

Hepatitis A virus in surface water in South Africa: what are the risks?

J. M. E. Venter, J. van Heerden, J. C. Vivier, W. O. K. Grabow and M. B. Taylor

ABSTRACT

The aim of this study was to assess the potential risk of infection constituted by HAV to persons using surface dam and river water for domestic and recreational purposes. It estimates the potential risk using a deterministic exponential risk assessment model with mean values and conservative assumptions. Hepatitis A virus was detected in 17.5% of river and 14.9% of dam water samples tested. The number of indicator organisms in these sources exceeded drinking and recreational water quality guidelines set by the United States Environmental Protection Agency (US EPA), indicating possible health risks to recreational water users. Based on the available data and taking all the assumptions into consideration, the probability of infection (P_{inf}) to the higher socio-economic population using the river water for recreational purposes was 1.1×10^{-3} per day and 3.3×10^{-1} per annum if 100 ml was ingested per day. For recreation in the dam water the P_{inf} value was 1.2×10^{-4} per day and 4.2×10^{-2} per annum. For the lower socio-economic population, risk values for drinking purposes (2L day^{-1}) were ten-fold greater. These surface waters therefore did not conform to the US EPA guidelines of 1 infection per 10,000 consumers per year for drinking water or eight gastrointestinal illnesses per 1,000 bathers per day in environmental waters used for recreational purposes. This is the first risk assessment study addressing the risk of infection by HAV in surface water to different socio-economic populations in South Africa.

Key words | drinking water, hepatitis A virus, recreational water, risk assessment, surface water

INTRODUCTION

A wide spectrum of human enteric viruses excreted in faeces are potential water pollutants (Grabow 1996). Faecally polluted natural surface water used for recreational activity could therefore pose a potential health risk to the public (López-Pila & Szewzyk 2000; World Health Organization (WHO) 2003). Different analytical approaches, namely epidemiological studies and mathematical models based on dose-response relationships, have been applied to determine the risk of infection posed by viruses in recreational waters (Garin *et al.* 1994; Gammie &

Wyn-Jones 1997) and drinking water (Vivier *et al.* 2002; WHO 2004). Contaminated drinking water has been implicated in outbreaks of hepatitis A (Hunter 1997) and recreational exposure to faecally polluted water has unequivocally been linked to outbreaks of hepatitis A (Mahoney *et al.* 1992; Hunter 1997), with the risk of infection increasing with increased immersion in contaminated water (Taylor *et al.* 1995; Gammie & Wyn-Jones 1997). To date, a limited number of epidemiological studies have been applied to determine the risk posed by hepatitis A virus

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(HAV) infection after recreational exposure to polluted surface water sources (Phillip *et al.* 1989; Gammie & Wyn-Jones 1997). There is however a dearth of guidelines, both worldwide and in South Africa, as to what an acceptable risk of infection is for waterborne pathogens. Based on dose-response data for rotaviruses (Ward *et al.* 1986) and *Giardia* (Regli *et al.* 1991; Macler & Regli 1993) an acceptable risk of one waterborne infection per 10,000 consumers per year for drinking water was considered acceptable by the United States of America Environmental Protection Agency (US EPA) (Environmental Protection Agency (EPA) 1986; Regli *et al.* 1991; Macler 1993; Hunter *et al.* 2003). As there are very few data on the maximum acceptable limit for viruses in recreational water (Guidelines for Canadian Recreational Water Quality 1992), water quality guidelines and risk values are based on levels of indicator organisms, namely faecal coliforms, enterococci and *Escherichia coli* (EPA 1986; Guidelines for Canadian Recreational Water Quality 1992; López-Pila & Szewzyk 2000). An acceptable risk value of one illness per 1,000 swimmers has been suggested by Grabow (1996), but the acceptable risk recommended by the US EPA is eight gastrointestinal (GI) illnesses per 1,000 recreational water users for fresh water (EPA 1986). These indicator guidelines may be too lenient with regard to HAV infection as the burden of disease and economic impact of hepatitis A is substantial (Macler & Regli 1993; Centers for Disease Control & Prevention (CDC) 1999). It is therefore important to determine the risk posed by HAV to persons exposed to faecally polluted surface water.

