

Rotavirus



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Rotavirus

7. Rotavirus

7.1 Epidemiology of Rotaviruses

7.1.1. Hospital based surveillance of rotavirus disease and strains

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Rotavirus gastroenteritis is the major cause of severe dehydrating diarrhea in India. This study was undertaken to assess rotavirus epidemiology in Western India.

Objectives

- To estimate the proportion of rotavirus diarrhea
- To find out prevalent rotavirus strains among hospitalized children <5 years of age.

Work done

A total of 299 fecal specimens were collected from children <5 years of age, admitted for acute diarrhea in the local hospitals from Pune. Thirty percent hospitalized children showed presence of Group A rotavirus in their stools while none of the OPD patients were positive. Sixty ELISA positive specimens were subjected to multiplex PCR. Nearly 92% of rotavirus strains were typed into G1-G4, G9 and G10 with predominance of G1, G2 and G9. Eighty seven percent rotavirus strains were typed into P[4], P[6], P[8] and P[10] with higher prevalence of P[8] and P[4]. Rotavirus was detected by ELISA in 5.9% children hospitalized for diarrhea in Govt Medical College, Aurangabad..

Nearly 24% of ELISA negative specimens were positive for Rota RNA indicating that RT-PCR was useful for detection of Group A rotaviruses shed in low concentration. Earlier studies on rotavirus viremia were continued. VP6 gene PCR products derived from serum samples were sequenced. Analysis of the sequence data showed genetic relatedness to TK126 and TK159 strains reported from Kolkatta. The data obtained on paired stool and serum samples indicated G2 P[4] and G2 P[4] P[8]. In this study serotype G12 P[8] was detected for the first time in western India.

7.1.2. Detection and characterization of rotaviruses in adolescent and adults cases of acute gastroenteritis

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Although Rotavirus infections in adults are milder than those in children, deaths due to rotavirus have been reported. In India, limited studies conducted in adults indicate 5-7% prevalence of rotavirus diarrhea. However, role of rotaviruses as a pathogen in adults has long been under appreciated.

Objectives

- To characterize the rotavirus strains recovered from adolescent and adult cases of diarrhea.

Work done

Retrospective analysis of 134 fecal specimens collected during 2004–05 was carried out by ELISA, RNA PAGE and RT-PCR. Antigen was detected in 6% specimens. Group A rotavirus specific RNA pattern was noted in 4 of 81 specimens tested and Rota RNA was detected in 17 of 23 specimens. Among these specimens G2 P[4] and dual infections of G1 G9-P[4] P[8], G2G4-P[4] P[8] and G1G2 P[8] were detected.

Seven group B rotavirus strains recovered from adult diarrhea patients were PCR amplified and sequenced. Percent nucleotide identity of these strains was in the range of 89–95% with that of ADRV (China) strain for the genes 4,5 and 9.

Fecal samples were collected from 56 adolescents and adult cases of acute gastroenteritis visiting OPD during April, 2005 - March, 2006. ELISA / RNA PAGE positivity for rotavirus was detected in 7.14% specimens. Among these specimens (G4 P[4], G9 P[4]P[8], G1 P[8] and G2,G4 genotypes were detected.

7.2 Seroepidemiology of rotaviral infection

7.2.1. Anti-rotavirus IgA levels in follow-up serum /milk samples of mothers and infants of higher socio-economic group (HSG) and lower socio-economic group (LSG) after delivery

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Data on rotavirus specific immunoglobulins among mothers in the postpartum serum and milk samples may provide useful information to assess the extent of exposure to rotavirus and subsequent development/maintenance of anti-rotavirus antibody in infants from different socio-economic groups.

Objectives

- To estimate rotavirus specific IgA levels among Indian mothers and occurrence of rotavirus infections among their infants up to six months of age.

Work done

Higher rate of rotavirus specific IgA in sera was found among the LSG infants(65%) compared to HSG infants (41%). Mothers' sera from both the groups showed similar positivity at delivery as well as at 6 months post partum (~60%) indicating exposure to rotavirus in the past. IgA GMTs and percent positivity was higher in colostrum and milk samples upto 3 months among LSG mothers but the difference was not statistically significant (Table1).

