Rotavirus Diarrhoea in Children and Animals of Urban and Rural Nepal Sherchand JB^a and Haruki K^b

Abstract

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Introduction	Rotavirus, an etiologic agent of causing diarrhea, was identified in 25-40 percent of children with diarrhea in urban Kathmandu valley of Nepal, but the data in remote rural areas was inadequate. It was not clear whether domestic animals possessed Rotavirus and could become a source of infection in annual epidemic period. The current study described one-year prevalence of rotavirus in human beings and domestic animals in rural communities of Nepal.
Objectives	The purpose of this study was to reveal the association between domestic animal Rotavirus and child diarrhoea in the rural communities of Nepal.
Methods	Human faeces were collected from children under 5 years of age who developed diarrhoea and that from non-diarrhoeic children of equivalent ages. Animals' faecal samples were collected monthly from cows, buffaloes, pigs and dogs in the village. All samples were made 20 percent suspension in PBS and stored at -30°C. The study was undertaken from October 2001 to November 2002. Indirect sandwich ELISA with minor modification was applied for Rotavirus assay.
Results	A total of 714 stool samples from children and 772 from domestic animals were tested. Among children group, 116(16.2%) were found ELISA positive for Rotavirus whereas 8(1.03%) were positive among animal groups. The percentage of ELISA positive in hospital samples (30.6%) was significantly higher than in village (4.1%). The highest rate of rotavirus positive was also found in diarrhoeic stools (30.4%) compared to normal stools (0.87%) (P<0.05). The high incidence of diarrhea and Rotavirus was observed in winter. Rotavirus was found in 6 months to 2 years age groups more frequently than other groups while only three samples of normal feces contained Rotavirus. The incidence of Rotavirus in animals was 0.41 percent (1 of 242) of buffaloes, 2 of 254(0.78%) dogs and 3.62 percent (5 of 138) from diarrhoeal samples of pigs.
Conclusion	Rotavirus was found predominantly in winter and attacked 6 months to 2 years old children. The study indicated that the infected animals and hospitalized diarrhoeal children were possible source of Rotavirus infection in Nepal.
Keywords	Rotavirus, Diarrhoea, Children, Animal, Nepal.

Introduction

Rotavirus has been recognized as an etiologic agent of diarrhoea in infants, young children¹⁻³ and animals⁴⁻⁶. The household contact leading to infection was documented in different literatures⁶⁻⁸. Rotavirus was found in 25-40 percent of children with diarrhea in urban Kathmandu valleys of Nepal^{9,10}, but the data remote rural areas was inadequate. Furthermore, it was not clear whether domestic animals possessed rotavirus and could become a source of infection in annual epidemic period. The current study described one-year prevalence of Rotavirus in human beings and domestic animals in rural communities of Nepal. The purpose of this study was to reveal the association between

domestic animals Rotavirus and child diarrhoea in urban hospitals and rural communities of Nepal.

Methodology

Human faeces were collected from children under 5 years of age who developed diarrhoea and that from non-diarrhoeic children of equivalent ages. Animal's faecal samples were collected monthly from cows, buffaloes, pigs and dogs in the village. All samples were made in 20 percent suspension in PBS and stored at -30° C. the study was undertaken from October 2001 to November 2002

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Additional information such as age, sex, clinical symptoms and history of medication were collected. The stool samples were also examined the presence of parasitic infection using standard normal saline and Iodine preparation methods.

An Indirect sandwich ELISA⁷ with minor modification was applied for Rotavirus assay. The test used the rabbit anti-rotavirus coated micro plate, monoclonal anti-rotavirus reagent and peroxides-conjugated rabbit anti-mouse antiserum. All positive samples were confirmed by ELISA-blocking test.

Results

A total of 714 stool samples of children were collected from two areas in which 388 from villages and 326 from hospitals. Total Rotavirus positive from these two areas were 116(16.2%), in which 16 cases of Rotavirus positive (4.1%) samples were from villages and 100 cases (30.6%) from hospitals. The percentage of ELISA positive in hospital samples (30.6%) was significantly higher than in village (4.1%).

The high incidence of diarrhoea and Rotavirus positive cases by months was observed in winter December, January and February (Figure 1).