Hepatitis A virus is endemic in South Africa with epidemiological features of both the developed and developing countries being present (Martin *et al.* 1994; Schoub *et al.* 2000). Routine vaccination for HAV is not included in the childhood immunization schedule currently recommended in South Africa (Department of Health 1995), consequently in the high density, low socio-economic communities where sanitation is inadequate, nearly 100% of children acquire immunity before the age of ten years (Martin *et al.* 1994; Taylor *et al.* 2001). However, with the current trends in urbanization, and with the provision of clean water and improved sanitation in the rural areas, a decrease in the endemic level, with a concomitant increase in the epidemic vulnerability, can be expected (Martin 1992; Taylor *et al.*

2001). This could result in an increase in the incidence of symptomatic hepatitis A in the adult population with associated economic impact (Grabow 1997). The sporadic pattern of disease is seen in the urbanized, higher and predominantly white socio-economic community where the prevalence and severity of clinical HAV infection increases with age (Martin 1992), and by 40 years of age 50–70% of this community is immune to HAV infection (Taylor *et al.* 2001).

Although there are little data on the incidence of water-related viral illnesses in South Africa (Grabow 1996), contaminated river water was identified as the possible source of HAV infection in canoeists (Taylor *et al.* 1995). Hepatitis A virus has been detected in surface river and dam (impoundment) water used for recreational and domestic purposes in South Africa (Taylor *et al.* 2001). These water sources are used by the non-immune higher socio-economic communities for recreational activities while the predominantly immune lower socio-economic population uses the same water for domestic, irrigation and recreational purposes. Data regarding the burden of HAV infection and disease in South Africa is inadequate (Schoub *et al.* 2000), consequently the contribution of treated and untreated drinking water, and recreational water to the burden of HAV infection in South Africa is unknown. In this study the possible risk of infection constituted by HAV to individuals in different socio-economic communities using the same surface water sources for domestic and recreational purposes was determined. To quantify the possible risk of infection posed by HAV, a risk assessment approach based on the following four steps was applied: (1) hazard identification, (2) dose-response analysis, (3) exposure assessment, and (4) risk characterization (Hunter *et al.* 2003).

MATERIALS AND METHODS

Hazard identification

Hepatitis A virus is a small (27 nm in diameter), icosahedral, non-enveloped, single-stranded, positive-sense RNA virus belonging to the family Picornaviridae (Hollinger & Emerson 2001). The two biotypes of HAV, namely human HAV and simian HAV, are the only members of the genus *Hepatitisvirus* (King *et al.* 2000). There is only one serotype

(Hollinger & Emerson 2001), with infection conferring lifelong immunity (Hollinger & Emerson 2001). Hepatitis A virus is predominantly spread by the faecal-oral route with person-to-person contact being the most important route of infection (Ryder 1999). Enteric viruses are excreted in high numbers (10^5 – 10^{12} per gram) in water during recreational activities (Gerba 2000). Maximal faecal excretion of HAV occurs two to three weeks prior to the onset of clinical symptoms (Zuckerman & Zuckerman 1999) and remains infectious for three to four weeks after the alanine aminotransferase (ALT) levels peak (Polish *et al.* 1999), facilitating the spread of the virus. The infectious dose of HAV is unknown, and although Grabow (1997) suggested that one virion can cause infection, the infectious dose is presumed to be of the order of 10 to 100 virions (US Food and Drug Administration/Center for Food Safety and Applied Nutrition 2004) which implies that even low levels of faecal pollution could pose a risk of infection.

High risk populations include the elderly, immunocompromised patients, intravenous drug abusers, and individuals living in close proximity together such as families, young children and the staff in day care centres, military personnel, and institutionalized individuals (Hollinger & Ticehurst 1996; Feinstone & Gust 2002). Children experience asymptomatic infections in >90% of cases (Zuckerman & Zuckerman 1999), and serve as a reservoir of infection for adults who are more likely to experience clinically apparent and more severe infection (Termorshuizen *et al.* 2000; Hollinger & Emerson 2001), with a fatality rate of 1.5% in persons over the age of 64 (Martin 1992; Hollinger & Ticehurst 1996). Recent data indicates that faecal excretion of HAV may be prolonged in HIV-infected individuals thereby serving as an additional reservoir of infection (Ida *et al.* 2002).

