

Assessment of the risk of infection associated with Coxsackie B viruses in drinking water

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Abstract The risk of infection constituted by enteroviruses detected in drinking water supplies analysed in this study were assessed. Coxsackie B viruses (CBV) were used as a model in these assessments. A high proportion of Coxsackie B virus infections are asymptomatic. However, clinical manifestations may range from mild, undifferentiated febrile illness or upper respiratory tract infection to a severe, systemic and sometimes fatal disease of sensitive populations. Dose-response studies suggested that an exponential model best describes infectivity of CBV. The analysis of 172 samples of treated drinking water supplies described in this study revealed the presence of CBVs in 11% (water treatment unit A) and 16% (water treatment unit B) of the samples. This incidence of CBV was used as a basis for risk assessment. The results indicated that the drinking water supplies concerned constitute a risk of CBV infection of 3.91×10^{-3} (unit A) and 7.4×10^{-3} (unit B) per year. The estimated risk of infection are about an order of magnitude higher than the yearly acceptable risk of one infection per 10,000 consumers proposed for drinking water supplies.

Keywords Coxsackie B viruses; drinking water; risk assessment

Introduction

The potential for human disease, contracted from exposure to waterborne microorganisms is a major public health concern in countries at all levels of development. Drinking water has been recognised as a potential vector for the transmission of communicable diseases (Grabow, 1996; Payment *et al.*, 1997). Although concentrations of pathogenic microorganisms may be quite low, drinking water reaches every member of a population, so that even a low risk of infection may affect a significant number of consumers.

The detection, quantification and characterisation of pathogens in drinking water is essential for quantitative risk assessment and is now considered useful, if not yet essential, for monitoring the quality and microbiological safety of source and treated waters (Haas *et al.*, 1999). These data are essential for the assessment of public health risks. The United States Environmental Protection Agency (US EPA) has recommended the following acceptable risk for drinking water: Not more than one infection per 10 000 consumers per year (Macler and Regli, 1993).

Coxsackie B virus infection may result in serious or even fatal disease such as acute myocarditis and aseptic meningitis (Muir and van Loon, 1997). Neonates are at particular risk of infection as well as the elderly and immunocompromised. Hospitalisation rates of 50–364 infants per 100,000 live births per year with a mortality of 3.9 per 100 000 live births per year have been reported (Kaplan, 1988). There is evidence that enteroviruses contribute to common chronic diseases, including insulin-dependent diabetes mellitus (Roivainen *et al.*, 1998) and dilated cardiomyopathy (Kandolf *et al.*, 1999).

Several studies have documented the presence of enteroviruses in raw and treated water (Pallin *et al.*, 1997; Reynolds *et al.*, 1997; Abbaszadegan *et al.*, 1999; Grabow *et al.*, 2001). The objective of this study was to assess the risks of infection constituted by CBVs, detected in the drinking water supplies analysed in this study. The quality of the raw surface water source, treatment by coagulation, sedimentation, filtration and disinfection by chlorine,

complied with international specifications for drinking water supplies (WHO, 1996, 1997). The drinking water supplies were monitored for a one year period for the presence of enteroviruses (Vivier *et al.*, 2001). Enteroviruses (predominantly Coxsackie B viruses) were detected in 11% (water treatment unit A) and 16% (water treatment unit B) of the drinking water samples by an integrated cell culture/n-PCR approach (Vivier *et al.*, 2001).

The risk assessment approach followed in this study involves four basic steps: (1) hazard identification; (2) dose-response assessment; (3) exposure assessment; and (4) risk characterisation (Haas *et al.*, 1999). These steps were used to assess the risk of contracting a Coxsackie B virus infection from the consumption of drinking water.

Hazard identification

Coxsackie B viruses belong to the enterovirus group and are members of the family Picornaviridae (Melnick, 1996). There are six serotypes of these small nonenveloped, single-stranded RNA viruses (CBV1–6) (Melnick, 1996). In temperate climates, infections are predominantly spread during the late summer–early fall, especially in young children (Cromwell and Landau, 1997). Coxsackie viruses are ubiquitous agents and can spread rapidly within the community causing epidemics (Pallansch, 1997). Although a high proportion of these virus infections are subclinical, presentation may range from mild, undifferentiated febrile illness or upper respiratory tract infection to a severe, systemic and sometimes fatal disease of neonates (Baboonian *et al.*, 1997). Reports have associated CBV infection with various diseases and syndromes. These associations are based on studies of outbreaks of enteroviral infections (Pallansch, 1997). Some are based on one or several clinical case descriptions associated with large numbers of people with similar symptoms who showed evidence of infection with the same serotype of CBV (Pallansch, 1997). CBV infection has been ascribed in these studies as the cause of aseptic meningitis, encephalitis, pleurodynia, myocarditis, and pericarditis (Pallansch, 1997). The most common outcome of CBV infection is asymptomatic infection, an undifferentiated febrile illness or mild upper respiratory symptoms (Pallansch, 1997).