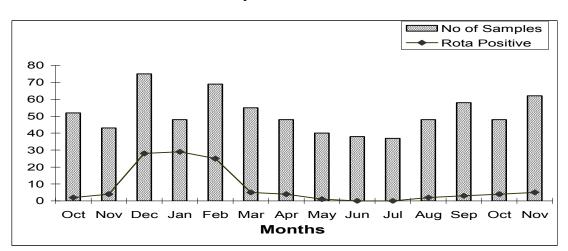


Figure 1: Month-wise distribution of Rotavirus positive cases in diarrhoeic and normal stools

Diarrhoeic (371) and normal (343) stool samples were tested for the presence of rotavirus. Results and age-wise distribution of Rotavirus infection were described in Table 1 and 2. Rotavirus was found more frequently in 6 months to 2 years age group than other age groups. The highest rate of Rotavirus positive was

also found in diarrhoeic stools (30.4%) compared to normal stools (0.87%) (P<0.05). The percentage of Rotavirus positive cases from diarrhoeal stools in the hospitals 38.6 percent was significantly higher than that found in the villages (12.8%) (P<0.05) as presented in table 1.

Table 1: Age-wise distribution of Rotavirus infection in diarrheic children

	Diarrhoeic stools, No. of positive/ No. of tested(%)		
Age	Villages	Hospitals	Total
1-5 months	2/21(9.5)	31/84(36.9)	33/105(31.4)
6-11 months	6/26(23.07)	43/88(48.86)	49/114(42.9)
1-2 years	5/32(15.6)	22/54(40.74)	27/86(31.4)
3-5 years	2/38(5.26)	2/28(7.14)	4/66(6.06)
Total	15/117(12.8)	98/254(38.6)	113/371(30.4)

	Normal stools/No. of positive/No. of tested(%)			
Age	Villages	Hospitals	Total	
1-5 months	0/44(0.0)	0/14(0.0)	0/58(0.0)	
6-11 months	0/56(0.0)	1/16(6.25)	1/72(1.4)	
1-2 years	1/66(1.5)	0/18(0.0)	1/84(1.2)	
3-5 years	0/105(0.0)	1/24(4.16)	1/129(0.8)	
Total	1/271(0.36)	2/72(2.8)	3/343(0.87)	

Table 2: Age-wise distribution of Rotavirus infection in normal children

In order to identify natural reservoir of Rotavirus, normal and diarrhoeal animals' faeces (138 samples of cows, 242 buffaloes, 138 pigs and 254 dogs) were collected monthly from the villages and tested. It was found that one of 242(0.41%) Rotavirus positive from buffaloes samples, 5 of 138(3.62%) Rotavirus positive from pigs and 2 of 254(0.78%) from diarrhoeal samples of dogs (Table 3). Of the 8 positive samples were collected in winter (December, January and February).

Table 3: Distribution of Rotavirus in domestic animals' stools

Domestic Animals	Number of samples	Rotavirus positive(%)
Cows	138	0
Buffaloes	242	1(0.41)
Pigs	138	5(3.62)
Dogs	254	2(0.78)
Total	772	8(1.03)

Discussion and Conclusion

Rotavirus is a major cause of pediatric gastroenteritis and responsible for causing half of the cases to be suffered with acute diarrhoeal illness among hospitalized patients of 6 to 24 months of age^{9,11,12} which is similar to the studies conducted in other countries^{13,14}.

Rotavirus illness is also common in community and family settings¹⁵. In the study, Rotavirus was predominant in winter, particularly in December to February, it accounted for more than 60 percent of paediatrics diarrhoea (figure 1). However, the incidence in village samples was found relatively lower than that found in the hospitals (Table 1 and 2). This might be due to the population density, which was low in village and high in urban areas. The present results strongly suggested the causative role of Rotavirus in child diarrhoea. Though we did not collect sufficient stool samples from non-diarrhoeic children from the hospitals, 2.8 percent of the children were found to be positive for Rotavirus infection. Thus, the study

indicated that there was a possibility of Rotavirus infection among non-clinical or diarrhoeal children. It was known that Rotavirus occasionally caused travellers' diarrhoea in adult and diarrhoea in immunocompromised (including AIDS) patients, parents of children with Rotavirus diarrhoea, and the elderly. It was stressed that Rotavirus diarrhoea in young children was characterized by an acute onset, vomiting, watery diarrhoea, and hence a rapid development of dehydration and acidosis^{16,17}.

The clinical symptoms often were more severe in rotavirus diarrhoea than that caused by other enteropathogens. It was also known that Rotavirus cases usually dominate in hospital-based survey in children^{3,9,18}. The present study showed a highest rate of infection among hospitals children (38.6%) than village children (122.8%). In the study, Rotavirus infection was found in domestic animals such as pigs, dogs and Buffaloes. Though the animal viruses did not produce disease in human; group B and C Rotaviruses identified in humans appeared to be quite distinct from those found in animals.

