Real-time PCR quantitative assessment of hepatitis A virus, rotaviruses and enteroviruses in the Tyume River located in the Eastern Cape Province, South Africa

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ABSTRACT

We applied real-time RT-PCR (reverse transcription-polymerase chain reaction) to assess the incidence of hepatitis A virus, rotaviruses and enteroviruses in the Tyume River, an important water resource in the impoverished Eastern Cape Province of South Africa. Detection of noroviruses was done using conventional semi-nested RT-PCR. Water samples were collected once monthly from 6 sampling sites over a 12-month period starting in August 2010 and ending in July 2011. Hepatitis A virus was detected in 13% of the samples in concentrations ranging between 1.67×10^3 genome copies/ℓ and 1.64×10^4 genome copies/ℓ while rotaviruses were detected in 4% of the samples with concentrations ranging from 9×10^1 genome copies/ℓ to 5.64×10^3 genome copies/ℓ. Enteroviruses were not detected in any of the samples, while noroviruses were detected in 4% of the samples. All hepatitis A and rotaviruses positive samples were from the upstream sections of Tyume River while noroviruses were detected in samples from downstream sections only. Statistical analysis showed that occurrence of the viruses in Tyume River was sporadic. Risk analysis showed that hepatitis A virus posed greater risk than rotaviruses for both recreational and domestic water uses. Because of the low infectious dose of enteric viruses, the detection of even low concentrations of hepatitis A virus, rotaviruses and noroviruses in surface water poses a significant risk to public health.

Keywords: Hepatitis A virus, rotaviruses, noroviruses, TaqMan real-time PCR, reverse transcription, Tyume River, public health

INTRODUCTION

Ground and surface water sources may be subjected to faecal contamination from a variety of sources, including sewage treatment plant effluents, on-site septic waste treatment discharges, land runoff from urban, agricultural and natural areas, and leachates from sanitary landfills (Abbaszadegan, 2001). Consequently, millions of people throughout the world do not have access to microbiologically safe water for domestic, recreational and other general purpose uses (Gibson et al., 2011). Health risks associated with surface water use, either raw or treated, include infectious diseases predominantly caused by human and animal enteric pathogens, most notably viruses. RNA viruses constitute the most abundant group of pathogens in man, animals and plants (Bustin and Mueller, 2005). Surveillance of source waters for viral pathogens is therefore necessary to protect public health. The culture-propagation procedure is still the best method to enumerate viruses and demonstrate their infectivity. However, for the detection of noroviruses (NoVs), hepatitis A virus (HAV) and other enteric viruses like enteroviruses (EVs) for which appropriate cell cultures are not available, slow or limited (Hong et al., 2011), molecular techniques become the most viable option. Molecular techniques have been successfully applied on environmental samples, allowing a rapid and specific detection of human enteric viruses (Bosch et al., 2008; De Paula et al., 2007; Costafreda et al., 2006). The ability of real-time reverse transcription polymerase chain reaction (qRT-PCR) to generate accurate quantitative data has had a huge impact on the study of viral agents of infectious disease (Schutten and Niesters, 2001).

Hepatitis A virus has a worldwide distribution and is the aetiological agent of hepatitis, an acute, usually self-limiting infection of the liver. On average, about 1.5 million cases of clinical hepatitis are recorded world-wide each year (WHO, 2003). Hepatitis A virus belongs to the genus *Hepatovirus* (Feinstone, 1996) and has a positive-sense, single-stranded RNA genome which is 7.5 kb in length (Murray et al., 2005). The virus is shed in the faeces of persons with both asymptomatic and symptomatic infection and under favourable conditions HAV may survive in the environment for months (CDC, 1999). Hepatitis A disease is rarely fatal but may represent a substantial economic burden, particularly in countries with low and intermediate incidence rates where a larger portion of the adult population do not have immunity against the disease (Grabow, 1997). Infected persons are infective from 14 to 21 days before the onset of jaundice and up to 7 to 8 days after jaundice has resolved (Tong et al., 1995). Food and water have been identified as the main vehicles for HAV transmission (Koopmans et al., 2002). Possible routes of infection with HAV include close personal contact with infected persons (Adhami and Carey, 2010), recreational exposure to faecally polluted surface water (Hunter, 1997), as well as consumption of contaminated food and water (Koff, 1995).

On the other hand rotaviruses (RVs) are responsible for the majority of acute gastroenteritis infections occurring in young children worldwide (Jothikumar et al., 2009). The genus *Rotavirus* belongs to the family Reoviridae and can be divided into 7 groups, A–G, with the majority of human infections being caused by viruses of Group A (Adlhoch et al., 2011). Rotaviruses are non-enveloped RNA viruses (Fritzinger et al.,

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