Food and water have been identified as important vehicles of HAV infection worldwide (Grabow 1997; Koopmans *et al.* 2002), with outbreaks linked to faecally contaminated treated and untreated drinking water (Gerba & Rose 1990) and recreational water sources (Taylor *et al.* 1995; Gammie & Wyn-Jones 1997). Hepatitis A virus has been shown to be resistant to concentrations of free residual chlorine of 0.5–1.5 mg L⁻¹ for 1 h, and exposure to 2–2.5 mg L⁻¹ for at least 15 min is recommended to inactivate any infectious HAV (Hollinger & Ticehurst 1996; Feinstone & Gust 2002). Hepatitis A virus can

withstand temperatures of 60–80°C for a minimum of 1 h (Koopmans *et al.* 2002), low relative humidity ($\pm 25\%$ for 7 days) (Mbithi *et al.* 1991) and pH values as low as pH 1 (King *et al.* 2000; Feinstone & Gust 2002). Hepatitis A virus has been shown to survive for months in experimentally contaminated fresh water, seawater, marine sediments, wastewater, soils, and oysters (Hollinger & Emerson 2001) and depending on conditions, can be stable in the environment for months (CDC 1999).

Exposure assessment

Surface water samples

Over a period of three years (June 1997–June 2000) weekly water samples, ± 190 L and ± 25 L, were collected from the same sites from a dam and river in Gauteng, South Africa, respectively. The dam water is used as source water for a water purification facility as well as by the higher socio-economic community for recreational purposes, while the river and dam water is used by the lower socio-economic communities for domestic and recreational purposes. Hepatitis A virus was detected in 27/154 (17.5%) river and 23/154 (14.9%) dam water samples by an integrated cell culture-reverse transcriptase-polymerase chain reaction (RT-PCR)-oligonucleotide probe hybridization assay as described previously (Taylor *et al.* 2001). In addition to HAV, the water samples were routinely analysed for selected indicator organisms, namely total and faecal coliforms and F-RNA coliphages.

Exposure Analysis

The exposure analysis was based on: (1) the concentration of HAV in the two sources respectively; (2) the efficiency of the recovery technique used; (3) the viability of the virus; and (4) the average volume of water consumed during recreational activities or drinking purposes per individual in the different socio-economic populations. Since the higher socio-economic population do not use the surface water for drinking purposes, only possible health risk values for recreational activities were calculated.

Average concentration (C₀). The integrated cell culture-RT-PCR-oligonucleotide probe hybridization assay used to

detect HAV in the water samples gives qualitative and not quantitative results. In order to determine the concentration of HAV in the different water sources, a random distribution of viruses within and between samples is assumed and described by a Poisson distribution. The Poisson parameter, λ , was calculated for the dam and river water sources respectively (Table 1). The mean concentration of HAV L^{-1} was calculated to be 7.94×10^{-3} in the river water and 8.53×10^{-4} for the dam water (Table 1).

Corrected HAV concentration (C). To calculate the corrected mean concentration of HAV in water, the efficiency of recovery value plays an important role. The calculation for corrected viral concentration per litre of water is:

$$C = C_0 \times (1/R) \times I$$

where: C = Corrected concentration of HAV in water samples (viruses L^{-1})

C_0 = Average concentration of HAV in water samples (viruses L^{-1})

R = Efficiency of recovery (%)

Values to calculate the corrected mean HAV concentration in the water sources investigated are summarized in Table 1 and 2.

Efficiency of the recovery. This represents the fraction of pathogenic microorganisms recovered. Viruses present were recovered using a glass wool adsorption-elution technique (Grabow & Taylor 1993) and secondarily concentrated by precipitation with polyethylene glycol 6,000 in the presence of sodium chloride (Vilaginès *et al.* 1997;

Taylor *et al.* 2001). Based on in-house efficiency of recovery studies (unpublished data), an overestimate efficiency of recovery value of 40% was used in calculations.

Viability. Recovered viruses were isolated and amplified on FRhK-4R, passages 60–100 (kindly supplied by Prof. Dr B Flehmig, Hygiene-Institut der Eberhard Karls Universität, Tübingen) and the PLC/PRF/5 (ATCC CRL 8024), passages 80–100, cell lines. Cell monolayers in 25 cm^2 flasks were infected in duplicate and harvested after 21 days prior to analysis using a RT-PCR-oligonucleotide probe hybridization assay. Amplification of the nucleic acid in cell culture is considered to be an indication of the potential viability and hence infectiousness of the virus since *in vitro* amplification requires infection of a host cell and the activation of the replication cycle (Deng *et al.* 1994; Taylor *et al.* 2001). Since naked viral nucleic acids do not adsorb to glass wool (Grabow *et al.* 2001), and will degrade rapidly in the environment (Pallin *et al.* 1997), it can be concluded that the viruses detected in this study are intact, viable and potentially infectious.

