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Rotavirus, astrovirus and adenovirus associated with an outbreak of gastroenteritis in a South African child care centre

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SUMMARY

An outbreak of gastroenteritis in the infant–toddler unit of a child care centre (CCC) in Pretoria, South Africa, was investigated for possible viral enteropathogens. Rotavirus was found in association with seven (70%) diarrhoeal episodes. Co-infection with rotavirus and human astrovirus (HAstV) was demonstrated in two of these episodes, and rotavirus, HAstV and enteric adenovirus (EAd) co-infection in another. Rotavirus occurred alone in four of the diarrhoeal episodes, while HAstV and EAd were each detected alone in one episode. Two HAstV and one rotavirus asymptomatic infection episodes also occurred. Overall, 8 of 10 children had rotavirus infections, of which 7 were symptomatic, 6 of 10 children had HAstV infections (4 symptomatic), and 2 of 10 children had EAd infection, both symptomatic. These results highlight the diversity of viral enteropathogens that may be associated with a diarrhoeal outbreak in a CCC and emphasize the need to investigate the possibility that multiple enteropathogens may simultaneously cause a single outbreak of diarrhoea.

INTRODUCTION

Acute diarrhoea is a major health problem throughout the world and a leading cause of death among children in developing countries [1–3]. In 30–70% of cases no pathogen is identified, although in infants and small children endemic viral agents such as enteric adenovirus (EAd), human astrovirus (HAstV) and human calicivirus (HuCV) are probably responsible for many of these episodes [1]. Housing of susceptible individuals in settings such as child care centres (CCC) and care centres for the elderly increases the risk of exposure to viral gastroenteritis pathogens [1, 4–6]. Studies in CCC have shown that children not yet toilet-trained are at greatest risk of transmitting diarrhoea and other viral diseases spread by the faecal–oral route [7]. Consequently outbreaks of infectious diarrhoea, associated with many bacterial, parasitic and viral enteropathogens, are common among infants and toddlers in CCC [4, 5, 7–13].

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Rotavirus and *Giardia lamblia* are the most frequently identified pathogens during outbreaks of gastroenteritis in CCC [5, 10, 13]. Multiple enteropathogens, either simultaneously or sequentially, have also been demonstrated [7–12] and outbreaks where rotavirus and EAd [10], HAstV and rotavirus [11], and HAstV and EAd [11] were co-pathogens have been documented. Electron microscopy (EM) provides the only 'catch-all' method for viral detection in diarrhoeal stool specimens [14] and application of this technique has improved our knowledge and understanding of viruses associated with childhood diarrhoea [15]. Specific and more sensitive assays for individual pathogens, namely enzyme immunoassays (EIA) and the reverse transcriptase–polymerase chain reaction (RT–PCR), have greatly improved detection of HAstV [11, 13, 16], EAd [10, 12] and HuCV [8, 17] infections and facilitated a greater understanding of the importance of these viruses in outbreaks of gastroenteritis in CCC. This study reports the application of EM, EIA and RT–PCR to the virological

investigation of an outbreak of diarrhoea in an infant-toddler unit in a CCC, Pretoria, South Africa (SA).

SUBJECTS AND METHODS

The outbreak began 23 April 1996, and continued to 3 May 1996. Stool specimens were collected from both healthy and symptomatic children, 7–18 months of age. Unusually loose stools, with at least twice the normal daily frequency, were considered to be diarrhoeal. For the purposes of this investigation a diarrhoeal episode was defined as the occurrence of one or more sequential diarrhoeal stools with no more than 1 day between diarrhoeal stools. The infants and toddlers were predominantly from the higher socio-economic group and while attending the CCC were not in contact with children of other age groups. Stool specimens were examined by direct EM [18] and tested by specific EIA for rotavirus (Rotaclone®, Cambridge Biotech, Worcester, MA), EAd serotypes 40 and 41 (Adenoclone® – Type 40/41 Adenovirus detection kit, Cambridge Biotech, Worcester, MA) and HAstV [16, 19]. An optimized RT-PCR assay [20] using a published set of primers [13], followed by oligonucleotide probe hybridization [20], was also used for the detection of HAstV.