Coxsackie viruses are transmitted by the faecal-oral route, thus exposure to contaminated food, water or fomites can result in acquisition of an infection (Melnick, 1996). Coxsackie viruses can be shed in the faeces as long as three months after acquiring an infection (Hirschman and Hammer, 1974). Numerous common source outbreaks of the Coxsackie virus have been documented including two documented waterborne outbreaks (Hawley *et al.*, 1973; Dennis *et al.*, 1974). Coxsackie viruses have been found in raw sewage, recreational waters and drinking water (Lucena *et al.*, 1985; Payment *et al.*, 1985; Krikelis *et al.*, 1986; Dahling *et al.*, 1989; Vivier *et al.*, 2001). Coxsackie viruses are reported to be more resistant to chloramines and ultraviolet light disinfection than polio viruses (Payment *et al.*, 1985; Battigelli *et al.*, 1993) and are very persistent in the environment (Lo *et al.*, 1976). Lo *et al.* (1976) found that the Coxsackie virus type B5 was more stable than echo virus type 6 and polio virus type 1 at any temperature.

Exposure assessment

Drinking water supplies

Eleven (unit A) and sixteen (unit B) percent of drinking water samples ($n = 172$), monitored over a one-year period, were positive for Coxsackie B viruses (Vivier *et al.*, 2001). The supplies were derived from acceptable quality surface water sources using treatment processes which conform to international specifications for the production of safe drinking water (WHO, 1996, 1997; SABS, 2001). The water treatment units supply water to approximately 10 million consumers in South Africa (Grabow *et al.*, 2001).

Exposure analysis

The exposure analysis was based on: (1) concentration of CBV in the treated drinking water; (2) efficiency of the recovery technique; (3) the viability of the viruses; and (4) the average volume of treated water consumed per individual. The daily exposure (N) was determined using the following equation (Teunis *et al.*, 1997; Haas *et al.*, 1999):

$$N = C \times \frac{1}{R} \times I \times 10^{-DR} \times V_c \quad (1)$$

where:

- C = concentration of pathogenic microorganisms (CBV) in treated drinking water
 R = efficiency of the recovery method
 I = fraction of the detected pathogens that is capable of infection (viability)
 DR = removal or inactivation efficiency of the treatment process ($DR = 0$ when concentrations in drinking water are used)
 V_c = daily individual consumption of unboiled drinking water

Concentration

The polymerase chain reaction gave qualitative, not quantitative, values. In order to determine the concentration of Coxsackie viruses in treated drinking water quantitatively, we assumed a random distribution of organisms within and between samples, which is described by a Poisson distribution. The Poisson parameter λ was determined for water treatment units A and B (Table 1).

Due to the uncertainty associated with sampling, a random number of possible samples was simulated by means of bootstrapping (@RISK, version4, Pallisade Corporation, 2000). This simulates another possible test result in any volume of the original sample, using the concentrations estimated in Table 1. The concentration data for units A and B have been fitted with a normal and log logistic distribution, using the Chi-square test. The mean concentration of Coxsackie B viruses was $4.67 \times 10^{-4} \text{ L}^{-1}$ for unit A and $8.90 \times 10^{-4} \text{ L}^{-1}$ for unit B (Table 2).

Efficiency of recovery

The efficiency of a recovery method is the fraction of the pathogenic microorganisms detected by the method used. On the basis of published data (Vilaginès *et al.*, 1997) and unpublished findings in our laboratory, the recovery of the glass wool adsorption–elution method used in this study was conservatively estimated as 40%.

Table 1 Determination of the Poisson parameter λ and concentration for water from treatment units A and B

	Equation	Unit A	Unit B
Poisson parameter (λ)	$\lambda = -1 \ln[P(0)]$	0.0953	1.422
Mean volume (V)	$C = \lambda / V$	204.3523	162.5723
Concentration (C)		0.0005	0.0009

Table 2 Summary of Monte Carlo trials. Probability distribution of bootstrap values for enterovirus concentrations in water treatment units A and B

Statistic	Enterovirus concentration (Unit A)	Enterovirus concentration (Unit B)
Mean	4.67×10^{-4}	8.9×10^{-4}
Median	4.67×10^{-4}	8.86×10^{-4}
Standard deviation	1.63×10^{-4}	6.398×10^{-5}
Lower 95% confidence limit	2.00×10^{-4}	7.937×10^{-4}
Upper 95% confidence limit	1.00×10^{-3}	9.993×10^{-4}

Viability

Test samples were inoculated onto cell cultures prior to RT-PCR screening to amplify viral nucleic acid. This amplification of viral nucleic acid gives an indication of the viability and infectivity of viruses because in vivo amplification of nucleic acid requires infection of host cells and activation of the replication cycle (Egger *et al.*, 1995; Reynolds *et al.*, 1997; Grabow *et al.*, 2001; Seidel *et al.*, 2000). The viruses detected in this study were considered viable and infectious.