Although more studies are needed to clarify the direct link between Rotavirus infection and domestic animals, the current study indicated that the infected animals and hospitalized diarrhoeal children are possible source of Rotavirus infection in Nepal.

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References

- 1. Cukor G, Perron DM, Hudson R and Blacklow NR. Detection of Rotavirus in Human stools by using monoclonal antibody *Journal of Clinical Microbiology* 1984; 19:888-92.
- Kapikoan AZ, Kim HW, Wyatt RG, Cline W, Arrobio JO, Brandt CD, Rodriguez SA, Sack RM and Parrot RH. Human rotavirus like agent as the major pathogen associated with winter Gastroenteritis in hospitalized infants and young children. New England Journal Medicine 1976; 294:965-72.
- 3. Hart CA, Cunliffe NA and Bresee JS. Diarrhoea caused by viruses. In "Manson's Tropical Diseases" Cook JC, Zumla A, eds. WB Saunders, London 2003; 823-30.
- Yolken RH, Babour B, Wyatt RG, Kalica AR, Kapikian AZ and Chanock RM. Enzyme-liked immunosorbent assay for identification of Rotaviruses from different animal species. Sciences 1978; 201:259-62.
- Hoshino Y, Wyatt RG, Greenberg HB, Flores J and Kapikian AZ. Serotypic similarity and Diversity of Rotavirus of mammalian and avian origins as studies by plaque-reduction neutralization. *Journal Infectious Disease* 1984; 149: 694-702.
- Wasi C, Lousirirotchanakul S, Thakerngpol K, Satrasook S, Surakhaka M, Varavithya W and Thongcharoen P. The epidemiological study of viral diarrhoea in Thailand. *Journal Medical* Association Thailand 1984; 67:369-76.
- 7. Yolken RH. Enzyme Immunoassays for the detection of infectious antigens in body fluids: Current limitations and future prospects. Reviews in Infectious Diseases 1982; 4:35-68.
- 8. Nakagomi O, Koshimura Y and Nakagomi T. Rotavirus vaccines in Japan and Australia. *Lancet* 1999; 353:1275.
- 9. Sherchand JB, Larsson S, Rana BJ, Dixit H, Bam DS, Adhikari RK, Sharma PR, Shrestha MK and Shrestha MP. On the incidence of Rotavirus and enteric adenovirus diarrhoea in children attending

- the outpatient department of Kanti Children's Hospital and general practitioners in the Kathmandu area. *J Nepalese Med Assoc* 1992; 30:149-53.
- Sherchand JB, Shrestha MP, Hommel M and Ohara H. Hospital based study Southern Nspal on morbidity of malaria. Khoj-Bin: Journal Nepal Health Research Council 1998; 2:18-22.
- Echeverria P, Ho MT, Blacklow NR, Quinnan G, Portnoy J, Olson R, Conklin R, DuPont HL and Cross JH. Relative importance of viruses and bacteria in the etiology of pediatric diarrhoea in Tiawan. *Journal Infectious Disease* 1977; 136:383-90.
- 12. Parashar UD, Hummelmann EG, Bresee JS, Miller MA and Glass RI. Global illness and deaths caused by rotavirus disease in children. *Emerg Infect Dis* 2003; 9:565-72.
- 13. Bishop RF. Epidemiology of diarrhoeal disease caused by rotavirus. In: Development of vaccines and Drugs against Diarhoea, 11th Nobel Conference, Stockholm 1985, Eds: *Journal Holmgren Lund* 1986; 11:158-70.
- 14. Parashar Ud, Bresee JS, Gentsch JR and Glass RI. Rotavirus. *Emerg Infect Dis.* 1998; 4:562-70.
- 15. Hendricks MK, Cuevas LE and Hart CA. Rotavirus diarrhoea in Thai infants and children. *Ann Trop. Paediatr.* 1995; 15:147-52.
- 16. Maki MA. A prospective clinical study of Rotavirus diarrhoea in young children. *Acta Paediatr Scand* 1981; 70:107-13.
- 17. Cunliffe NA, Gentsch JR, Kirkwood CD, Gondwe J, Dove W, Nakagomi O, Nakagomi T, Hoshino Y, Bresee JS, Glas RI, Molyneux ME, and Hart CA. Molecular and serologic characterization of novel serotype G8 human rotavirus strains detected in Blantyre, Malawi. *Virology* 2000; 274:309-20.
- 18. Louisirirotchanakul S, Wasi C, Satrasook S, Surakhaka M and Thongcharoen P. Rotavirus in paediatric diarrhoea at Siriraj Hospital, Bangkok, Thailand. *Southeast Asian Journal Tropical Medicine and Public Health* 1984; 15:348-53.