Consumption. For the purpose of this study, an assumed value of 100 ml was taken as the volume ingested per day for recreational exposure (one or more exposures per day) (Haas & Eisenberg 2001), and that an individual consumed an estimated 2 L of unboiled water per day for drinking purposes (Macler & Regli 1993; Haas & Eisenberg 2001). This assumption represents an overestimate of water consumed and will not result in an underestimated risk value.

Table 1 | Results used to calculate the mean hepatitis A virus (HAV) concentration per litre during the three year investigation period

	Calculation	River	Dam
Mean volume of water analysed	Average volume of 154 water samples	24.3 L	190 L
Mean HAV detected	Fraction of positive HAV results from 154 water samples (RT-PCR)	17.5%	14.9%
Negative	1 – (mean HAV detected)	8.25×10^{-1}	8.51×10^{-1}
Poisson parameter (λ)	$-\ln$ (negative)	1.93×10^{-1}	1.62×10^{-1}
Concentration viruses (C_0)	λ / mean volume of water	7.94×10^{-3} viruses L^{-1}	8.53×10^{-4} viruses L^{-1}

Table 2 | Summary of the model parameters used in the deterministic model to estimate probable health risk posed by hepatitis A virus (HAV)

Model parameters	River	Dam	Dimension
HAV Concentration in water (C_0)	7.94×10^{-3}	8.53×10^{-4}	Virus L^{-1}
Recovery (R)	40%	40%	
Decimal reduction by treatment (DR)	0	0	
Infectivity (I)	1	1	
Volume consumed (recreational) (V_r)	0.1	0.1	$L \text{ day}^{-1}$
Volume consumed (drinking) (V_d)	2	2	$L \text{ day}^{-1}$
Dose response parameter (r)	0.549	0.549	

The daily exposure (N) was determined using the following equation (Teunis *et al.* 1997):

$$N = C \times 1/R \times I \times 10^{-DR} \times V_c$$

where: C_0 = Average concentration of HAV in the water samples (viruses L^{-1})

R = Efficiency of recovery (%)

I = Fraction of the detected pathogens capable of infection (viability)

DR = Removal or inactivation efficiency of treatment processes

(DR = 0 since surface water is untreated)

V_c = Daily individual consumption of water ($L \text{ day}^{-1}$)

Hazard characterization

Dose response model

For this investigation, an exponential model was applied to estimate the risk constituted by HAV in water to consumers. Since this is the first mathematically-based risk analysis done to determine the probable risk of infection constituted by HAV in surface water, many uncertainties and variables were identified, consequently, this deterministic model was chosen in an effort to minimize the various uncertainties. The model uses mean values and works on the basis of an overestimate, so as to represent the worst-case scenario. The daily risk of infection with HAV was calculated as

follows:

$$P_{\text{inf/day}} = 1 - \exp(-rN)$$

where $P_{\text{inf/day}}$ = probability of becoming infected

N = number of HAV particles ingested

r = dose response parameter

The estimated annual risk of infection ($P_{\text{inf/year}}$) was calculated as follows:

$$P_{\text{inf/year}} = 1 - (1 - P_{\text{inf/day}})^{365}$$

Uncertainties

Since point estimates are used, the degree of uncertainty in the risk determination is not represented. These uncertainties include variable consumption volume, socio-economic status, population behaviour, exposure patterns, immune competency, age, etc. Sensitivity analyses demonstrate the model's response to various input parameters such as variant recovery values and consumption volumes. Virus concentrations, and therefore the risk of infection can change depending on recovery, isolation and detection techniques applied.

Dose-response parameter and probabilities

The dose-response parameter, r (0.549), used in this investigation was that reported by Haas & Eisenberg

(2001). To calculate the estimated probability of becoming clinically ill from an infection (P_{illness}) on a daily ($P_{\text{illness/day}}$) and annual basis ($P_{\text{illness/year}}$), morbidity values for the selected socio-economic groups had to be taken into consideration, e.g.:

$$P_{\text{illness/day}} = P_{\text{inf/day}} \times \text{morbidity value for socio-economic group}$$

$$P_{\text{illness/year}} = P_{\text{inf/year}} \times \text{morbidity value for socio-economic group}$$

Depending on age, the morbidity for the high socio-economic groups can be between 11% and >70% as reported for the US (CDC 1999). An arbitrary and overestimate value of 45% was therefore used in the model to cover all age groups within the heterogeneous higher socio-economic population. For the lower socio-economic population the morbidity value used was 10% as infection occurs predominantly in children where approximately 90% infections are asymptomatic (Feinstone & Gust 2002). Mortality rates for HAV vary from 0.5–1.5% (CDC 1999), depending on age, immune status and socio-economic impact, and an average of 1% was used throughout the risk model. This value was multiplied with the probability value of becoming ill from infection to calculate the risk of death (P_{mort}) from a clinical infection. The parameters used in the model are presented in Table 2.