Consumption

As a default number, 2 L per person per day was used in this study to estimate drinking water exposure. This value (2 L/person) was used in studies conducted in the USA (Haas *et al.*, 1993; Macler and Regli, 1993). A survey conducted in the greater Cape Town area (consumers analysed by sex, age, population group, income and season) found differences in water consumption between population groups (Bourne *et al.*, 1987). The average water consumption for the one population group was 2.19 L per day and for the other population group 1.26 L per day. The assumption of 2 L per day represents the higher consumption rate of the population groups and will therefore not underestimate risks (Genthe and Rodda, 1999).

Hazard characterisation

The exponential risk assessment model was used (Haas, 1983) to estimate the daily risk of infection related to the daily ingested dose of Coxsackie B viruses:

$$P_i/\text{day} = 1 - \exp(-rN) \quad (2)$$

where:

- P_i = probability of becoming infected
- N = number of organisms
- R = dose response parameter

The naïve estimate for the annual risk was simply estimated as:

$$P_\chi = 1 - (1 - P_i/\text{day})^\chi \quad (3)$$

where:

- P_χ = probability (risk) of one or more infections over period c
- χ = number of days of exposure
- P_i/day = daily risk

Estimated risk of infection, morbidity and mortality

The dose response parameter (7.75×10^{-3}) used in this study was estimated based on dose response experiments with Coxsackie virus (type B4 and A21 strains pooled) (Suptel, 1963; Crabtree *et al.*, 1995; Haas *et al.*, 1999). The probability of becoming ill from that infection as well as the probability of mortality were determined. The probability of clinical illness was calculated by multiplying P_i (the probability of infection) by the morbidity rate of 0.75 reported for Coxsackie virus (Cherry, 1981). The probability of death from an infection was calculated by multiplying P_i morbidity rate (0.75) by case/fatality rate. The mortality rate used in this risk assessment was 0.0059 (Gerba and Haas, 1988). Point estimates for the daily risk and yearly risk of infection as well as the risks for morbidity and mortality for both treatment units are presented in Table 3. The yearly risk estimates from

treatment units A and B were 6.59×10^{-3} and 1.25×10^{-2} respectively. This is considerably higher than the mean yearly risk of 10^{-4} recommended by the EPA for treated drinking water (Macler and Regli, 1993).

Uncertainty analysis

Using the distributions of various parameters in Table 4, the variation in risk of infection was estimated by sampling from the distributions for each of the factors (concentration and drinking water consumption).

The Latin hypercube sampling method was employed in a simulation run of 1,000 model iterations. Summary statistics on 1,000 iterations of the Monte Carlo model are shown in Table 5. The risk of infection from units A and B followed a lognorm2 and gamma distribution, respectively. The mean individual daily risk for treatment units A and B was 1.02×10^{-5} and 1.95×10^{-5} , respectively (Table 5).

The naïve estimate for the annual risk for units A and B was 6.59×10^{-3} and 1.25×10^{-2} , respectively. This assumes that for a given individual, the daily risk is constant throughout the year. A more realistic simulation is to estimate 365 independent values. The yearly risk was calculated as a product of all estimated daily risks over a one year period. The yearly risk of CBV infection from unit A was 3.91×10^{-3} and from unit B was 7.4×10^{-3} .

Discussion

The risk of infection associated with exposure to Coxsackie B viruses in two drinking water supplies has been outlined in this study. The estimated risks of infection of 3.91×10^{-3} (unit A) and 7.4×10^{-3} (unit B) (determined with the probabilistic model) were an order of magnitude higher than the acceptable risk of one infection per 10 000 consumers per year as proposed by the US EPA (Macler and Regli, 1993). Risks of infection constituted by enteroviruses, which exceed this acceptable risk, have also been reported for other drinking water supplies. Crabtree *et al.* (1995) calculated that Coxsackie viruses in drinking water supplies in the USA and Canada constituted a risk of infection of 2.55×10^{-4} (CBV concentration of 5×10^{-5} PFU.L⁻¹) and 7.30×10^{-1} (CBV concentration of 0.31 PFU.L⁻¹) per year. Previously reported data on the concentrations of Coxsackie viruses in drinking water

Table 3 Model parameters used in the deterministic model to estimate risk associated with water consumption from treatment units A and B