Table 1: Rotavirus specific IgA GMTs and IgA percent positivity in follow-up Mother/Infant pairs of HSG and LSG

Samples (n=17)	IgA GMTs		IgA No. positive (% positivity)	
	HSG	LSG	HSG	LSG
Mothers serum (after delivery)	60	92	10(58.82%)	11(64.7%)
Mothers serum (at 6 months)	92	96	10(58.82%)	11(64.7%)
Child serum (at 6 months)	67	133	7(41.17%)	11(64.7%)
Colostrum (at 1-2 days)	46	97	7(41.17%)	11(64.7%)
Milk (at 4-6 days)	45	50	5(29.41%)	9(52.94%)
Milk (at 3 months) ^a	31	39	3(11.11%)	7(31.81%)
Milk (at 6 months)	42	36	6(35.29%)	5(29.41%)

^an=27 for HSG & n=22 for LSG

7.2.2. Detection of anti CH2 rotavirus antibody in field chicken sera

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Rotaviral infections are extensively studied in humans and animals. However, limited studies have been carried out to determine the extent of exposure of birds to avian and human rotaviruses.

Objectives

- To determine the prevalence of avian rotavirus antibodies in birds.

Work done

IgG antibody capture ELISA was used to screen 120 field serum samples from chicks. Specific immuno conjugate prepared in the laboratory was used as a probe to detect the captured virus at 1: 200 dilution. Cut off was calculated as Mean NC ± 3SD. Sera from captivated day old chicks (n = 8) and four days old SPF chicks (n = 20) served as negative control for the test. Sixty percent (72/120) were found to be antirotavirus antibody positive indicating exposure of birds to avian rotavirus or similar agent that is circulating in Pune city.

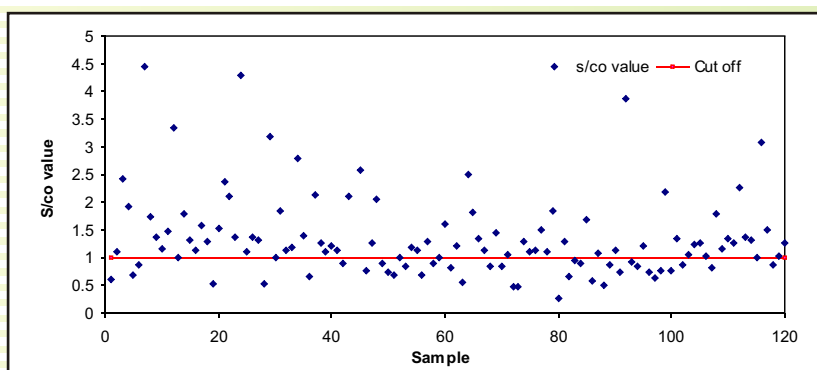


Fig. 1 : Scatter diagram of S/Co values indicating antirotavirus antibodies in chicken sera.

Future Plan

ELISA positive sera from birds will be investigated for neutralizing activity against rotaviruses.

7.3 Characterization of rotaviruses

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The rotavirus diversities pose a challenge in development of vaccine and other preventive measures. Characterization of local rotavirus strains is required to find out the distribution of sero/genotypes. Their comparison with those from other regions is useful in identification of variations such studies representing western region of India, were carried out at NIV, Pune.

Objectives

- To characterize at molecular level rotavirus strains categorized as non-typeable, multi-reactive, dually reactive and typeable on the basis of reactivity to monoclonal antibodies against human rotavirus serotypes G1-G4, G6 and G10.

Work done

Ninety fecal specimens collected from acute diarrhea patients in the years 1990-95 and 2000-02 were undertaken for the study. All specimens showed Group A rotavirus specific RNA pattern on polyacrylamide gel electrophoresis. All specimens were tested by RT-PCR using VP7 and VP4 gene specific primers. This was followed by sequencing of PCR products. The G and P type distribution among 90 specimens was P[8]G1 (36.66 %), P[4]G2 (34.4%), P[8]G3 (4.4%), P[8]G4 (5.5%). Nineteen percent specimens showed unusual combination of G and P types. These unusual rotavirus strains include, G1P[6], G1P[4], G4P[6], G9P[8], G3P[4] and G1P[19]. The presence of human-porcine reassortant-P[19]G1 was detected for the first time in India.