RESULTS

Microbial indicator analysis. Somatic and F-RNA coliphages were detected, by qualitative presence/absence tests, in all dam and river water samples analysed. In the river water faecal coliform counts ranged from 130 to 66,000 colony forming units (cfu) 100 ml⁻¹, with more than 99.99% of samples exceeding 200 cfu 100 ml⁻¹. In the dam water samples faecal coliform counts ranged from 4 to 3100 cfu 100 ml⁻¹, with counts <100 cfu 100 ml⁻¹ in 85.7% (132/154) of the samples. In 7.8% (12/154) of dam water samples the faecal coliform counts were >100 cfu 100 ml⁻¹,

and in 6.5% (10/154) of samples counts exceeded 200 cfu 100 ml⁻¹.

Corrected HAV concentration. The corrected mean concentration of HAV per litre of water was 2.13×10^{-3} and 1.99×10^{-2} for the dam and river water respectively. These values could be a gross underestimate of the actual concentration of HAV in the water as the isolation and detection of HAV in cell culture is influenced by factors such as viral strain, cell type, incubation time and incubation temperature.

Sensitivity analysis. The efficiency of recovery of the glass wool adsorption-elution technique used in this study was taken as 40%. The effect of varying recovery values on the probable risk of HAV infection per day for the different socio-economic populations in the dam water is demonstrated in Figure 1. From the graph it is clear that the more efficient the recovery, the lower the P_{inf} rate as the fraction of 1/R in the calculation becomes smaller. The same applies to the P_{illness} , since P_{inf} is used to calculate P_{illness} . A similar trend was observed for the river water (results not shown).

The effect of varying dose-response parameter input values on the P_{inf} per day for river water being used by the different communities is demonstrated in Figure 2. The efficiency of recovery value used in calculations was 40% and volumes included were constant at 100 ml for recreational activity and 2 L for drinking purposes. The same trend was observed for the dam water investigated (results not shown).

Sensitivity analysis of the influence of the volume (V) of water ingested during either recreational or domestic activity on the P_{inf} of the two economic groups, for river and dam water, demonstrated that the larger the volume ingested, the higher the probability of becoming infected with HAV (results not shown).

Risk to higher socio-economic populations. The estimated daily and annual risks of HAV infection, morbidity and mortality are presented in Table 3. These results indicate that for the higher socio-economic group the estimated daily risk of infection during recreational activity in the river water is 1.1 infection per 1,000 recreational users per

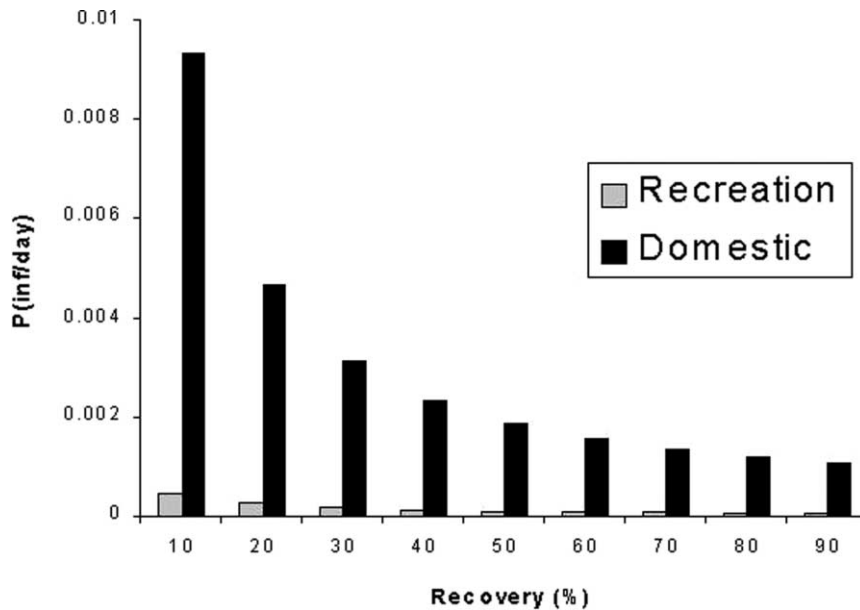


Figure 1 | Effect of viral efficiency of recovery (R-values 10-90%) on the $P_{\text{inf}} \text{ day}^{-1}$ to individuals using dam water for recreational and drinking purposes.