Model parameters	Mean value (A)	Mean value (B)	Dimension
Concentration in drinking water (C)	4.67×10^{-4}	8.90×10^{-4}	Viruses/L
Recovery (R)	4.00×10^{-1}	4.00×10^{-1}	
Infectivity (I)	1	1	
Volume consumed (V _c)	2	2	L/day
Dose response parameter (CBV4)	7.75×10^{-3}	7.75×10^{-3}	

Table 4 Model parameters used in the probabilistic model to estimate risk associated with water consumption from treatment units A and B

Model parameters	Median value		Probability distribution	
	Unit A	Unit B	Unit A	Unit B
Concentration in drinking water (C)	4.67×10^{-4}	8.86×10^{-4}	Bootstrap	Bootstrap
Recovery (R)	0.4	0.4	Fixed	Fixed
Infectivity (I)	1	1	Fixed	Fixed
Volume consumed (V _c)	1.13	1.13	Lognorm2 (0, 0.5)	Lognorm2 (0, 0.5)
Dose response parameter (CBV4)	7.75×10^{-3}	7.75×10^{-3}	Fixed	Fixed

Table 5 Summary of Monte Carlo Trials. Daily and yearly risks of Coxsackie B virus infection (probabilistic model)

Statistic	Treatment unit A		Treatment unit B	
	Individual daily risk	Individual yearly risk	Individual daily risk	Individual yearly risk
Mean	1.02×10^{-5}	3.7×10^{-3}	1.95×10^{-5}	7.01×10^{-3}
Median	8.56×10^{-6}	3.12×10^{-3}	1.75×10^{-5}	6.37×10^{-3}
Standard deviation	6.68×10^{-6}	2.42×10^{-3}	9.96×10^{-6}	3.61×10^{-3}
Lower 95% confidence limit	2.28×10^{-5}	8.3×10^{-3}	3.86×10^{-6}	13.99×10^{-3}
Upper 95% confidence limit	2.975×10^{-6}	1.08×10^{-3}	7.26×10^{-6}	2.65×10^{-3}

(Hejkal *et al.*, 1982; Payment *et al.*, 1985) were used in their assessment. The results of risk assessment carried out on a drinking water supply in South Africa indicated that the risk of enterovirus infection was as high as 4×10^{-1} (one infection per 40 consumers) per year (Genthe and Rodda, 1999).

Both deterministic and probabilistic models were used for the estimation of the risk of infection in this study. The purpose of the probabilistic model was to quantify the uncertainty associated with the risk of infection by Coxsackie B viruses. Variability in recovery efficiency, viability and dose-response was not accounted for, because of lack of data, but can be included to refine the risk assessment model, if the data become available. Lack of information on the dose-response parameters is a major shortcoming. A deterministic dose response parameter, which does not reflect variability has been used.

Calculations carried out in this study indicated that the risk of infection constituted by the CBVs in these drinking water supplies exceeds acceptable risks. These findings warrant further investigation. This would include further details on the variables which affect the accuracy of risk assessment. More accurate data on the risk of infection constituted by the viruses in the drinking water supplies concerned would facilitate decisions about upgrading the treatment and disinfection processes to obtain levels of viruses within acceptable risks of infection.

Sensitivity analysis indicated that variation in the input parameters (recovery, drinking water consumption and dose-response) have a significant influence on the model output value. Dose-response data for CBV-infection used were based on experiments carried out on healthy, normal individuals (Gerba *et al.*, 1996). However, a substantial component of consumer populations may be more susceptible to infection as well as at risk to develop clinical illness (Gerba *et al.*, 1996). These highly susceptible individuals would include the very young, the elderly, malnourished individuals, pregnant women and immunocompromised people notably organ transplant patients, cancer patients and AIDS patients (Gerba *et al.*, 1996). In many parts of the world this highly susceptible component of populations would appear to increase (Gerba *et al.*, 1996). This would include South Africa, where an exceptionally high incidence of HIV infection in many communities (Editorial, 1999) may increase dose-response figures. An increase in the dose-response parameter would increase the risk of infection constituted by CBVs in drinking water supplies.

In view of available information, the figures used for the above variables tended to be rather conservative, which implies that the actual risk of infection constituted by the water supplies may actually be lower than indicated. Therefore more detailed experiments need to be conducted on recovery efficiency of the glass wool adsorption–elution procedure and drinking water consumption in South Africa to determine these parameters more accurately.

Risk assessment can be expanded to give an indication of economic impact, which is determined by integrated measures of health, such as Disability Adjusted Life Years

(DALY) (Havelaar *et al.*, 1999). The loss in healthy life years in a population is measured in DALY's and weighed with a factor between 0 and 1 for the severity of the disability. This assessment requires more data and is outside the scope of this investigation.

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