**EXECUTIVE SUMMARY OF RESEARCH ON**

# ASSESSMENT OF THE RISK OF INFECTION ASSOCIATED WITH VIRUSES IN SOUTH AFRICAN DRINKING WATER SUPPLIES

Report to the Water Research Commission

by

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# 1. INTRODUCTION

## 1.1. Background

The project was motivated by the impact of waterborne diseases worldwide. Details on waterborne diseases are described in many literature sources. This includes evidence that South Africa is no exception to the rule in this regard. The global impact of waterborne diseases is reflected by data such as those recently released by the World Health Organization (WHO) according to which every year there are 1,7 million deaths related to unsafe water, sanitation and hygiene, mainly through infectious diarrhoea. The vast majority of these deaths are among children under five years of age. An estimated 4 billion cases of diarrhoea annually account for over 82 million Disability Adjusted Life Years (DALYs), representing 5,7 % of the global burden of disease and placing diarrhoeal diseases as the third highest cause of morbidity and sixth highest cause of mortality (Prüss and Havelaar, 2001). In addition, waterborne disease is a major threat to millions who are displaced or otherwise affected by conflicts and disasters. Although the developing world is hardest hit by waterborne diseases, developed countries are also affected by these diseases. For instance, the largest outbreak of a waterborne disease on record occurred in 1993 in Milwaukee, a highly developed modern city in the USA (Grabow, 1996).

In South Africa, diarrhoeal diseases rank as the fifth most important cause of mortality in the entire population, after HIV/AIDS, Homicide/Violence, Tuberculosis and Road traffic accidents (Editorial, 2003a). In females, diarrhoeal diseases actually rank second only after HIV/AIDS as cause of death. Obviously, diarrhoeal diseases are not only associated with polluted water, but also with other sources of infection such as food. The mortality rate associated with diarrhoeal diseases is generally lower than that of certain other infectious diseases. The mortality data do, therefore, not reflect the large number of infected individuals who suffer from clinical manifestations that range from mild discomfort to severe illness, with far-reaching socio-economic implications (Pegram *et al*, 1998).

## 1.2. Waterborne diseases

Drinking water supplies are a major source of waterborne diseases all over the world. A wide spectrum of pathogens, including bacteria, protozoan parasites and viruses, is involved. Historically the role of bacterial pathogens is well known and well established. Bacteria typically associated with waterborne disease include *Vibrio cholerae* and *Salmonella typhi*, that cause cholera and typhoid fever, respectively. These diseases are clinically well defined and the diagnosis of patients is relatively easy. The transmission of disease by drinking water was confirmed for the first time in 1876 by John Snow. He associated cholera infections with drinking water derived from a hand pump in Broad Street, London. This pioneering discovery was made possible largely by the easy diagnosis of infected individuals. For many years to come, waterborne diseases were almost exclusively associated with bacterial pathogens.

## 1.3. Waterborne viruses

More recently epidemiological data began to reveal that pathogens other than bacteria, notably viruses, may also be transmitted by water. The typical example was hepatitis A virus (HAV). Bacterial pathogens transmitted by water are relatively easy to recover from water using methods such as membrane filtration, and to detect by cultivation on selective growth media. The

recovery of viruses from water, and the detection of these viruses, provides a totally different challenge and research approach. Recovery of viruses by filtration is generally not a practical option because the pore size of membranes suitable for the recovery of viruses from water is so small that they tend to clog rapidly. The detection of viruses recovered from water constitutes another major challenge. Strictly speaking, viruses are not living organisms, because they cannot replicate themselves. Viruses multiply by infecting (penetrating) a specific host cell. Once inside the host cell, the virus releases its nucleic acid that contains genetic information which instructs the host cell to produce new viruses identical to itself. Certain viruses typically transmitted by water are known as cytopathogenic viruses. This implies that in the process of replication the host cells become damaged. In an infected human being this damage to cells is the primary cause of clinical disease.

## 1.4. Detection of viruses in water

### 1.4.1. Early studies

Research on viruses in water started in the 1940s. One of the pioneers in the field was Joseph L Melnick in the USA. He started his work on the detection of polioviruses in the East River where it flowed through New York City. He used vervet monkeys to detect the polioviruses. After ingestion the viruses infect the epithelial cells of the gastrointestinal tract of the monkey. These cells release replicated viruses into the blood stream of the monkey and via this route they reach central nervous cells in the brain and spinal cord of the monkey. These cells are highly susceptible to infection by polioviruses. Replication of polioviruses in these cells causes a typical cytopathogenic effect (CPE) which results in readily detectable paralysis of the monkey. In addition, the damaged cells are clearly visible by microscopic analysis of brain and spinal cord autopsy specimens of the monkey. Monkeys inoculated with test samples were observed daily over a few weeks for signs of paralysis. Those which displayed paralysis, were sacrificed for microscopic analysis of brain and spinal cord specimens.

Melnick expressed the detection of polioviruses in river water by this method in "monkey infectious doses" of polioviruses. Despite this very tedious, time-consuming and labour intensive procedure in which large numbers of vervet monkeys were used, Melnick made some fundamentally import observations on the behaviour of viruses in water. One of these was that faecal bacteria, such as *Escherichia coli*, generally used to indicate the potential presence of waterborne pathogens, are poor indicators for the presence of viruses in water. This is because the incidence and behaviour of viruses in water differs for a number of reasons from that of faecal bacteria.

### 1.4.2. Cell culture propagation

The next major step forward in the development of techniques for the detection of viruses was the establishment of procedures for the cultivation of mammalian cells in test tubes. This implied that cell cultures could be infected with viruses and the CPE caused by virus replication was readily detectable by microscopic analysis of the cells. During the 1950s the cell culture detection of viruses became established as a routine procedure for the virological analysis of water. Cell cultures retained the role of fundamentally important tools in research on viruses to this day, and will probably carry on playing that role for a long time to come.

Despite attractive features, cell cultures have important shortcomings for the detection of waterborne viruses. This is related to the exceptional host specificity of viruses typically transmitted by water. This group of viruses is collectively referred to as enteric viruses because they primarily infect certain cells of the gastrointestinal tract. A small selection of enteric viruses, notably polio-, some coxsackie-, some echo-, some entero- and some adenoviruses, as well as reoviruses, readily infect cells in culture and cause a distinctive CPE in these cells. Monkey kidney cells are exceptionally susceptible to most of these viruses, and are commonly used for the detection of the viruses. A number of other cell cultures of animal and human origin, each with their own advantages and disadvantages, are also used for this purpose.