day. This translates to a P_{illness} of 0.41 illnesses per 1,000 recreational water users per day. For dam water recreation, the risk for infection is 0.12 per 1,000 recreational users with a P_{illness} value of 0.053 illnesses per 1,000 recreational users per day. Water-sportspersons, e.g. swimmers and canoeists, who may accidentally ingest water and who are exposed to the river water on a daily basis, will have a 14.8%

annual risk of becoming clinically ill from HAV infection should 100 ml be ingested per day. For recreational exposure to the dam water the annual risk of becoming clinically ill with HAV would be 1.9% if 100 ml of this water is ingested per day.

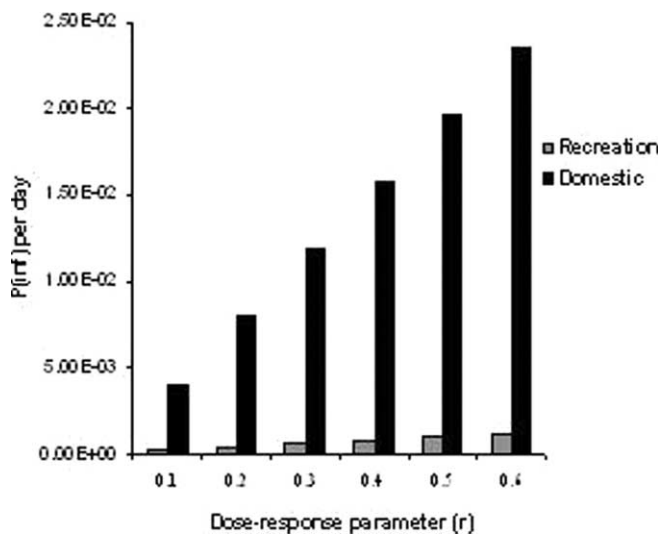


Figure 2 | Effect of varying dose-response parameter values ($r = 0.1$ to 0.6) on the daily risk of infection to communities using the river water for recreational and drinking purposes.

Risk to lower socio-economic populations. The $P_{\text{inf/day}}$ for the lower socio-economic communities, often from informal settlements, who use these untreated water sources directly from the source for drinking purposes, proved to be higher (Table 3). For the river water the daily risk of HAV infection was calculated to be 220 per 10,000 consumers, with an annual risk of 100%. Communities using dam water for drinking (2 L day^{-1}) purposes have a daily risk of infection of approximately 23 per 10,000 and an annual risk of 5,800 per 10,000 consumers.

DISCUSSION

This investigation evaluates the potential risk of infection constituted by HAV to populations exposed to selected surface water sources in South Africa. To our knowledge this is the first study applying a mathematical model to assess the

Table 3 | Calculated risk values for the higher socio-economic (HSE) and lower socio-economic (LSE) groups in South Africa during recreational and drinking use of surface water sources

Population group	River water		Dam water	
	Per day	Annual	Per day	Annual
HSE* (Recreation[†])				
P_{inf} [‡]	1.1×10^{-3}	3.3×10^{-1}	1.2×10^{-4}	4.2×10^{-2}
$P_{illness}$ ^{**}	4.1×10^{-4}	1.5×10^{-1}	5.3×10^{-5}	1.9×10^{-2}
P_{mort} ^{††}	4.1×10^{-6}	1.5×10^{-3}	5.3×10^{-7}	1.9×10^{-4}
LSE[†](Recreation)				
P_{inf}	1.1×10^{-3}	3.3×10^{-1}	1.2×10^{-4}	4.2×10^{-2}
$P_{illness}$	1.1×10^{-4}	3.3×10^{-2}	1.2×10^{-5}	4.2×10^{-3}
P_{mort}	1.1×10^{-6}	3.3×10^{-4}	1.2×10^{-7}	4.2×10^{-5}
LSE (Drinking[§])				
P_{inf}	2.2×10^{-2}	1	2.3×10^{-3}	5.8×10^{-1}
$P_{illness}$	2.2×10^{-3}	1×10^{-1}	2.3×10^{-4}	5.8×10^{-2}
P_{mort}	2.2×10^{-5}	1×10^{-3}	2.3×10^{-6}	5.8×10^{-4}

*Higher socio-economic population.

