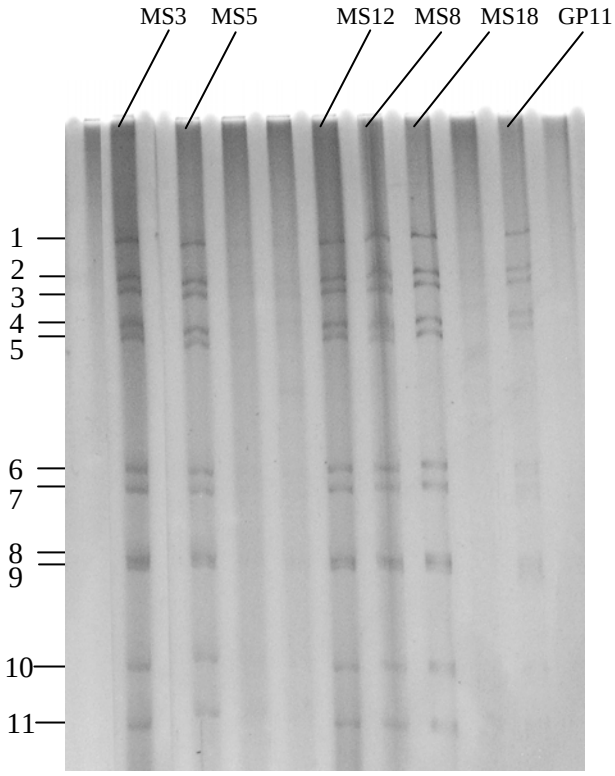

Fig. 1. Electrophoretic migration pattern of avian rotavirus dsRNA in polyacrylamide gel. In the left side, 1-11 numbers are indicated the dsRNA segments. In the upper portion of the figure, MS3-GP11 indicated the sample numbers.

In this study, all rotavirus infected birds were found diarrhoeic, dehydrated, anorectic and low body weight. These observations are in conformity with the earlier reports of McNulty (2003) and Tamehiro *et al*. (2003) who reported that in field conditions, rotavirus infections in poultry might induce subclinical manifestations, or they might be associated with enteritis, dehydration, anorexia, unrest, litter ingestion, low weight gain and increased mortality.

From the findings, it may be concluded that the prevalence of avian rotavirus infection was 13.81% in chickens in Bangladesh. Rotavirus infection was high in young birds and gradually became low in older birds. The prevalence of avian rotavirus infection was high in Barisal district and low in Chittagong district. Avian rotavirus infection was high in summer season. Only group D rotavirus was found during the study period in Bangladesh. Therefore, there is a possibility of happening higher rate of rotavirus infection in chickens of Bangladesh in near future.

REFERENCES

1. Ahmed MS and Ahmed MU (2006). Detection of avian rotavirus-like virus in broiler chickens in Bangladesh. *Bangladesh Journal of Veterinary Medicine* 4 (2): 73-77.
2. Ansari SA, Springthorpe VS and Sattar SA (1991). Survival and vehicular spread of human rotaviruses: Possible relation to seasonality of out breaks. *Reviews of Infectious Diseases* 13: 448-461.
3. Bergeland ME, McAdaragh JP and Stotz I (1977). Rotaviral enteritis in turkey poults. *Proceedings of the 26th Western Poultry Diseases Conference*. pp. 129-130.

4. Decaesstecker M, Charlier G and Meulemans G (1988). Epidemiological study of enteric viruses in broiler chickens: Comparison of tissue culture and direct electron microscopy. *Avian Pathology* 17: 477-486.
5. Dimitrov DH, Graham DY, Lopez J, Muchinik G, Velasco G, Stenback WA and Estes MK (1984). RNA electropherotypes of human rotaviruses from North and South America. *Bulletin WHO* 62 (2): 321-329.
6. Estes MK, Graham DY and Dimitrov DH (1984). The molecular epidemiology of rotavirus gastroenteritis. *Progress in Medical Virology* 29: 1-22.
7. Estes MK, Palmer EL and Obijeski JF (1983). Rotaviruses: a review. *Current Topics in Microbiology and Immunology* 105: 123-184.
8. Laemmli UK (1970). Cleavage of structural proteins during the assembly of the bacteriophage T_4. *Nature* 227: 680-685.
9. McNulty MS, Allan GM and McFerran JB (1984). Prevalence of antibody to conventional and atypical rotaviruses in chickens. *Veterinary Record* 114: 219.
10. McNulty MS (2003). Rotavirus infections. In: Saif YM, Barnes HJ, Glisson JR, Fadly AM, Mcdougald LR, Swayne DE (eds.), *Diseases of Poultry*. 11th edn., Ames, Iowa State University Press. pp. 308-317.
11. McNulty MS, Allan GM, Todd D, McFerran and McCracken RM (1981). Isolation from chicken of a rotavirus lacking the rotavirus group antigen. *Journal of General Virology* 55: 405-413.
12. Pedley S, Bridger JC, Chasey D and McCrac MA (1986). Definition of two new group of atypical rotaviruses. *Journal of General Virology* 60: 131-137.
13. Saif LJ, Saif YM and Theil KW (1985). Enteric viruses in diarrrhoeic turkey poults. *Avian Diseases* 29: 798-811.
14. Steele AD and Alexandar JJ (1987). Molecular epidemiology of rotavirus in black infants in South Africa. *Journal of Clinical Microbiology* 25 (12): 2384-2387.
15. Tamehiro CY, Alfieri AF, Medict KC and Alferi AA (2003). Segmented double-stranded genomic RNA viruses in fecal samples from broiler chicken. *Brazilian Journal of Microbiology* 34 (4): 344-348.
16. Theil KW and Saif YM (1987). Age related infections with rotavirus, rotavirus-like virus and atypical rotavirus in turkey flocks. *Journal of Clinical Microbiology* 25: 333-337.
17. Theil KW, Reynolds DL and Saif YM (1986). Genomic variation among rotavirus-like viruses detected by polyacrylamide gel electrophoresis. *Avian Diseases* 30 (4): 829-834.
18. Villareal LYB, Uliana G, Valenzuela C, Chacon JLV, Saidenberg ABS, Sandches AA, Brandao PE, Jerez JA and Ferreian AJP (2006). Rotavirus detection and isolation from chickens with or without symptoms. *Rev. Bras. Cienc. Avic.* 8 (3): 187-191.
19. Yason CV and Schat KA (1984). Isolation and characterization of avian rotaviruses. *Avian Diseases* 29 (2): 499-508.