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PREVALENCE OF ROTAVIRUS, ADENOVIRUS AND ASTROVIRUS INFECTION IN YOUNG CHILDREN WITH GASTROENTERITIS IN GABORONE, BOTSWANA
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ABSTRACT

Objective: **To determine the prevalence of three enteric viruses, namely rotavirus, adenovirus and astrovirus, as agents of diarrhoea in and around Gaborone, Botswana.**
Design: **The sample were categorised into four groups according to the age of the patient: 0-3 months, 4-6 months, 7-12 months and 25-60 months. Total monthly samples across age groups formed basis for calcultating seasonal prevalence of rotavirus infection.**
Setting: **Stool samples were collected from three medical laboratories in Gaborone and one in the town of Mochudi. These were collected from children under the age of five years with gastroenteritis.**
Subjects: **Stool samples were collected between March 2001 and February 2002 from 346 children less than five years of age suffering from gastroenteritis. These samples had been sent to medical laboratories for microbiological examination.**
Methods: **The samples were screened for rotavirus (RV), adenovirus (Ad) and astrovirus (AsV) antigens using commercially available ELISA kits. The Ad positive samples were further analysed by commercially available group specific Ad type 40/41 Enzyme Immuno Assays (EIA).**
Results: **Shedding of RV was detected in 9.2%, Ad in 7.8% and AsV in 2.7% of the samples analysed. The enteric Ad (types 40 and 41) were detected in 2% of the samples and the remaining 5.8% of Ad positive samples were non-enteric Ad. An increase of RV was noted in the autumn-winter season but no seasonal pattern was observed in Ad shedding. Seasonal prevalence of AsV could not be determined. The average age of children infected with these agents was less than one year.**
Conclusion: **The incidence of rotavirus infection amongst children in Botswana appears to be relatively low. The prevalence rate of adenovirus and astrovirus is similar to other studies in parts of Southern Africa. However, continued enteric virus surveillance and epidemiology amongst this group is required.**

INTRODUCTION

Diarrhoeal diseases affect millions of people around the world and have the greatest impact on children, especially those in developing countries. Almost every child contracts a diarrhoeal disease during the first five years of their life, on an average several times per year (1). Diarrhoea is one of the leading causes of death in developing countries, responsible for 25-30% of deaths among children younger than five years of age (2,3). In these countries the incidence of diarrhoeal cases varies between 2.5-3.9 episodes per child per year. In Africa, about 2.5 episodes per child per year are reported mainly among children between 6-11 months of age, corresponding to the introduction of weaning foods(1).

Diarrhoea can be caused by a number of different agents, including viruses, bacteria, parasites and toxins. However, during the past two decades, viruses have been firmly established as aetiological agents of acute gastroenteritis (GE)(1). There are four major virus groups aetiologically linked to diarrhoea ; rotavirus, astrovirus, adenovirus and calicivirus(1). In both developed and developing countries, rotavirus is responsible for the greatest proportion of severe childhood diarrhoea(1).

In Botswana, a high incidence of childhood diarrhoea accounts for 8-12.5% of all deaths in children less than five years of age(4). In this age group the estimated mean number of episodes of diarrhoea are 3.2 per child per year. However, in children below one year of age this number increases to 4.5 per child per

year. In addition, within the past 14 years there has been a 40% increase in diarrhoeal cases, increasing from 1959/10,000 episodes in 1983 to 2756/10,000 episodes in 1999(6).

Very few studies have been performed in Botswana to determine the aetiological agents of diarrhoeal diseases. General laboratory analysis in referral hospitals and clinics mainly report *Shigella,* a bacterial agent, as the causative agent of diarrhoea(1). Thus, most diarrhoeal cases (30-70%) are not aetiologically elucidated, particularly those linked to virus infection (5). Studies by Tlagae(7) and BIDPA(4) concentrated on diarrhoea incidences and risk factors. Recently Urio *et al.*,(8) carried out a study on *Shigella* and *Salmonella* strains where they isolated *Shigella* from 43/221 (21%) and *Salmonella* from 8/221 (3%) of rectal swabs from children under five years of age with diarrhoea. However, studies by Sebunya *et al.*(9) and Kasule(10) have confirmed rotavirus as an important viral agent causing diarrhoea (about 17%) in children below five years of age. Hence, there is a need to establish the contribution of enteric viruses associated with diarrhoeal disease in Botswana. This will add to the data on viral diarrhoeal agents in sub-Saharan Africa. This study looks into the prevalence of three viral agents of diarrhoea, namely rotavirus, astrovirus and adenovirus, in children less than five years of age in Gaborone and surrounding areas.

MATERIALS AND METHODS

Location of Study: This study was conducted in Gaborone city and in Mochudi, a town 32 km from Gaborone. These two sites were chosen as they cover two health districts in Botswana; namely the Gaborone district and the Kgateleng district respectively. The samples were collected from four laboratories: The National Health Laboratory (Gaborone); Gaborone Private Hospital Laboratory; Diagnofirm Laboratory (Gaborone) and Deborah Retif Memorial Hospital Laboratory (Mochudi).