†Lower socio-economic population.

‡100 ml ingested per day.

§2 litre consumed per day.

†Probability of infection.

** Probability of illness from infection.

††Probability of mortality from infection.

risk of HAV infection in an epidemiologically heterogeneous population exposed to contaminated surface water.

Based on the US EPA guideline of an acceptable risk of one waterborne infection per 10,000 consumers per year for drinking water the use of these untreated waters for drinking purposes was unacceptable as >50% (5,800 per 10,000) of consumers per annum were at risk of infection. However, since it is persons from the lower socio-economic, predominantly immune communities living in high density informal settlements with close proximity to these water sources that utilise the water for drinking purposes, the risks are minimal for individuals older than 10 years of age. These risk values, however, will be of concern for the very young (children <10 years),

immunocompromised or non-immune individuals using these water sources for drinking purposes. The faecal coliform numbers in these waters exceeded acceptable levels for drinking water, i.e. 0 counts per 100 ml (Department of Water Affairs and Forestry (DWAF) 1996; WHO 1996) and persons using these waters for drinking purposes are at risk of infection (DWAF 1996). F-RNA coliphages, used as surrogates for enteric viruses in water environments (Wade *et al.* 2003) and indicators of faecal pollution (Grabow 1996), were also demonstrated in all of the dam and river water samples tested, implying that enteric viruses, including HAV, could have been present. The risk of infection as determined in the model was therefore corroborated by the microbial indicator data.

Applying an acceptable risk level of eight GI illnesses per 1,000 swimmers/recreational water users (EPA 1986) to HAV, would result in a minimal risk (0.053 to 0.41 illnesses per 1,000 recreational users per day) of developing clinical hepatitis A in the higher socio-economic, predominantly non-immune, population using these surface waters for recreational activities. For adolescents and adults from the lower socio-economic communities, the risk of developing hepatitis A after recreational exposure to the same water sources is well within the acceptable limits suggested by the US EPA (EPA 1986). Although the calculated annual risk of mortality appears to be high (Table 3), it must be borne in mind that the high socio-economic group, who use these waters for recreational activities, are not exposed on a daily basis. The lower socio-economic group who use the same water for drinking purposes develop immunity at a very young age, and this seemingly high annual risk of mortality would therefore only be significant for non-immune very young, elderly or immunocompromised individuals who consume 2 L of untreated water on a daily basis.

The water samples were not tested for *E. coli* or enterococci, hence the US EPA water quality criteria for freshwater bathing waters, i.e. 126 *E. coli* cfu 100 ml⁻¹ or the 33 enterococci cfu 100 ml⁻¹, which are generally applied as predictors of gastrointestinal illness (EPA 1986; Wade *et al.* 2003) could not be applied to assess the risk of HAV infection. The river water did not conform to the US EPA bacterial criteria of 200 cfu 100 ml⁻¹ faecal coliforms for bathing waters (Wade *et al.* 2003), hence on the basis of this criterion, non-immune persons using the river water for

recreational purposes were at risk of infection. If these bacterial criteria were applied to the dam water, the risk of infection during recreational activity would be minimal as only 6.5% of the dam water samples exceeded the 200 cfu 100 ml⁻¹ limit for faecal coliforms.

In the most recent WHO water quality guidelines (WHO 2003), a volume of 20–50 ml for estimating risk of infection to recreational water users was proposed. Should these volumes, instead of 100 ml, have been applied in the model, the calculated risk of infection would have been proportionately lower. As the volume of water ingested per day could differ, depending on the extent of exposure, an estimated volume of 100 ml was used to determine the risk per day for recreational water. The annual risk of HAV infection calculated for water-sportspersons exposed to these water sources on a daily basis, i.e. 330 per 1,000 recreational water users per day, is supported by sero-epidemiological data. In a country-wide investigation of a cohort of South African canoeists, predominantly from the higher socio-economic group, 37% seropositivity to HAV could be attributed to canoeing in waters with different microbiological and physical qualities from various geographical areas in South Africa, including the water sources investigated in this study (Taylor *et al.* 1995).