Unfortunately, however, the great majority of the wide spectrum of enteric viruses fail to infect cell cultures with the production of a detectable CPE. The reasons are not altogether clear. The most important reasons are probably related to host specificity of the viruses, and relevant features of the cells under the artificial conditions in culture. In other words, it has not yet been possible to culture the specific host cells susceptible to many of the viruses concerned, or in culture the host cells have lost their susceptibility to the viruses concerned. The latter may be related to failure to produce the virus receptor sites in culture. Failure to produce cytopathogenic viruses may also be related to failure in producing enzymes or ingredients required for the assembly of complete viral particles which leads to a CPE. This possibility is actually supported by sound evidence. Certain enteric viruses succeed in infecting cells in culture, and they make some progress towards the production of cytopathogenic viruses, but they fail to complete the production process. Typically some viruses proceed as far as the replication of their nucleic acid, but they fail to produce cytopathogenic viruses. Infection of cell cultures by these viruses can actually be determined by the detection of viral nucleic acid in the cell cultures which fail to display a cytopathogenic effect. Many enteric viruses display this feature in cell cultures. However, there are some enteric viruses which seem to even fail to infect presently available cell cultures, or replicate their nucleic acid in these cells. This includes the large group of noroviruses which play a major role in waterborne gastroenteritis. The notorious Norwalk virus is a member of this group. Since noroviruses are so difficult to detect, much of the information on these viruses was derived from research in which human volunteers were used to detect the virus.

### 1.4.3. Molecular techniques

The next landmark breakthrough in progress on the development of methods for the detection of viruses unfolded in the1960s when techniques for the detection of viruses by molecular techniques were established. These techniques are based on the detection of the nucleic acid of viruses by a diversity of procedures including gene probe hybridisation, the polymerase chain reaction (PCR) and reverse transcriptase-PCR (RT-PCR). These techniques have most valuable benefits. For instance, they can be used to detect any virus for which the nucleotide sequence of the nucleic acid is known. In addition, the techniques are highly specific and sensitive. Generally, they yield results in a shorter period of time than the isolation of viruses by cell culture propagation. Unfortunately, they also have important shortcomings. Among these are the failure to distinguish between viable and non-viable viruses. Also, it is difficult to obtain quantitative data on the numbers of viruses detected by molecular techniques. The tests are relatively complicated and require well trained staff and appropriate laboratory facilities to be carried out. False positive results due to contamination of test specimens, and laboratory environments contaminated with amplicons of viral nucleic acid, are major risks. Research on the improvement and modification of molecular techniques is a high priority in many laboratories worldwide, and many of the shortcoming may be resolved in the not too distant future.

The question of viability of viruses detected by molecular techniques is to some extent resolved by inoculating cell cultures with test samples in which viruses are to be detected. The inoculated cell cultures are incubated for a few days to allow the viruses to infect the cells and replicate their nucleic acid. The replicated nucleic acid is then detected by molecular techniques. There is reason to believe that viruses detected by this procedure are viable because they were able to infect cell cultures and replicate their nucleic acid. However, the ability to infect cell cultures does not confirm the ability to cause clinical disease in an infected human being.

Although presently available techniques for the detection of viruses still have many shortcomings, they made it possible to obtain most valuable information on the incidence and behaviour of viruses in water, and on the health risk constituted by viruses in drinking water supplies.

### 1.5. Resistance of viruses to drinking water treatment and disinfection processes

A common feature of viruses and the waterborne cysts and oocysts of protozoan parasites is that they are more resistant than bacteria to unfavourable conditions such as those prevailing in water environments, and water treatment and disinfection processes. This is reflected by data on the epidemiology of waterborne diseases in the United States since the 1940s. The data show a meaningful increase in the prevalence of waterborne diseases caused by protozoan parasites and viruses, and a corresponding decrease in waterborne diseases caused by bacterial pathogens. This is ascribed to a selection for the more resistant pathogens by treatment and disinfection processes for drinking water supplies. The conclusion is supported by data on the detection of waterborne pathogens in drinking water supplies (Grabow, 1996; Grabow et al, 2001b).

### 1.6. Numbers of viruses in drinking water

Data on the incidence and behaviour of viruses in waste waters, water resources, and treated drinking water supplies, have been described in the literature. Likewise there is extensive information on the epidemiology of waterborne viral diseases. Important features of available information includes the following: there is little if any quantitative information on viruses in drinking water. This is because the numbers of viruses in treated drinking water are relatively low, and the quantitative enumeration of these low numbers of viruses by means of currently available techniques is virtually impossible. Furthermore, detection of the low numbers of viruses in treated drinking water requires recovery of the viruses from large volumes of water. These recovery techniques have shortcomings of their own. Their efficiency of recovery (EOR) ranges from an estimated average of 10 % to perhaps 80 % under optimal conditions. Obviously it is impossible to calculate with meaningful accuracy numbers of viruses in the water under investigation from results for viruses in test samples without meaningful data on the quantitative efficiency of the technique used to recover the viruses from the water.

Consequently most quantitative data on viruses in drinking water supplies are restricted to the presence or absence of viruses in a particular volume of water. These results are subject to a spectrum of variables including the efficiency of the recovery technique used, the sensitivity of the virus detection method used, the spectrum of viruses detected by the method used, clumping of virus particles, turbidity of the water under investigation, and the extent of injury to viruses. The latter is rather important because there is sound evidence that unfavourable conditions in water environments, notably ultraviolet irradiation, and unfavourable conditions during water

treatment and disinfection that includes exposure to strong oxidising agents such as chlorine, injures many viruses to the extent that they fail to infect cell cultures, or complete their replication cycle to produce a CPE. This is confirmed by findings that a high percentage of normally cytopathogenic viruses in water environments fail to produce a CPE in cell cultures, but their nucleic acid is detectable in the cell cultures (Grabow, 1996). Without exception all the variables referred to imply an underestimate of the number of viruses present. In combination the cumulative effect of all the variables mentioned is likely to result in a major underestimate of the true number of viruses in water under investigation. No attempt has yet been made to determine how much this underestimate may be. It is unlikely that a meaningful answer to this question may be available shortly, because at the moment it is just not clear how this can be determined.

### 1.7. Health risk constituted by viruses in drinking water

Despite shortcomings, the application of available methods generated valuable information on the incidence and behaviour of viruses in drinking water supplies. It is now evident that viruses are detectable in many drinking water supplies by means of molecular techniques. The health risk constituted by these viruses in drinking water supplies remains to be established. Some observations indicate that these viruses constitute a meaningful health risk. A comprehensive epidemiological study carried out in Canada revealed that an estimated 35 % of gastroenteritis cases in communities investigated may be associated with drinking water supplies which had been treated and disinfected by internationally accepted procedures (Payment et al, 1997). Although the causes of gastroenteritis were not diagnosed, the epidemiology and clinical symptoms strongly suggest that the majority of infections were caused by viruses. A similar study in the USA failed to confirm the Canadian observations (Editorial, 2003b). Unfortunately epidemiological data of this kind are extremely limited. Reasons include the cost and labour required to carry out statistically meaningful investigations. Also, the definition of "gastroenteritis" or "diarrhoea" is difficult because in many infected individuals clinical symptoms of disease are hardly detectable. Another complicating factor is that many, if not most, people do not only drink water at home but also at work, school and many other places.