Specimen Collection: A total of 346 stool samples were collected from March 2001 to February 2002 from children less than five years of age presenting with gastroenteritis. These samples were sent for microbiological screening at the pathology laboratories. The samples were collected in sterile specimen collection bottles and transported to the University of Botswana in iceboxes and stored in the cold-room (5-10°C). Each sample was then diluted to 10% with sterile distilled water and stored at 4°C.

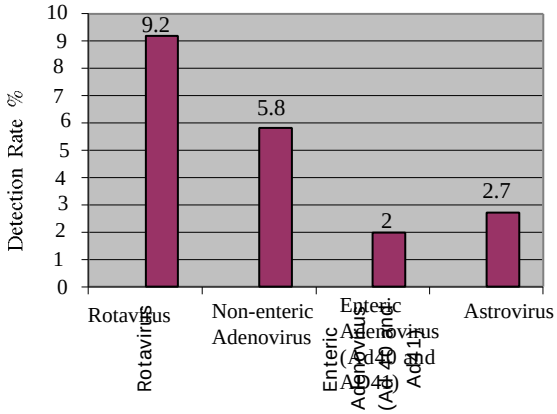
ELISA Detection of Rotavirus, Astrovirus and Adenovirus antigens: The presense of viruses in stool samples was determined using enzyme immunoassay (EIA) according to the manufacturer's protocols. Results were interpreted against controls included in each plate. Group A rotaviruses and astroviruses were detected using commercial ELISA kits (IDEA™) Rotavirus or Astrovirus, DAKO Ltd., Denmark House, Germany). Adenoviruses were screened using a commercial ELISA (Ridascreen Adenovirus, R-Biopharm, Germany). Adenovirus positive samples were subjected to further screening for the enteric adenovirus types 40 and 41 with a species specific EIA (Premier Adenoclone® Type-40/41 EIA (Meridian Biosciences).

RESULTS

All of the 346 children with GE were screened for rotaviruses and adenoviruses while only 219 of these were screened for astroviruses. The incidence of rotavirus infection was the highest at 9.2% (32/346). Non-enteric adenoviruses were detected in 5.8% (20/346) and enteric adenoviruses were detected in 2% (7/346), while astroviruses were detected in 2.7% (6/219) of the stool samples (Figure l).

Figure 1

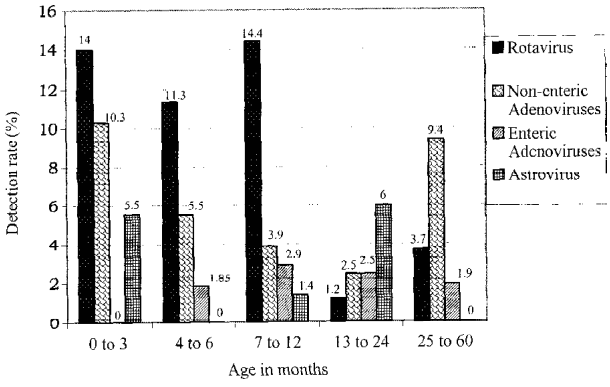
Incidence of enteric virus infection in children less than five years of age with gastroenteritis



The figure shows the detection rate in percentage of rotavirus, non-enteric adenovirus, enteric adenovirus (Ad 40 and Ad41) and astrovirus in children less than five years of age suffering from gastroenteritis.

Figure 2

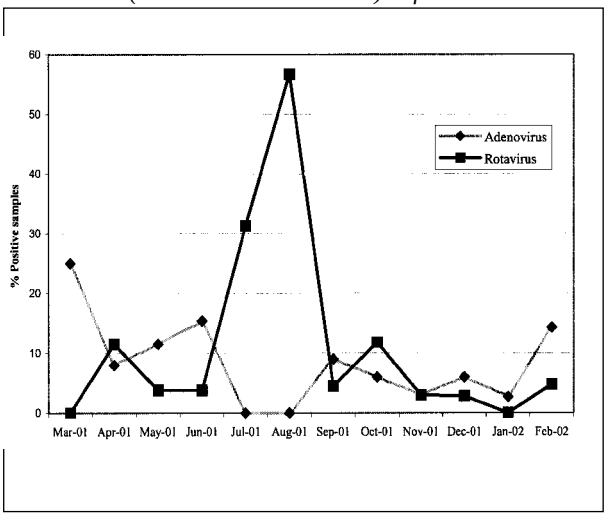
Comparative infection rates of rotavirus, adenovirus (enteric and non-enteric) and astrovirus in children less than five years of age



The figure shows the comparative infection rates of rotavirus, adenovirus (enteric and non-enteric) and astrovirus in children less than five years of age suffering from gastroenteritis.

