

A comparison of two sets of primers for the RT-PCR detection of astroviruses in environmental samples

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Abstract

Human astroviruses (HAstV) are associated with sporadic cases and outbreaks of diarrhoea. The faecal-oral route is the predominant mode of transmission and contaminated drinking water and shellfish have been implicated as vehicles of transmission. Conventional diagnostic techniques have limited sensitivity and in this study two primer pairs, designated *Jon* and *Mon*, were compared for the detection of HAstV in environmental specimens by the reverse transcriptase-polymerase chain reaction (RT-PCR). Both primer pairs yielded positive RT-PCR products for the cell culture adapted HAstV-1 positive control. The *Jon* primers, however, also yielded positive results for other viruses as well as for a number of water samples. These data suggest that the regions amplified by the *Jon* primers are not unique to HAstV. The *Mon* primer pair yielded positive RT-PCR results only for HAstV serotypes 1 to 4 and some environmental samples. The results obtained using the *Mon* primer pair could be confirmed by either hybridisation with an oligonucleotide probe specific for HAstV or a HAstV specific enzyme immunoassay (EIA). RT-PCR, using the *Mon* primers, proved more sensitive than electron microscopy (EM), immune electron microscopy (IEM) and EIA for the direct detection of HAstV in river water. Cell culture amplification using the PLC/PRF/5 human primary liver carcinoma cell line improved the sensitivity of HAstV detection by EIA and RT-PCR, but not EM and IEM. The sensitivity of the RT-PCR assay system was enhanced by prior viral recovery by a glass wool adsorption-elution technique.

Introduction

Human astroviruses (HAstV) are associated with both sporadic episodes and outbreaks of human gastroenteritis. Although HAstV infections mainly occur in young children and the elderly, people of all age groups may be affected (Oishi et al., 1994). Eight serotypes of HAstV have been associated with human infections, with HAstV serotype 1 (HAstV-1) being the most prevalent (Jonassen et al., 1995). HAstV are typically transmitted by the faecal-oral route, and outbreaks have been associated with the consumption of contaminated water and food, particularly oysters (Kurtz and Lee, 1987). Routine diagnostic methods rely on electron microscopy (EM) (Appleton and Higgins, 1975), immune electron microscopy (IEM) (Lee and Kurtz, 1994) and enzyme immunoassays (EIA) (Herrmann et al., 1990; Moe et al., 1991). The detection limit of EM is about 10^6 viral particles/ml of test suspension, and the reverse transcriptase-polymerase chain reaction (RT-PCR) has been proven to be more sensitive than EIA for the detection of HAstV in clinical specimens (Mitchell et al., 1995). RT-PCR would therefore be more suitable for research on the low titres of HAstV which may occur in faecally polluted water environments. RT-PCR has successfully been used to detect HAstV in stool specimens (Jonassen et al., 1993; Mitchell et al., 1995), sea water (Myint et al., 1994) and environmental water samples (Marx et al., 1995).

In a previous study, using a set of nested primers reportedly highly specific for HAstV-1, we found that approximately 70% of the environmental samples tested yielded positive RT-PCR results (Marx et al., 1995). This 70% incidence of HAstV was higher than expected for environmental samples, consequently the evaluation of another set of primers was indicated to confirm or negate these findings. We report on a comparison of the

sensitivity and specificity of these two sets of primers for the detection of HAstV in environmental samples. The efficacy of a glass wool adsorption-elution viral recovery procedure and the effect of cell culture amplification on the detectability of HAstV by RT-PCR were also examined.

Experimental

Samples

Samples (500 ml) were collected from water environments in the Pretoria area over a period of four months. These samples included sludge from a sewage plant, hospital effluent collected from the HF Verwoerd hospital sewage system, water from a river that flows through a suburban area (River II) and source water for a water purification system (River I). Viruses were recovered from 20 to 40 l of river water (River I) using a glass wool adsorption-elution procedure (Grabow and Taylor, 1993).

Viruses and bacteriophages

The following laboratory strains of viruses were used to determine the specificity of the primer sets: HAstV-1 to -4 (TW Lee, Public Health Laboratory, John Radcliffe Hospital, Oxford, UK); poliovirus 1 (polio 1)(vaccine strain LSc 2ab); coxsackievirus B1 to B6 (cox B1-6), coxsackievirus A9 (cox A9), and echovirus 1 (echo 1)(National Institute of Virology, Johannesburg); cytopathogenic hepatitis A virus strain pHM-175 (HAV) (Cromeans et al., 1989); reovirus (reo); simian rotavirus SA-11 (rota SA-11)(ATCC VR-899); human rotavirus HRV-3 (rota HRV3); adenovirus type 40 (Ad40) strain Hovi-X (Grabow et al., 1992) and type 41 (Ad41) strain 23341-77 (Grabow et al., 1992); somatic coliphage VI (Grabow et al., 1984); and male specific coliphage MS2 (ATCC 15597-B). Unless otherwise specified, the origin of these viruses has been documented previously by Grabow et al. (1995).

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