### 1.8. Waterborne protozoan parasites

The history of waterborne protozoan parasites, notably *Cryptosporidium* and *Giardia* species, is even shorter than that of viruses. The waterborne transmission of these pathogens was discovered only in the 1970s. Their importance as waterborne pathogens escalated dramatically and rapidly. Compared to viruses, the cysts and oocysts of protozoan parasites are detected in drinking water by relatively simple and inexpensive techniques because the spectrum is basically restricted to *Cryptosporidium* and *Giardia* oocysts and cysts. These are large particles readily visible under a light microscope, and recoverable from drinking water by a variety of filtration processes using filters with relatively large pore sizes. As with viruses, determination of the viability and infectivity of cysts and oocysts is a challenge.

### 1.9. Quality guidelines

Quality guidelines and standards for viruses in drinking water have been recommended ever since Joseph Melnick's first findings on polioviruses in water sources during the 1940s. Most of these specify the absence of viruses from a certain volume of drinking water. More naive guidelines simply just recommend the absence of viruses from drinking water. Very few of the guidelines specify a certain number of viruses in a volume of water. This is because of the shortcomings in

methods for the quantitative enumeration of viruses in drinking water mentioned earlier. One exception to the rule is SABS-241:2001 (SABS, 2001), which refers to numbers of viruses. In the case of cytopathogenic viruses the numbers may possibly be determined by quantitative plaque assays. However, there is no indication of how numbers of non-cytopathogenic viruses are supposed to be determined.

### 1.10. Risk assessment

The first attempts at calculating the risk of infection constituted by viruses in drinking water have been carried out (Haas, 1983; Gerba and Haas, 1988; Crabtree et al, 1995; Gale, 1996; Gerba et al, 1996; Genthe and Rodda, 1999; Haas et al, 1999; Regli et al, 1999; Benford, 2001; Fewtrell and Bartram, 2001; Vivier et al, 2002). In view of a wide spectrum of variables these must be seen as early attempts subject to many shortcomings. The variables include the quantitative numbers of viruses in the drinking water, the infectivity of the viruses detected, the susceptibility of the consumers to the viruses concerned, and the volume of water consumed by the users. Although the results obtained may have many shortcomings, they give a valuable indication at least of potential health risks. This has important benefits for purposes such as the formulation of water quality guidelines, strategies for water quality control, and burden of disease calculations (Fewtrell and Bartram, 2001).

### 1.11. Burden of disease

Risk assessment data have been used to calculate the burden of disease associated with waterborne pathogens. The burden of disease has been expressed quantitatively in terms of DALYs, and the data thus obtained proved most valuable to assess the impact of waterborne diseases and to evaluate public health priorities (Prüss and Havelaar, 2001; Havelaar and Melse, 2003). This project had no mandate to carry out burden of disease assessments for the viruses detected in drinking water supplies, but this should certainly be a high priority for follow-up work.

### 1.12. Rationale of project

The objective of this project was to obtain for the first time a comprehensive indication of the virological quality of drinking water supplies in South Africa. These data were then used to assess the risk of viral infection associated with the drinking water supplies using statistical models not previously used in the country. Water supplies were selected to represent supplies in developed communities, developing communities, rural communities and informal settlements. The results were used to recommend revision of current guidelines and specifications for viruses in drinking water supplies.

## 2. AIMS

The aims of the project were to:

2.1. Gather information on the incidence of a comprehensive spectrum of enteric viruses in selected representative drinking water supplies in South Africa.

2.2. Assess the risk of viral infection associated with drinking water supplies in typical developed, developing and rural communities.

2.3. Establish new data essential for guidelines on drinking water quality and monitoring programmes, and water treatment and disinfection processes.

2.4. Develop new techniques for the detection of small numbers of viruses in large volumes of drinking water, and investigate the value of indicators for assessment of the virological quality of drinking water.

2.5. Transfer technology to historically disadvantaged educational institutions, and train manpower for future research on waterborne viruses, and for monitoring the virological safety of drinking water supplies in all user communities

## 3. METHODOLOGY

### 3.1. Recovery of viruses

Attempts were made to improve the efficiency of recovery (EOR) of the glass wool adsorption-elution procedure by modification of the pH level and volume of the elution buffer used. Facilitating the release of viruses adsorbed to glass wool by disintegrating the packed glass wool from filters and sonicating the glass wool in elution buffer, was investigated.
Improvement of the EOR by secondary concentration using polyethylene-glycol (PEG) precipitation and ultrafiltration has been investigated.
The EOR of positively charged membranes used by some laboratories in the UK for the adsorption-elution recovery of viruses from water, has been investigated. A vaccine strain of poliovirus was used as model in these recovery experiments, which is in line with standard procedures followed world-wide.

### 3.2. Detection of viruses

Cell culture amplification and molecular techniques for the detection of selected viruses have been developed and optimised. This includes the selection and evaluation of appropriate cell cultures and RT-PCR primer pairs. Viruses concerned included entero, adeno, astro, rota, noro, and hepatitis A and E viruses.

### 3.3. Typing of viruses

The following techniques for the typing of viruses have been established and optimised: Molecular techniques based on PCR and gene probe hybridisation, nucleotide sequence analysis, and restriction enzyme analysis.

### 3.4. Virological analysis of drinking water

A spectrum of drinking water supplies representing different types and quality of water supplies used for drinking purposes has been selected. This included conventional supplies derived from a variety of raw water sources and treatment by different utilities according to international specifications for the treatment and disinfection of drinking water. Drinking water directly reclaimed from wastewater by a comprehensive train of treatment and disinfection processes has also been included. The spectrum included untreated water sources used for drinking purposes by developing communities. Untreated water sources were selected in consultation with co-workers at historically disadvantaged education and research facilities. The water sources were

selected to represent typical examples of untreated water used for drinking purposes. The co-workers collected samples from the sources at regular intervals for shipment to the Pretoria laboratory for virological analysis. The co-workers kept part of the samples for analysis in their own laboratories, predominantly for indicator organisms. The water supplies selected for the study were designated:
Treated drinking water:
Gauteng (samples supplied by a collaborating utility in Gauteng)
Northwest (samples supplied by a collaborating utility in Northwest)
Free State (samples supplied by a collaborating utility in Free State)
Reclaim (samples of reclaimed drinking water supplied by a collaborating utility)
Untreated drinking water:
Bloemfontein River (samples supplied by collaborators at the Free State Technikon)
Bloemfontein Borehole (samples supplied by collaborators at the Free State Technikon)
Fort Hare River (samples supplied by collaborators at the University of Fort Hare)
Fort Hare Borehole (samples supplied by collaborators at the University of Fort Hare)
Umgeni Spring (samples supplied by collaborators at Umgeni Water)
Umgeni Borehole (samples supplied by collaborators at Umgeni Water)
Venda River (samples supplied by collaborators at the University of Venda)
Venda Borehole (samples supplied by collaborators at the University of Venda)
Samples were collected during the period 2001-01-02 to 2002-10-31.