Figure 3

Monthly distribution of rotavirus and adenovirus (enteric and non-enteric) infection



The figure shows the monthly distribution of the rotavirus and adenovirus positives that were detected over the period of 12 months from March 2001 to February 2002.

The age distribution and virus detection rate is shown in Figure 2. From this data, the mean ages of infection in children less than five years of age for rotavirus, non-enteric adenovirus, enteric adenovirus and astrovirus were determined to be 9.7, 16.8, 16 and 11.3 months respectively.

Rotavirus and adenovirus infections appear to occur all year round with a distinct peak in rotavirus infection during autumn and the cool dry winter season (June to August) (Figure 3). Adenovirus infection was slightly more during the first half of 2001.

DISCUSSION

In this study, group A rotaviruses were detected in 9.2% of the stool samples of children less than five years with GE. This figure is much lower than the published median rate of 24% (reviewed in 43 published studies of rotavirus epidemiology in Africa)(11). This 12-month survey of enteric virus infection in and around Gaborone indicates that rotaviruses were circulating within the community throughout the year, with infection peaking in the cooler and drier months of July (31.25%) and August (56.7%). These results are in accordance with the other studies conducted in Botswana by Sebunya *et al.*(9) and Kasule(10). These results also support other southern American studies from Zambia, Zimbabwe and South Africa(11). Published data indicated that the highest rotavirus prevalence rates occurred in children aged 6-18 months with severe infections occurring more frequently in younger children aged 6-12 months in developing countries(12). In addition, infection often occurs in children less than three months of age (13,14). In this study, the majority of the rotavirus infections (91%) were observed in children less than two years of age, emphasising the fact that rotavirus infection occurs early in life. It is reported that multiple rotavirus infection is common during the first two years of life.

A Venezualan study by Perez-Schael *et al.*(15) reported the generation of rotavirus-specific IgA antibodies following rotavirus infection. By the age of 11 months about 85% of the infants had acquired rotavirus IgA antibodies. Neutralising IgG antibodies also increased markedly as the children reached a year in age, reflecting exposure to natural infection. As expected, infection in this age group is generally mild, with few deaths reported worldwide. In the present study, a low prevalence to rotavirus was noted in older children between 2 to 5 years of age (3.7%). This could be explained by the acquisition of neutralising antibodies to rotavirus early in life as a result of multiple exposures to rotavirus infection.

Adenoviruses were detected in 27/346 (7.8%) of the samples. These results are comparable to other studies where the incidence of enteric adenoviruses was reported as: 6.5% in South Africa (20), 6.7% in Iran (21) and 13% in Mexico (22). These figures are high in comparison to other reports from Brazil at 1.55% (16), 3.1% in Australia (17), 3% in South Africa (18) and 3% in France (19). Most of the adenovirus positives (77.7%) were detected from children less than two years of age. Majority of the adenoviruses detected were non-enteric ones (5.8%) while only 2% of the detected adenoviruses were enteric adenoviruses (type Ad40 and Ad41). Most of the non-enteric adenoviruses were detected in children less than three months of age and in older children between 25 to 60 months. Non-enteric adenoviruses, like Adl2, Adl8 and Ad31 (sub-species A), have a tropism for the alimentary canal and have been reported to cause diarrhoea, mainly in children(23). They may also represent the strains responsible for respiratory infections. As a systemic infection, respiratory adenoviruses may also cause diarrhoea(24). It appears that infection with enteric adenoviruses (Ad40 and Ad41) was low in the study group and was not detected in children less than three months of age. Seroprevalence studies have shown that 50% of children less than four years of age have antibodies to enteric adenoviruses (16,20). This indicates that there is an initial exposure to adenovirus infection in infants and in toddlers.

The prevalence of astrovirus was 6/219 (2.7%) of the samples. This is similar to other studies showing an incidence of astrovirus infection in children with GE of 2% in India (25), 3% in Brazil(26), 4.2% in Australia(27). However, in some studies, higher rates of astrovirus infection have also been reported. These include 6.3% in France (19), 6.8% in USA (28), and 7% in South Africa (18). The majority of the astrovirus positive samples (75%) were observed in children less than two years of age and no positive cases were found in children over two years of age. In this regard, seroprevalence studies in England reported that by the age of 5 to 10 years, astrovirus antibodies are found in 75% of the children(29). This suggests that older children may have acquired antibodies against astroviruses early in life due to natural exposure to this virus.

In conclusion, comparison of the detection rates for

rotavirus, non-enteric adenovirus, enteric adenovirus and astrovirus in these children indicates that rotavirus infection is mainly observed in children under two years of age with the rate of disease declining thereafter. The relatively low prevalence rates of these enteric viruses in children as compared to studies elsewhere in the developing world could be attributed to a relatively low incidence of these viral infections within urban settings, such as Gaborone. Studies are required to confirm the incidence rates of these viral agents within rural communities.

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