# CHARACTERIZATION OF AVIAN REOVIRUS ds-RNA BY PAGE MIGRATION PROFILES

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## ABSTRACT

Avian reovirus field isolates were characterized by polyacrylamide gel electrophoresis with the migration pattern of double stranded RNA (dsRNA) segments. Our study demonstrates characterization and classification of individual ARV isolates with obtained significant electrophoretic migration profile of the dsRNA segments among different isolates by RNA-PAGE.

**KEYWORDS:** Avian Reovirus, ds- RNA genome, Characterization, PAGE.

## INTRODUCTION

Reoviruses are important infectious agent among human, plant and animal populations with a vast array of manifestations. Reoviruses belongs to reoviridae family, representatives of non-enveloped double stranded RNA (dsRNA) virus. Family Reoviridae characterized by a genome comprising 10 segments (Spandidos and Graham, 1976, Naveca et al., 2007, Tran et al., 2009, Chi et al., 2013, Borodavka et al., 2015, Lawson et al., 2015).

An array of disease states are associated with ARV infection in chicken viz., gastroenteritis, respiratory and enteric involvement, hydropericardium, myocarditis and pericarditis in chickens, infectious enteritis in young turkeys, intermittently met with mortality, hepatitis, runting-stunting syndrome, however, ARV infections are best recognized by arthritis and tenosinovitis. Apparently healthy birds may shed virus in tracheae and faeces. Definitely, the manifestation differs with the serotypes of the infecting virus on the basis of tissue tropism (Gouvea and Schnitzer 1982, Lawson et al., 2015, Borodavka et al., 2015).

Susceptibility of day-old chicks to oral infection by avian reovirus was inversely related to the immune status of their dams and concluded that breeder flock vaccination might be an important method of controlling avian reovirus infection in broilers (Ide and Dewitt, 1979).

Mixed infections of vvIBDV, cvIBDV and ARV causing proventriculitis and other suggestive lesions of both diseases are common in poultry flocks throughout the world (Kutkat et al., 2010). The identification, characterization and classification of the viruses is crucial in view of epidemiological study, improving knowledge of circulating genotype (Kort et al., 2013) in Indian flocks, and thereby, for further management and prevention and control strategies.

In the present research the ARV isolates, isolated from samples collected from different poultry farms in Maharashtra were subjected to RNA-PAGE for detection and to obtain electrophoretypes to obtain electrophoretypes on the basis of their migration patterns by RNA-PAGE.

## MATERIAL AND METHODS

The nine field isolates, isolated from samples collected from different poultry farms in Maharashtra and maintained at Microbiology department, Bombay Veterinary College, Mumbai were used for study. The genome of virus was detected and analysed by RNA-PAGE using discontinuous buffer system without SDS as described by Laemmli (1970).

**Extraction of viral RNA:** The RNA was extracted from concentrated virus isolates and chorio-allantoic membrane (CAM) (Makadiya, 2004) by TRI reagent. 250 µl of allantoic fluid or supernatant of triturated tissue was added to 750 µl TRI reagent and vortexed, incubated 5 min at room temperature. 200 µl Chloroform was added and mixed vigorously for 15 sec, incubated 15 min at room temperature. The mixtures were phase separated by centrifugation at 12,000 rpm for 20 min at 4ºC. 500 µl isopropanol was added to the aqueous phase and left at -20ºC for 60 min to precipiotate RNA, centrifuged at 12,000 rpm for 30 min to obtain pellets, then washed twice with 75% ethanol (v/v), air dried and dissolved in DEPC treated double distilled water and stored at -20ºC.

**Casting of the polyacrylamide gel:** Gel casting plate's borders were sealed with 2% agarose. Resolving gel (8%) and stacking gel (5%) (Sambrook and Russsel, 2001) were prepared. Resolving gel solution was poured between the gel casting plates (15x10 cm) and 1 ml distilled water in 1 ml quantity was overlaid on to the top. The water layer was completely removed after gel polymerization. The stacking gel was carefully overlaid on to the resolving gel, comb was inserted in stacking gel and removed after polymerization.

**Electrophoresis:** Extracted RNA pellet (5 µl) was mixed with 5µl of 2X RNA-PAGE loading buffer and loaded in the wells for vertical electrophoresis system in tris-glycine buffer (TGB) 1X (pH 7.8) at a constant 120 Volt (8-10 Volt/cm).

**Gel Staining:** Silver nitrate staining of the gel was carried as per Tamehiro *et al.* (2003). Gel was taken out, marked at the lower right corner and fixed in the fixative solution for 30 min with intermittent gentle shaking, followed by staining for 30 min in staining solution with gentle shaking. Staining solution was drained off; gel was quickly rinsed twice with double distilled water and freshly prepared developer solution was then added with gentle shaking. RNA bands were observed for desired contrast and developer was removed immediately and reaction was stopped by adding the stop solution. Gel was stored in 10 % ethanol.