### 3.5. Selection of statistical risk assessment model

A five-day Workshop was organised for 8 to12 May 2000 to evaluate risk assessment models and to select a model most appropriate for the purposes of this project. Experts in the field were invited to participate in the process. These included Dr AH Havelaar, Rijksinstituut voor Volksgezondheid, The Netherlands, who was the chairman of the Task Force invited by the WHO to evaluate risk assessment models for the same purpose. Other experts included Dr PJ Becker (Head, Department of Biostatistics, Medical Research Council), Prof ACM de Wet (Head, Department of Statistics, University of Potchefstroom), Prof P Rheeder (Head, Department of Clinical Epidemiology, Faculty of Health Sciences, University of Pretoria) and Dr JE van Wyk (Biostatistician, Rand Water). All postgraduate students and members of staff involved in the project took part. After the objectives of risk assessment for this particular project had been clearly defined, potentially suitable risk assessment models were debated and evaluated, and eventually the model of choice was selected.

### 3.6. Risk assessment

The suitability of selected viruses as models for risk assessment has been investigated.
The model of choice, and the results of virological analyses, were used to assess the risk of infection for viruses in the above drinking water supplies.

### 3.7. Assessment of indicator organisms

Drinking water supplies analysed for viruses were also tested for a selection of indicator organisms. Results obtained were compared to those for viruses in order to assess the value of the indicators to indicate the presence of viruses.

### 3.8. Assessment of quality guidelines and specifications

Assessment of quality guidelines and specifications was based on the following two considerations:

(a) The extent to which quality guidelines and specifications ensured expectations with regard to the absence of viruses from drinking water supplies;
(b) The extent to which risk assessment indicated an acceptable risk of viral infection for drinking water supplies which were treated and disinfected by approved processes and met quality guidelines and specifications for indicator organisms.

## 4. RESULTS

### 4.1. Recovery of viruses

Experiments described in detail by Jongman et al (2001) and Jongman (2002) confirmed that the optimum pH for the glycine buffer used to elute polioviruses from the glass wool filters was 9,5. Higher or lower levels failed to improve the EOR. The volume of buffer generally used in the laboratory to elute enteric viruses from standard size glass wool columns is 200 ml. Evidence has been presented that this volume released most, but not all, of test polioviruses from the column. Small numbers of additional poliovirus were recovered by eluting with an additional volume of 100 ml buffer. Likewise, the sonication of glass wool released additional polioviruses from the glass wool.

The EOR of secondary concentration using polyethylene-glycol (PEG) or ultrafiltration were both close to 100 %. However, the ultrafiltration membranes are extremely expensive.

The EOR of commercial positively-charged membranes for the recovery of poliovirus from 100 litre-volumes of tap water was lower than that of the glass wool filters. Elution of viruses from the filters displayed features similar to the elution of viruses from glass wool filters with regard to the volume of buffer required for maximum elution of viruses. In addition, the positively-charged membranes were much more expensive than the glass wool filters.

Further details on the recovery of viruses from water were described by: entero (Grabow et al, 2001; Jongman et al, 2001; Jongman, 2002; Pavlov et al, 2002); astro (Nadan et al, 2001, 2002; Naus, 2002); hepatitis A virus (Taylor et al, 2000, 2001; Venter et al, 2001; 2002a; Naus, 2002); adeno (Van Heerden et al, 2002a,b; 2003); rota (Van der Linde et al, 2001; Van der Linde, 2002; Van Zyl et al, 2003); hepatitis E (Williams, 2004).

### 4.2. Detection of viruses

Important progress included the following:
New techniques for the detection of hepatitis A (Taylor et al, 2001; Venter et al, 2001, 2002a; Naus, 2002) and E (Williams, 2004) viruses, and adeno (Van Heerden et al, 2002a,b; 2003), rota (Van der Linde et al, 2001; Van der Linde, 2002; Williams et al, 2002; Van Zyl et al, 2003), entero (Vivier et al, 2000, 2001, 2002a; Ehlers et al, 2003b; Pavlov et al, 2003) and astro (Taylor et al, 2000; Nadan et al, 2001; 2002; Nadan, 2002) viruses.

### 4.3. Typing of viruses

Typing procedures were based predominantly on molecular techniques using the polymerase chain reaction or gene probe hybridization, nucleotide sequence analysis, and restriction enzyme analysis.

Particularly valuable progress has been made with typing procedures for entero (Vivier, 2001; Vivier et al, 2000, 2001, Pavlov et al, 2003; Pavlov, 2004), hepatitis A (Venter, 2004), adeno (Van Heerden, 2004), astro (Nadan, 2002; Nadan et al, 2003) and rota (Van Zyl et al, 2003) viruses.

### 4.4. Virological analysis of drinking water

Results for the virological analysis of selected drinking water supplies are summarised in Tables 1 to 3. Tests for a spectrum of indicator organisms were carried out at collaborating laboratories, predominantly by students.

### 4.5. Selection of statistical risk assessment model

At the workshop held on 8 to 12 May 2000 it was agreed to use the risk assessment model recommended by the World Health Organization (WHO) for assessment of the risk of infection constituted by viruses in drinking water. Dr PJ Becker of the Medical Research Council took part in this workshop. Details of the model have been described by Haas et al (1999), Fewtrell and Bartram (2001), Haas and Eisenberg (2001), and Vivier et al (2002a).

### 4.6. Risk assessment

An explanation of the risk assessment model, and its application in assessment of the risk of infection constituted by viruses detected in drinking water supplies under investigation in this Project, are presented in the Discussion section of this report.

### 4.7. Assessment of indicator organisms

Assessment of the value of indicator organisms for indicating the presence of viruses in drinking water supplies was based on the results of conventional tests for indicator organisms on water supplies in which viruses were detected. Table 1 shows that viruses were detected in a substantial number of drinking water supplies which yielded negative results for indicator tests, including highly sensitive qualitative presence-absence tests for somatic and F-RNA coliphages on 500 ml samples. In the case of untreated drinking water supplies indicator organisms generally indicated unacceptable quality for water in which viruses were detected.