RESULTS

Twelve episodes of diarrhoea were recorded in 7 of 10 (70%) children in the unit during the outbreak (Fig. 1). The duration of diarrhoea was 1–4 days and symptoms included fever, vomiting, decreased appetite and abdominal pain. None of the children required hospitalization. A total of 37 stool specimens were collected from the 10 infants, with 19/37 specimens being classified as diarrhoeal and 18/37 as asymptomatic. No protozoal or bacterial pathogens were detected, by an independent laboratory, in an acute phase stool specimen from the index case. Rotavirus was detected in 12 (63%), HAstV in 7 (37%) and EAd in 2 (11%) of the diarrhoeal specimens, while rotavirus was evident in 3 (17%) and HAstV in 4 (22%) of the non-diarrhoeal specimens. Small round viruses (SRVs) were detected by EM in two specimens, one diarrhoeal and the other non-diarrhoeal. No viral enteropathogens were detected in four of the diarrhoeal specimens. There was a strong correlation between EM and EIA for the detection of rotavirus in both diarrhoeal and non-diarrhoeal specimens. In only one diarrhoeal sample could rotavirus be demonstrated by EIA and not by EM, while in one of the non-diarrhoeal stool specimens rotavirus was detected by EM and not by EIA. Adenovirus and SRVs were detected by EM, and EAd by EIA, in one of the diarrhoeal stool specimens, while in another the occurrence of EAd was only evident by EIA. With the detection of astroviruses a strong correlation was also noted between EM and EIA, especially in the diarrhoeal stool specimens where 100% concordance was demonstrated. However in the non-diarrhoeal stool specimens HAstV was seen by EM in one of the specimens where HAstV was only detected by RT–PCR, while in another asymptomatic stool specimen SRVs were noted where HAstVs were demonstrated by both EIA and RT–PCR. RT–PCR followed by oligoprobe hybridization proved to be the most sensitive HAstV detection method.

DISCUSSION

Rotavirus, HAstV and EAd occurred, either alone or as co-pathogens, in the diarrhoeal episodes (Fig. 1). Rotavirus infection, either alone or in association with other viral enteropathogens, was demonstrated in at least one diarrhoeal episode from each of the seven symptomatic children, and alone in three of the non-diarrhoeal stool specimens. Rotavirus and HAstV co-infection was noted in three sequential diarrhoeal stool samples from the index case, child no. 2, and in a single diarrhoeal stool specimen from one of the other children, child no. 4. Multiple pathogens, namely HAstV, EAd and rotavirus, were noted in the diarrhoeal episode of child no. 1. In another child, child no. 9, rotavirus and EAd were associated with sequential episodes of diarrhoea. HAstVs, either alone or in co-infections, were therefore associated with 4 (33%) of the diarrhoeal episodes of 4 (57%) of the symptomatic children. On the second day of the outbreak, HAstV was detected in non-diarrhoeal stool specimens from 2 of the children, 1 of whom, child no. 7, subsequently developed rotavirus-associated diarrhoea. In this investigation simultaneous infections by multiple viral pathogens occurred in 3 of 5 younger children $\leqslant 14$ months of age, while sequential infections by different pathogens occurred in older children, 15–18 months of age.

In this investigation, rotavirus, HAstV and EAd were identified as co-pathogens in a diarrhoeal outbreak in the infant-toddler unit of a CCC in Pretoria, SA. Rotavirus was detected most frequently. Rotavirus has been a common viral enteropathogen

