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 $3.91 \times 10^{-3}$ (unit A) and $7.4 \times 10^{-3}$ (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):

\[
N = C \times \frac{1}{R} \times I \times 10^{-DR} \times V_c
\]

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 \( \lambda \) 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, version 4, 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 (Vilaginës 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>
<thead>
<tr>
<th>Table 1</th>
<th>Determination of the Poisson parameter ( \lambda ) and concentration for water from treatment units A and B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equation</td>
<td>Unit A</td>
</tr>
<tr>
<td>Poisson parameter ( (\lambda) )</td>
<td>[ \lambda = -\ln[P(0)] ]</td>
</tr>
<tr>
<td>Mean volume ( (V) )</td>
<td></td>
</tr>
<tr>
<td>Concentration ( (C) )</td>
<td>[ C = \frac{\lambda}{V} ]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Summary of Monte Carlo trials. Probability distribution of bootstrap values for enterovirus concentrations in water treatment units A and B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Statistic</td>
<td>Enterovirus concentration (Unit A)</td>
</tr>
<tr>
<td>Mean</td>
<td>( 4.67 \times 10^{-4} )</td>
</tr>
<tr>
<td>Median</td>
<td>( 4.67 \times 10^{-4} )</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>( 1.63 \times 10^{-4} )</td>
</tr>
<tr>
<td>Lower 95% confidence limit</td>
<td>( 2.00 \times 10^{-4} )</td>
</tr>
<tr>
<td>Upper 95% confidence limit</td>
<td>( 1.00 \times 10^{-3} )</td>
</tr>
</tbody>
</table>
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:

\[ P_i/\text{day} = 1 - \exp (-rN) \]  

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:

\[ P_x = 1 - (1 - P_i/\text{day})^\chi \]  

where:
- \( P_x \) = probability (risk) of one or more infections over period \( \chi \)
- \( \chi \) = number of days of exposure
- \( P_i/\text{day} \) = daily risk

Estimated risk of infection, morbidity and mortality
The dose response parameter (7.75 \( \times 10^{-3} \)) 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 $6.59 \times 10^{-3}$ and $1.25 \times 10^{-2}$ 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 $1.02 \times 10^{-5}$ and $1.95 \times 10^{-5}$, respectively (Table 5).

The naïve estimate for the annual risk for units A and B was $6.59 \times 10^{-3}$ and $1.25 \times 10^{-2}$, 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 $3.91 \times 10^{-3}$ and from unit B was $7.4 \times 10^{-3}$.

**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 $3.91 \times 10^{-3}$ (unit A) and $7.4 \times 10^{-3}$ (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 $2.55 \times 10^{-4}$ (CBV concentration of $5 \times 10^{-5}$ PFU.L$^{-1}$) and $7.30 \times 10^{-1}$ (CBV concentration of $0.31$ PFU.L$^{-1}$) per year. Previously reported data on the concentrations of Coxsackie viruses in drinking water

<table>
<thead>
<tr>
<th>Model parameters</th>
<th>Mean value (A)</th>
<th>Mean value (B)</th>
<th>Dimension</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration in drinking water (C)</td>
<td>$4.67 \times 10^{-4}$</td>
<td>$8.90 \times 10^{-4}$</td>
<td>Viruses/L</td>
</tr>
<tr>
<td>Recovery (R)</td>
<td>$4.00 \times 10^{-1}$</td>
<td>$4.00 \times 10^{-1}$</td>
<td></td>
</tr>
<tr>
<td>Infectivity (I)</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Volume consumed ($V_c$)</td>
<td>2</td>
<td>2</td>
<td>L/day</td>
</tr>
<tr>
<td>Dose response parameter (CBV4)</td>
<td>$7.75 \times 10^{-3}$</td>
<td>$7.75 \times 10^{-3}$</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Model parameters</th>
<th>Median value</th>
<th>Probability distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration in drinking water (C)</td>
<td>$4.67 \times 10^{-4}$</td>
<td>Bootstrap</td>
</tr>
<tr>
<td>Recovery (R)</td>
<td>0.4</td>
<td>Fixed</td>
</tr>
<tr>
<td>Infectivity (I)</td>
<td>1</td>
<td>Fixed</td>
</tr>
<tr>
<td>Volume consumed ($V_c$)</td>
<td>1.13</td>
<td>Lognorm2 (0, 0.5)</td>
</tr>
<tr>
<td>Dose response parameter (CBV4)</td>
<td>$7.75 \times 10^{-3}$</td>
<td>Fixed</td>
</tr>
</tbody>
</table>
(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 $4 \times 10^{-1}$ (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.

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

<table>
<thead>
<tr>
<th>Statistic</th>
<th>Treatment unit A</th>
<th>Treatment unit B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Individual daily risk</td>
<td>Individual yearly risk</td>
</tr>
<tr>
<td>Mean</td>
<td>$1.02 \times 10^{-5}$</td>
<td>$3.7 \times 10^{-3}$</td>
</tr>
<tr>
<td>Median</td>
<td>$8.56 \times 10^{-6}$</td>
<td>$3.12 \times 10^{-3}$</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>$6.68 \times 10^{-6}$</td>
<td>$2.42 \times 10^{-3}$</td>
</tr>
<tr>
<td>Lower 95% confidence limit</td>
<td>$2.28 \times 10^{-5}$</td>
<td>$8.3 \times 10^{-3}$</td>
</tr>
<tr>
<td>Upper 95% confidence limit</td>
<td>$2.975 \times 10^{-6}$</td>
<td>$1.08 \times 10^{-3}$</td>
</tr>
</tbody>
</table>
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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References