Valuable progress has been made in assessment of the application of F-RNA coliphages for distinction between the human and animal origin of faecal pollution in water (Schaper et al, 2002; Sundram et al, 2003).

### 4.8. Assessment of quality guidelines and specifications

The results in Table 1 show that entero, adeno, rota and hepatitis A viruses were detected in drinking water supplies which were treated and disinfected according to international recommendations, and which met international guidelines for indicator organisms in treated drinking water. The results of risk assessment (see Discussion section for details) indicated that coxsackie B viruses detected in these drinking water supplies exceeded recommended levels for acceptable risk of infection.

**Table 1. Enteric viruses and related indicators in treated drinking water supplies**

| Determinand | | Water Supply | | | |
|---|---|---|---|---|---|
| | | Gauteng | Northwest | Free State | Reclaim |
| n | | 402 | 23 | 29 | 32 |
| Plate count / 1 ml | (1) | < 100 | 225 | 37 | 215 |
| Total coliform / 100 ml | (1) | 0 | 0 | 0 | 0 |
| Faecal coliform / 100 ml | (1) | 0 | 0 | 0 | 0 |
| Enterococci / 100 ml | (1) | 0 | 0 | ND | 0 |
| Somatic phage / 500 ml | (2) | 0 | 0 | 0 | 0 |
| F-RNA phage / 500 ml | (2) | 0 | 0 | 0 | 0 |
| Enterovirus | (3) | 47 | 2 | 8 | 10 |
| Adenovirus | (3) | 69 | 4 | 11 | 7 |
| Astrovirus | (3) | 0 | 0 | 0 | 0 |
| Rotavirus | (3) | 0 | 0 | 2 | 0 |
| Hepatitis A virus | (3) | 0 | 0 | 1 | 0 |
| Total viruses | (3) | 116 | 6 | 22 | 17 |
| % samples with viruses | | 29 | 26 | 76 | 53 |

n = number of samples analysed
(1) = Average count
(2) = present in presence-absence test on 500 ml sample
(3) = present in volumes of test samples:
Gauteng = 200 L
Northwest = 50 L
Free State = 200 L
Reclaim = 10 L
ND = Not Done

**Table 2. Enteric viruses and related indicators in untreated drinking water supplies**

| Determinand | | Water Supply | | | |
|---|---|---|---|---|---|
| | | B River | B Borehole | FH River | FH Borehole |
| n | | 12 | 12 | 12 | 12 |
| Plate count / 1 ml | (1) | 750-3000<br>1800 | 50-1750<br>620 | 20-2900<br>735 | 10-340<br>150 |
| Total coliform / 100 ml | (1) | 4500-34000<br>15740 | 0-21000<br>5660 | 0-1000<br>360 | 0-122<br>19 |
| Faecal coliform / 100 ml | (1) | 80-5100<br>2250 | 0-1050<br>340 | 0-245<br>74 | 0-10<br>1 |
| Enterococci / 100 ml | (1) | 30-900<br>280 | 0-200<br>50 | 0-142<br>60 | 0-64<br>5 |
| Somatic phage / 500 ml | (2) | 10 | 6 | 10 | 3 |
| F-RNA phage / 500 ml | (2) | 9 | 7 | 7 | 1 |
| Enterovirus / 10 L | (3) | 2 | 2 | 4 | 3 |
| Adenovirus / 10 L | (3) | 1 | 1 | 0 | 1 |
| Astrovirus / 10 L | (3) | 3 | 1 | 0 | 1 |
| Rotavirus / 10 L | (3) | 0 | 2 | 0 | 2 |
| Hepatitis A virus | (3) | 1 | 0 | 1 | 0 |
| Total viruses / 10 L | (3) | 7 | 6 | 5 | 7 |
| % samples with viruses | | 58 | 50 | 42 | 58 |

n = number of samples analysed
(1) = range of counts followed by average
(2) = present in presence-absence test on 500 ml sample
(3) = present in analysis of 10 L sample

**Table 3. Enteric viruses and related indicators in untreated drinking water supplies**

| Determinand | | Water Supply | | | |
|---|---|---|---|---|---|
| | | U Spring | U Borehole | V River | V Borehole |
| n | | 12 | 12 | 12 | 12 |
| Plate count / 1 ml | (1) | 38-6350<br>2040 | 2-160<br>43 | 124-5550<br>1440 | 90-2635<br>690 |
| Total coliform / 100 ml | (1) | 0-140<br>64 | 0-28<br>3 | 63-5500<br>1520 | 0-41<br>19 |
| Faecal coliform / 100 ml | (1) | 0-30<br>11 | 0-1<br>0 | 0-175<br>61 | 0-5<br>1 |
| Enterococci / 100 ml | (1) | 0-140<br>14 | 0-10<br>1 | 80-360<br>274 | 0-13<br>3 |
| Somatic phage / 500 ml | (2) | 3 | 1 | 10 | 10 |
| F-RNA phage / 500 ml | (2) | 2 | 1 | 9 | 9 |
| Enterovirus / 10 L | (3) | 4 | 4 | 2 | 2 |
| Adenovirus / 10 L | (3) | 2 | 3 | 6 | 3 |
| Astrovirus / 10 L | (3) | 0 | 1 | 0 | 0 |
| Rotavirus / 10 L | (3) | 0 | 0 | 0 | 1 |
| Hepatitis A virus / 10 L | (3) | 0 | 1 | 1 | 0 |
| Total viruses / 10 L | (3) | 6 | 9 | 9 | 6 |
| % samples with viruses | | 50 | 75 | 75 | 50 |

n = number of samples analysed
(1) = range of counts followed by average
(2) = present in presence-absence test on 500 ml sample
(3) = present in analysis of 10 L sample

## 5. DISCUSSION

### 5.1. Recovery of viruses

Evidence has been presented that the glass wool adsorption-elution procedure is currently the most practical, economical and efficient method for the recovery of polioviruses from volumes of drinking water of 100 litres or more. The optimum volume of buffer required for the release of adsorbed viruses from the glass wool has been established. Although larger volumes of buffer marginally increased the number of viruses released, this benefit had to be weighed against the disadvantage of the larger volume of eluate in subsequent processing. Taking into consideration all the pros and cons, it was decided that elution with 200 ml buffer for standard size glass wool filters would best serve the objectives of this investigation and routine monitoring of drinking water supplies.

Positively-charged membranes proved less successful than glass wool with regard to the efficiency of recovery, the volume of water that can be filtered, and cost. This finding is in line with experience of others (Villaginès et al, 1997; Wyn-Jones, 2003).

Secondary concentration using polyethylene-glycol (PEG) precipitation or ultrafiltration both yielded an efficiency of recovery of close to 100 %. PEG precipitation was selected as the method of choice because of practical considerations and cost.