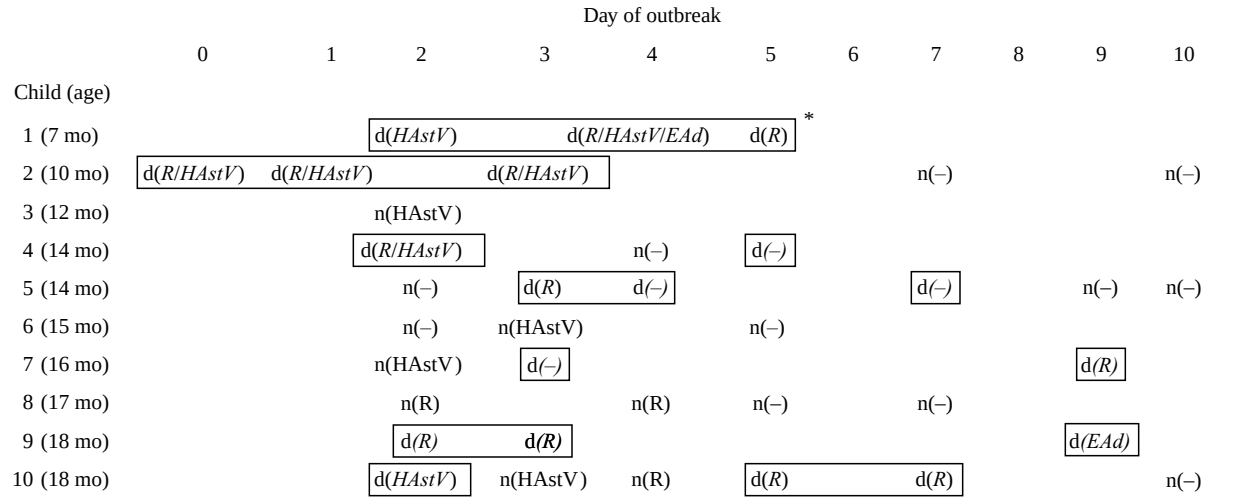

Fig. 1. Incidence and excretion of rotavirus (R), human astrovirus (HAstV) and enteric adenoviruses (EAd) in diarrhoeal (d) and non-diarrhoeal (n) stool specimens during an outbreak of gastroenteritis in an infant–toddler unit of a child care centre, Pretoria, South Africa. (–) no virus detected; * denotes diarrhoeal episodes.

associated with outbreaks of gastroenteritis in CCC [1, 4, 7]. Six (60%) children were also infected with HAstV. The occurrence of both viruses in stool specimens from the index case and the simultaneous presence of both viruses in subsequent cases suggests that rotavirus and HAstV were introduced into the CCC simultaneously. The viruses however did not distribute equally among the children. Both viral pathogens also occurred in asymptomatic contacts, consistent with findings in other studies where asymptomatic infection was shown to be common during outbreaks of viral diarrhoea in CCC [11, 21]. EAd also occurred during the outbreak, including in a diarrhoeal episode in which no other viral pathogens were detected, but was not detected in stool specimens from the index diarrhoea episode. The presence of SRVs in two stool specimens, as demonstrated by EM, may be co-incidental or possibly due to misclassification of astroviruses.

Direct EM is widely used for the detection of viruses associated with gastroenteritis [14, 22], but studies have indicated that viruses like HAstV may be misclassified [23, 24] or overlooked in the presence of larger viruses such as rotavirus and adenovirus [24]. Although EIAs have been found to be more sensitive than EM for the detection of HAstV in stool specimens [25, 26], a recent study of a HAstV-associated diarrhoea outbreak in a CCC proved RT-PCR to be even more sensitive than EIA for the detection of HAstV in stool specimens [13], a finding confirmed in this study. HAstV was detected in a total of 30% of the stool specimens by RT–PCR compared to the 16% detected by either EM and/or EIA. Using RT–PCR asymptomatic HAstV infection and rotavirus and HAstV co-infection could be detected in three stool specimens where HAstV infection would have gone undetected if only EM and/or EIA was used. The contribution of HAstV and possibly other viral enteropathogens could therefore be overlooked if sensitive and specific detection assays such as EIA and RT–PCR are not used.

The high rate of both rotavirus and HAstV infection noted in this study suggests uniform exposure of the children in the infant–toddler section of the CCC to the outbreak enteropathogens. Given the pattern of detection of the three viral enteropathogens the aetiology of the outbreak is clearly multiple. Whether bacterial and parasitic enteropathogens also occurred during the outbreak is uncertain as only the index case was assessed. This study emphasizes the need to use multiple detection methods for multiple enteropathogens, including bacteria and parasites, to investigate such outbreaks.

Further, they imply that diarrhoea outbreaks will remain common when intervention strategies specific for one pathogen, such as a rotavirus vaccine, are available. Preventative measures must therefore address reasons for common exposures to multiple pathogens.

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