## RESULT AND DISCUSSION

ARV genome is bisegmented (2 seg. ds RNA), which is distributed among 10 segments with molecular weight of 2.7 × 106, 2.6 × 106 , 2.4 × 106, 1.7 × 106 , 1.5× 106 ,1.3 × 106,1.2 × 106 ,0.80 × 106,0.74 × 106 ,and 0.68 × 106 for the largest (L1), to the smallest (S4) segments, respectively (Spandidos and Graham, 1976). Polyacrylamide gel electrophoresis (PAGE) of ARV ds-RNA genome has been used widely for detection, characterization and classification on the basis of the band/ migration pattern obtained (Gouvea and Schnitzer 1982, Rekik et al. 1990, Naveca et al., 2007).

The present study demonstrated RNA-PAGE for detection and characterization of ARV dsRNA (PLATE1), 10-11 segments of RNA with long electropherotype migration pattern indicated the presence of segmented RNA virus in all samples. Each of the 10 bands of ds RNA genome appearing in the gels were grouped into 3 classes as large (L1, L2, L3), medium (M1, M2, M3) and small (S1, S2, S3, S4) (PLATE 1) based on their electrophoretic mobility. RNA bands with 10 distinct pieces were obtained between the two avian strains (ARV strains, 2207/68 and Wi) by PAGE, thus their study permitted the definite classification among the virus strains (Nick et al., 1975) concordant with our results. In a similar study, Tamehiro et al., (2003) represented nine different electrophoretypes, however, Rekik et al., (1990) analysed electropherotypes of avian reovirus field isolates, and higher mobile segments viz, L1, S1, S2, S3 and S4 with no correlation between electrophoretype and geographic origin of isolates described emergence of electropherotypically distinct strains with the introduction of modified live reovirus vaccines.

A study by Ramig et al., (1977) presented 11 unique virus-specified polypeptides of ARV serotype 1, 2 and 3 with high-resolution PAGE in discontinuous buffer systems and they classified them into 3 classes as large (A), medium (A), and small (a-) as numbered in increasing order of electrophoric mobility. They described different electrophetic mobilities of RNA and corresponding polypeptides in each serotype. Our observations were also supported by the data discerned by Gouvea and Schnitzer (1982) who found marked polymorphism by migration patterns in all dsRNA segments among isolates of the same serotype as well as among different serotypes.

Denaturing gradient and constant denaturing PAGE was carried out to detect mutations in ARV ds-RNA (Naveca et al., 2007). A comparison study of PAGE and electron microscopy was introduced and band migration profiles were observed with reovirus in direct specimens (Lozano et al., 1992). Ide and Dewitt, (1979) demonstrated the susceptibility of day-old chicks to oral infection by avian reovirus which was inversely related to the immune status of their dams and concluded that breeder flock vaccination might be an important method of controlling avian reovirus infection in broilers.

## CONCLUSION

Thus, to the end, the method collectively might significantly guide to choose the appropriate vaccine entity/ strain for disease control and prevention. We conclude that segments of ARV could be detected well by RNA-PAGE followed by silver staining. PAGE could be the best preferred method for identification, characterization and classification of the various ds-RNA viruses, their isolates and different serotypes among them as well.

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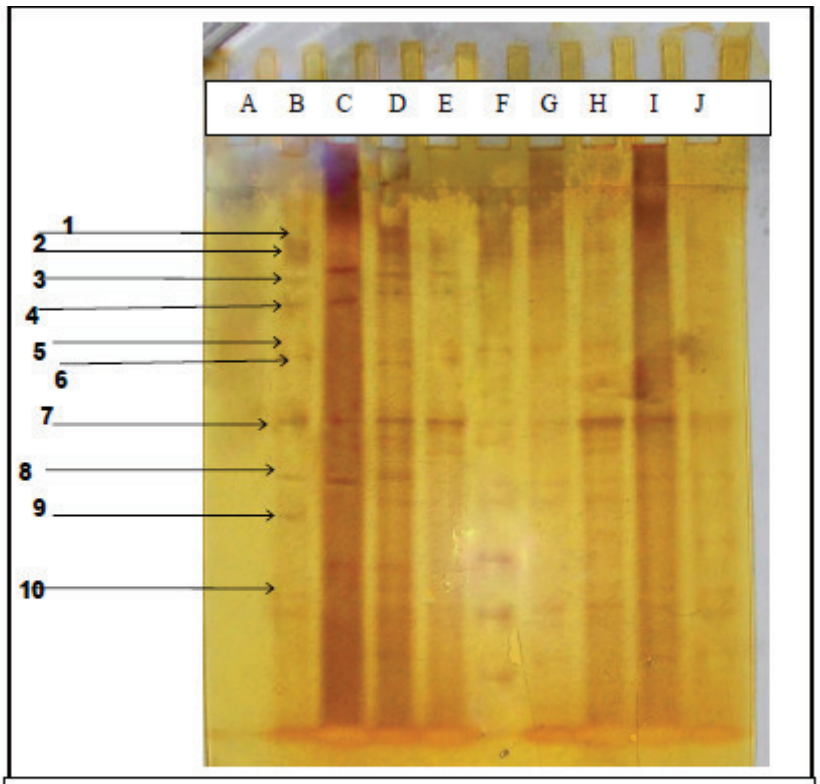
PLATE 1- RNA-PAGE for Avian Reovirus Isolates




Segments of ds RNA- 1) L1 3) L3 5) M2 7) S1 9) S3
2) L2 4) M1 6) M3 8) S2 10) S4

Lane A represent dye, lanes B- J represents sample no.9,1,3,4,14,8,18,10,15 respectively.