After careful consideration of all available results, information in the literature, and personal communication with experts in the field, it was concluded that glass wool adsorption-elution was the method of choice for the recovery of viruses from drinking water, and PEG precipitation the method of choice for the recovery of viruses from glass wool eluates. It should be noted that the great majority of experimental data on the recovery of viruses is based on vaccine strains of polioviruses used as model virus for the purpose of the investigations. On the basis of basic features of viruses, it is assumed the same applies to other enteric viruses. However, there may be exceptions, which remains to be investigated.

Results obtained in this study indicate that the efficiency of recovery (EOR) of the above procedure for the recovery of poliovirus from treated drinking water, as well as low turbidity untreated water, is about 50 % (Grabow et al, 2001; Vivier et al, 2002b). In the case of turbid untreated water, the EOR was estimated at 40 %. Vilaginès et al (1993, 1997) reported higher EOR values for the glass wool adsorption elution recovery of polioviruses from treated and untreated drinking water supplies, but in this study it was not possible to confirm these values. The EOR levels accepted in this project compare favourably with those reported for most other laboratories (Grabow, 1996), and proved sufficient for the purposes of the project.

Important for this Project was that available data on EOR were predominantly for polioviruses which are closely related to coxsackie B viruses (CBVs). This implies that assumptions for EOR very much apply to CBVs which have been selected as model viruses for risk assessment in this project.

### 5.2. Detection of viruses

Research on the improvement of existing techniques, and the development of new techniques, for the detection of enteric viruses in water is an ongoing activity of the Department. In summary,

substantial progress has been made with detection techniques for a number of enteric viruses, notably hepatitis A and E, adeno, rota, astro and entero viruses.

Despite potential for further improvement, particularly with regard to noroviruses, the techniques used for the detection of enteric viruses in this project represented a spectrum of viruses which was matched by perhaps one or two laboratories in the rest of the world. No other laboratory was known to apply this spectrum of viruses in the routine monitoring of drinking water supplies, or research on the quality of drinking water. The data on the virological quality of drinking water on record in this project are, therefore, unique in so far as that no comparable data are available for any drinking water supplies elsewhere in the world.

The data on the virological quality of the drinking water supplies under investigation in this project were considered adequate for meaningful assessment of the virological quality of drinking water supplies, and for meaningful assessment of the risk of infection constituted by the viruses in these drinking water supplies.

In view of the above discussion it should, however, be kept in mind that the data on enteric viruses in drinking water supplies reported in this project probably represent an underestimate. This is predominantly due to variables such as the restricted efficiency of recovery of enteric viruses from the drinking water, and failure of many viruses to infect the cell cultures used and replicate their nucleic acid in these cells. In addition, various compounds in the test samples may interfere with the molecular detection of viral nucleic acid, and the primer pairs used may fail to detect some species and strains of viruses. Some of the techniques were optimised during the course of the project, which implies that the detection of viruses became more sensitive and reliable towards the end of the project. This implied in particular to adeno, hepatitis A and rota viruses.

Particularly important for the purposes of this Project was that reliable and sensitive techniques were available for the detection of enteroviruses, including CBVs. The latter have been selected as model viruses for risk assessment in this project.

### 5.3. Typing of viruses

In this project valuable progress has been made with the typing of viruses. For instance, reliable techniques have been established for the typing of enteroviruses, which includes the accurate and reliable typing of CBVs, based largely on restriction enzyme analysis (Vivier et al, 2000, 2001). In addition, accurate techniques have been developed for the typing of polioviruses, which includes distinction between wild type and vaccine strains. The typing of polioviruses is based largely on molecular techniques and nucleotide sequence analysis (Pavlov et al, 2003; Pavlov, 2004). Most valuable progress has also been made with the typing of hepatitis A (Venter, 2004), adeno (Van Heerden, 2004), astro (Nadan, 2002; Nadan et al, 2003) and rota (Van Zyl et al, 2003) viruses.

In summary, reliable techniques are available for the typing of CBVs which have been selected as model viruses for risk assessment in this Project. Skills for the typing of other viruses will become particularly valuable when these viruses are being used for purposes of risk assessment and estimation of the burden of disease constituted by the viruses. The technology will also prove of fundamental importance in the distinction of species or strains of human and animal origin.

## 5.4. Virological analysis of water

As discussed in earlier sections, the data on viruses in drinking water supplies recorded in Tables 1 to 3 are the most comprehensive for drinking water supplies anywhere in the world. Despite shortcomings for many viruses, the results are relatively accurate for CBVs which have been selected as model viruses for risk assessment in this Project.

## 5.5. Selection of statistical risk assessment model

The risk assessment model for this project has been selected at a workshop of experts in the field held on 8 to 12 May 2000. Details on the statistical approach have been described in the literature and in section 5.6 on the application of the statistical model in this project.

## 5.6. Risk assessment

### 5.6.1. Objectives of risk assessment

The objective of risk assessment in this project was to obtain an indication of the risk of infection constituted by viruses in selected drinking water supplies. This information would enable an assessment of the extent to which the drinking water supplies under consideration conform to a proposed acceptable risk of infection for drinking water. It is important to note that the proposed acceptable risk clearly specifies infection, and not clinical disease of any kind or to any extent. This implies that the proposed acceptable risk of infection (1 infection per 10 000 consumers of a drinking water supply per year) includes infections which do not produce any clinical symptoms of disease. This would be the case for the great majority of enteroviruses such as CBVs (Pallansch and Roos, 2001).

For further details on the objectives of risk assessment in this study and their outcome, see Section 5.6.4.

### 5.6.2. Selection of model virus for risk assessment

CBVs were selected as model viruses for assessment of the risk of infection constituted by viruses in the drinking water supplies under investigation basically for the following reasons (Vivier et al, 2002a,b):

(a) CBVs occurred in relatively high numbers in the drinking water supplies which implied that quantitative assessment on counts of viruses was relatively reliable.
(b) CBVs are typical members of enteric viruses which implies that waterborne transmission is possible.
(c) Relatively reliable information was available on data required for exposure, dose-response and risk assessment.
(d) CBVs were detectable by simple, reliable and economic techniques.

Disadvantages of CBVs as model viruses for risk assessment include the following:

(a) Although waterborne transmission of CBVs has been described for recreational waters, CBVs are not typically associated with transmission by drinking water.

(b) CBVs are associated with a wide variety of clinical manifestations, which complicates hazard identification and risk characterization.

Further details on the selection of CBVs as model viruses for risk assessment are discussed in section 5.6.3.1.

Other enteric viruses in drinking water which have attractive features for risk assessment include adeno, rota and hepatitis A viruses, and possibly noroviruses.