7.4 Preparation of egg yolk antibodies against human rotaviruses

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The chicken egg is a complete diet for the developing embryo and a supplement for the first few days of life of chick. The birds vaccinated against human/poultry pathogens produce eggs having yolks with high level of antibody protein IgY.

Objectives

- To develop an ELISA specific for detection of avian rotavirus and its antibodies.
- To prepare immunoglobulins against human rotaviruses in egg yolk.

Work done

The human rotavirus (HRV1-4, HRV-9) stocks with titres between 1:320 and 1:640 were prepared by propagating KU, S2, YO, ST3 and F45 strains in MA104 cell line. The virus from each of the stocks was purified on sucrose gradient and protein was estimated.

Three groups of SPF hens, each consisting of 7 birds were immunized against human rotaviruses - HRV1, HRV2 and HRV9. Sera collected subsequently were tested by ELISA to detect anti-rotavirus antibodies. All birds generated anti-rota antibodies. Antibody titres in all 3 groups showed significant rise after 4th dose. Western blot carried out using HRV-9 showed presence of antibodies in bird's serum against major structural proteins (VP6, VP7, VP4) (Figs 2 and 3).

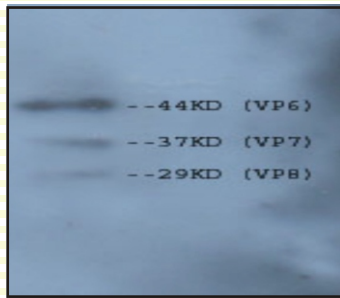
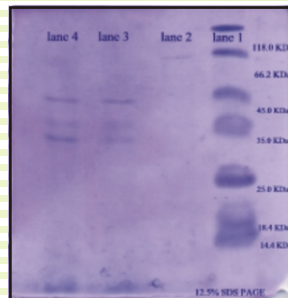


Fig 2.: Western blot analysis of bird's immune response to rotavirus (HRV9) polypeptides



Lane 1 - Protein Molecular weight Marker
Lane 2 - MA104 cell lysate
Lane 3 & 4 - HRV9

Fig. 3: Profile of purified HRV9 proteins stained with Coomassie Brilliant Blue on SDS PAGE

7.5 Cultivation of avian rotavirus in embryonated egg system

Cultivation of avian rotavirus strain CH2 in embryonated eggs was initiated using various routes namely yolk sac, amniotic, allantoic, allantoic + amniotic and chorioallantoic membrane of inoculation. Presence of virus in early passages was detected by antigen capture ELISA. With increase in passage level of virus, mortality of eggs increased. However, percent positivity to viral antigen in ELISA increased in a cyclic manner. Full virus particles were visualized by immuno electron microscopy (Fig. 4) while presence of viral RNA was detected by RT-PCR (Fig. 5) The propagation of virus was monitored upto passage 6, however, with low titres in comparison to that of cell culture. The virus was found infectious to normal MA104 cell line in infectivity assay.

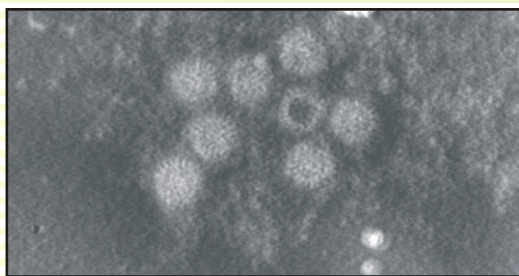


Fig. 4: Electron micrograph of chicken rotavirus Ch2 strain recovered from egg at passage 5

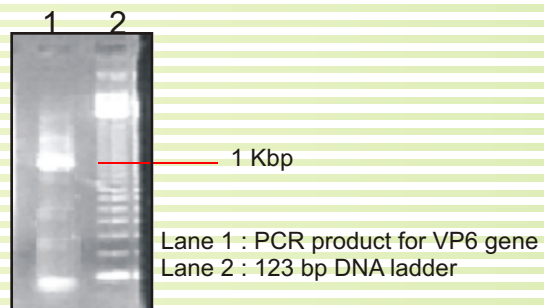


Fig. 5: Agarose gel electrophoretic migration pattern of PCR product derived from egg adapted Ch2 strain

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