Previous investigators have estimated the risk of infection in recreational waters by determining the ratio between the pathogen and indicator organisms (López-Pila & Szewzyk 2000; Wade *et al.* 2003). This could not be applied in this investigation due to the uncertainties associated with the isolation and detection of HAV. Even though the model was formulated to work on overestimates, due to the number of uncertainties and confounders identified in this investigation, the calculated risk of infection could still be an underestimate of the actual risk constituted by HAV. Confounders that include practical limitations in techniques for the recovery, isolation and detection of HAV, which clearly influence the virus concentration and subsequent calculations, were identified. Even though various primary and continuous cell cultures of primate origin support the replication of HAV, wild type (WT) HAV replicates slowly or not at all in conventional cell cultures (Hollinger & Ticehurst 1996). Selected cell culture systems, namely an African green monkey kidney cell line (AGMK), and the PLC/PRF/5 and FRhK-4R cell

lines, have been shown to be most susceptible for the isolation and propagation of WT HAV as well as cell culture-adapted strains of HAV (Daemer *et al.* 1981; Flehmig 1981; Taylor *et al.* 2001). Primary vervet monkey kidney cells and the Vero African green monkey kidney cell line have been used to a lesser extent (Daemer *et al.* 1981; Taylor *et al.* 2001). Incubation periods from two weeks up to twelve months (Crance *et al.* 1987) on various cell lines, with incubation temperatures ranging between 32°C and 37°C, have been reported (Crance *et al.* 1987; Dotzauer *et al.* 1994). In this study only the PLC/PRF/5 and the FRhK-4R cell lines and a single incubation temperature of 37°C for 21 days were used prior to molecular analysis, which could have limited the number of viruses detected. In addition, it must be borne in mind that HAVs excreted later in the infection cycle are less infectious to susceptible non-human primates, e.g. marmosets, than those excreted in the first few weeks before the onset of clinical symptoms (Polish *et al.* 1999), which could further affect the virus infectivity and detection. Another factor that could influence the viral concentration is the turbidity of the water investigated. The efficiency of recovery of the glass wool adsorption-elution technique for viruses has been found to be more efficient for less turbid waters (Vilaginès *et al.* 1997). In this study the turbidity of the water was not taken into account, thus the efficiency of recovery of 40% used in the calculations could also have been an overestimate of the actual recovery of HAV from the water sources. Sensitivity analyses indicated that variations in input parameters such as efficiency of recovery (R), consumption volumes (V) and different dose-response (r) input values have a significant effect on the model's output. Analysis performed with varying efficiency of recovery values demonstrated that more effective recovery techniques could result in the calculation of more accurate risk values for the different socio-economic communities (Figure 1). In this investigation a deterministic approach regarding efficiency of recovery (R = 40%), dose-response parameter (r = 0.549) and volumes ingested was followed where all individuals were assumed to be immunocompetent. As the HIV epidemic escalates and the immune status of individuals decreases, it is possible that a lower infectious dose of HAV will result in infection. This will result in higher dose-response values. This increase in the dose-response parameter will result in an increase in

the P_{inf} in both communities (Figure 2). More precise data regarding HAV sero-prevalence rates, the concentration of HAV in the water sources, the infectious dose and the efficiency of recovery is required for accurate and sensitive risk assessment. Future research should include multiple inputs for each of these parameters to adequately reflect the risk of HAV infection to the different communities exposed to these water sources during recreational and domestic activity.

A number of viruses, e.g. adenovirus, hepatitis E virus, HAV (and other picornaviruses), have been proposed as possible candidates for risk assessment models for pathogenic agents in drinking and recreational waters (Havelaar et al. 2001). As HAV represents a major health threat (Macler & Regli 1993), and has a clearly defined clinical picture with complete recovery, it is an attractive candidate for risk assessment studies. From this investigation it is evident that this may only be an option for countries where HAV is non-endemic and the burden of disease is clearly defined, e.g. the US (CDC 1999). In countries where HAV is endemic and the burden of disease is not clearly defined, all the confounders must be taken into consideration to facilitate accurate risk analysis. Therefore no risk assessment model is universally applicable (WHO 2003), and models need to be modified or adapted to take the micro-organism being assessed, and local or national demographics and socio-cultural behaviour into consideration. This study therefore only reflects the possible risk of infection constituted by HAV in two localised surface water sources in South Africa and the data cannot be applied universally.

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