Adenoviruses have the attractive features of being present in drinking water supplies in relatively high numbers, and they are detectable by relatively reliable and accurate techniques. However, the species *Human Adenovirus* (HAd) consists of 51 types which are associated with a wide spectrum of clinical manifestations which is an important disadvantage for meaningful hazard identification and risk characterization. In addition, the efficiency of techniques used for the recovery of adenoviruses from water has not been confirmed. Despite shortcomings, risk assessment has been carried out for adenoviruses detected in the water supplies under investigation in this Project (Van Heerden et al, 2004).

Rotaviruses have the attractive feature of clearly defined hazard identification as well as data for exposure, dose-response and risk assessment. Unfortunately rotaviruses are not typically associated with waterborne transmission, and techniques for estimating numbers of rotaviruses in drinking water supplies were not reliable. Despite shortcomings, rotaviruses are currently being used as model viruses for risk assessment by the WHO. In this project risk assessment was not carried out for rotaviruses because techniques for the detection of rotaviruses were optimised only towards the end of the project which implied that the reliability of results was not consistent throughout the period of investigation. Consequently there is reason to believe that the final results were a substantial underestimate of the incidence of rotaviruses in the drinking water supplies under consideration.

Principally HAV would probably be the model virus of choice for assessment of the risk of infection constituted by viruses in drinking water supplies. Reasons are that HAV has only one serological type which causes a clearly defined disease with unmistakable clinical symptoms. HAV is a typical waterborne virus. Reliable details are available for hazard identification, as well as dose-response and risk assessment. These features also imply that HAV would be ideally suited for calculation of the burden of disease. The only shortcoming of HAV is techniques for assessment of numbers of the viruses in water supplies. Although major progress has been made in this Project on the development of techniques for the detection of HAV in drinking water, the results obtained during the course of the study would not appear to meet the requirements of meaningful risk assessment. The WHO would prefer to use HAV as model for viral risk assessment, and keenly awaits progress on methods for detection of the viruses in water.

Noroviruses (previously known as "Norwalk-like viruses") have the important advantage as models for risk assessment of being typically associated with waterborne transmission. In addition, the disease caused by the viruses is well defined, and meaningful data are available for dose-response and risk assessment. Unfortunately it is extremely difficult to assess the numbers of these viruses in water. Another potential disadvantage is that the viruses are also often transmitted by personal contact and other means not associated with water. However, as progress is made with detection technology, these viruses, or species of the genus *Norovirus*, may emerge as useful models, at least for particular purposes.

CBVs represented 75 % of the total count of enteroviruses (Vivier et al, 2001a,b, 2002a,b). The majority of the remainder were vaccine strains of polioviruses. These findings are in agreement with those reported in studies carried out in other parts of the world (Crabtree et al, 1995; Reynolds et al, 1997).

### 5.6.3. Calculation of risk

Risk assessment involved the following four basic steps (Haas et al, 1999; Haas and Eisenberg, 2001; Vivier et al, 2002b): (1) hazard identification; (2) dose-response assessment; (3) exposure assessment; and (4) risk characterization. These steps were used to assess the risk of contracting a CBV infection from the consumption of the drinking water supplies under investigation.

#### 5.6.3.1. Hazard identification

The coxsackie B viruses (CBVs) are members of the group of enteroviruses, of the family Picornaviridae (Grabow, 1996). Members of this family are small, non-enveloped, single-stranded RNA viruses. The CBVs consist of six distinct serotypes, designated CBV1-6. The closely related coxsackie A viruses (CAVs) also belong to the group of enteroviruses and consist of 23 serotypes. The remaining members of the group of enteroviruses are the polioviruses (3 serotypes), echoviruses (33 serotypes) and the enteroviruses (4 serotypes). In temperate climates CBV infections predominantly occur during the late summer and early fall, mainly in young children (Cromwell and Landau, 1997). Coxsackie B viruses are ubiquitous agents and can spread rapidly in communities causing epidemics (Pallansch, 1997). Although a high proportion of CBV infections are subclinical, clinical manifestations 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). Information on the spectrum of clinical manifestations associated with CBV infections is based on data derived from epidemiological analysis of outbreaks of enteroviral infections (Pallansch, 1997). This includes clinical case descriptions associated with large numbers of patients with similar symptoms, all of which infected by the same CBV serotype (Pallansch, 1997). The results of these studies revealed various serotypes of CBV as the cause of conditions such as aseptic meningitis, encephalitis, pleurodynia, myocarditis, and pericarditis. The most common outcome of CBV infection is replication and excretion of the virus without clinical symptoms, an undifferentiated febrile illness, or symptoms of mild upper respiratory tract infection (Pallansch, 1997).

The group of enteroviruses belongs to the large group of "enteric viruses". This term refers to viruses which replicate in the gastrointestinal tract, are excreted in faeces, and are typically transmitted by the ingestion of food, water or other material contaminated with faeces containing the viruses, in other words, faecal-oral transmission. The group of enteric viruses also includes the noroviruses. In addition, the group of enteric viruses includes well-known viruses such as hepatitis A and E, astro, rota, reo and enteric adenoviruses. Membership of the groups of enteroviruses and enteric viruses, implies that CBVs are typically transmitted by the faecal-oral route, and that faecally-polluted water is an expected means of transmission. Although CBVs may by definition be waterborne, and waterborne transmission has indeed been confirmed in two outbreaks associated with recreational water (Hawley et al, 1973; Dennis et al, 1974), CBVs would not appear to be associated with waterborne transmission as frequently as certain other members of the group of enteric viruses such as hepatitis A and E, and noroviruses. It would seem that CBVs, like other enteroviruses, are predominantly based on faecal-oral transmission

with personal contact the most important means for the transmission of contaminated faeces.

The potential role of water in the transmission of CBVs is supported by the presence of large numbers of these viruses in waste waters, environmental waters such as rivers and impoundments, and raw and treated drinking water supplies. Evidence in this regard has been reported from many parts of the world (Lucena et al, 1985; Payment et al, 1985; Krikelis et al, 1986; Dahling et al, 1989; Grabow et al, 2001; Vivier et al, 2001). Infected individuals may excrete CBVs for as long as three months in the faeces (Hirschman and Hammer, 1974). Other features of CBVs which favour waterborne transmission include evidence that these viruses are more resistant to chloramines and ultraviolet light than polio viruses (Payment et al, 1985; Battigelli et al, 1993). According to Lo et al (1976), CBVs were exceptionally persistent in the environment, and CBV-5 was more stable than echo virus type 6 and poliovirus type 1 at any temperature.

#### 5.6.3.2. Exposure assessment

##### 5.6.3.2.1. Drinking water supplies

Results for drinking water supplies analysed appear in Tables 1 to 3.

##### 5.6.3.2.2. Exposure assessment

The exposure analysis was based on: (1) the count of CBVs in the drinking water supplies (C); (2) the efficiency of the recovery technique (R); (3) the viability of the viruses (I); and (4) the average volume of water consumed per individual (Vc). The daily exposure or dose (N) was determined by means of the formula presented in Table 4.

###### 5.6.3.2.2.1. Count of CBVs in water supplies

Average counts of CBVs in the drinking water supplies were calculated by the following procedure (Haas et al, 1999; Vivier et al, 2002b): A random distribution of viruses within and between samples, which is described by a Poisson distribution, was assumed. The Poisson parameter $\lambda$ was determined for each of the drinking water supplies using the formula

$\lambda = -\ln[P(0)]$

Average counts of viruses were then calculated by means of the equation

$C = \lambda/V$

where

C = Average count of viruses

$\lambda$ = Poisson parameter

V = Average volume of water tested

In the case of this particular study the calculation of average counts of viruses was simple because the volume of water of all samples tested for each drinking water supply was constant.

Results of calculated average counts of CBVs in the drinking water supplies are presented in Table 4.

###### 5.6.3.2.2.2. Efficiency of recovery

The efficiency of recovery (EOR) refers to the fraction of the CBVs in the water under investigation recovered by the recovery method used. On the basis of published data (Vilaginès et al, 1997) and unpublished findings of this project, the EOR of the glass wool adsorption-

elution method used in this investigation was conservatively estimated as 40 % for low turbidity treated drinking water and groundwater, and 30 % for high turbidity river water (Table 4).

#### 5.6.3.2.2.3. Viability

Suspensions of viruses recovered from water supplies were inoculated onto cell cultures to allow viruses to infect the cells and replicate their nucleic acid. The replication of viral nucleic acid confirmed that the viruses were viable in the sense that they were able to infect host cells in cell cultures and replicate their nucleic acid, which is generally accepted as evidence of viability and infectivity (Egger et al, 1995; Reynolds et al, 1997; Seidel et al, 2000; Grabow et al, 2001). This assumption is supported by sound evidence that viruses ingested by hosts infect natural host cells in vivo more readily than foreign host cells in the hostile environment of laboratory cell cultures. Consequently the viruses detected in this study were considered as viable and infectious. The replication of the viral nucleic acid had the additional benefit of increasing the sensitivity of the subsequent detection of the viral nucleic acid by PCR. However, in order not to overestimate the risk of infection constituted by CBVs in the drinking water supplies, it was assumed for the purposes of risk assessment in ths study that only 75 % of CBVs detected were viable and infectious (Table 4).

#### 5.6.3.2.2.4. Average volume of water consumed

For the purposes of this study the average volume of unboiled water consumed by consumers of the drinking water supplies was taken as one litre per day (Table 4). The determination of a meaningful indication of the average daily volume of water consumed by the members of the many communities concerned with the spectrum of drinking water supplies investigated was not part of the mandate of the project. The average volume of one litre per day was assumed for the purposes of this study in order not to overestimate the risk of CBV infection constituted by the drinking water supplies. In some other risk assessment studies carried out in the USA (Haas et al, 1993; Macler and Regli, 1993) and South Africa (Genthe and Rodda, 1999), the average daily volume of water consumed was taken as two litres. A survey carried out in the greater Cape Town area (consumers analysed by gender, 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.

### 5.6.3.3. Dose-response assessment

Dose response experiments carried out on human volunteers using a pool of CBV-4 and CAV-21 yielded a dose response parameter of $7{,}75 \times 10^{-3}$ (Suptel, 1963; Crabtree et al, 1995; Haas et al, 1995). The data derived from these studies were also used to assess the probability of becoming clinically ill from that infection as well as the probability of mortality. The probability of becoming clinically ill from that infection was calculated by multiplying $P_i$ (the probability of infection) by the morbidity rate of 0,75 reported for coxsackie viruses (Cherry, 1981). The probability of death from an infection was calculated by multiplying the morbidity rate (0,75) by the case/fatality rate, which yields a figure of 0,0059 (Gerba and Haas, 1988). These data in the literature were used for assessments in the present study. It was not an objective of this project to investigate dose-response data for the consumers of the drinking water supplies analysed.

### 5.6.3.4. Risk characterisation

Deterministic calculations were used in the exponential risk assessment model (Haas, 1983; Haas et al, 1999; Vivier et al, 2002) to estimate the daily risk of infection constituted by the CBVs in the drinking water supplies using the equation:
$P_i$ / day = 1 - exp (-rN)
where:
$P_i$ = probability of becoming infected
N = number of viruses
R = dose response parameter

The naïve estimate for the annual risk was simply calculated as:
$P_\chi = 1 - (1 - P_i / day)^{\chi}$
where:
$P_\chi$ = probability (risk) of one or more infections over period c
$\chi$ = number of days of exposure
$P_i$ / day = daily risk

Results of calculations of risk (probability) of infection constituted by the drinking water supplies under investigation, are presented in Table 5.

**Table 4. Exposure analysis for coxsackie B viruses in the drinking water supplies**

| Supply | C | R | I | Vc (Litre) | N |
|---|---|---|---|---|---|
| Treated | | | | | |
| Gauteng | 4,37E-4 | 40 | 0,75 | 1 | 8,20E-04 |
| Northwest | 1,27E-03 | 40 | 0,75 | 1 | 2,39E-03 |
| Free State | 1,13E-03 | 40 | 0,75 | 1 | 2,12E-03 |
| Reclaim | 2,62E-02 | 40 | 0,75 | 1 | 4,92E-02 |
| Untreated | | | | | |
| B River | 1,28E-02 | 30 | 0,75 | 1 | 3,19E-02 |
| B Borehole | 1,28E-02 | 40 | 0,75 | 1 | 2,39E-02 |
| FH River | 2,84E-02 | 30 | 0,75 | 1 | 7,10E-02 |
| FH Borehole | 2,01E-02 | 40 | 0,75 | 1 | 3,78E-02 |
| U Spring | 2,84E-02 | 40 | 0,75 | 1 | 5,32E-02 |
| U Borehole | 1,28E-02 | 40 | 0,75 | 1 | 5,32E-02 |
| V River | 1,28E-02 | 30 | 0,75 | 1 | 3,19E-02 |
| V Borehole | 1,28E-02 | 40 | 0,75 | 1 | 2,39E-02 |

Exposure analysis for coxsackie B viruses (CBVs) in drinking water supplies was calculated according to the following equation for daily exposure (N):

$N = C \times 1/R \times I \times Vc$
where:
C = Estimated Count of CBVs per one litre in drinking water
R = Efficiency of recovery method
I = Fraction of the detected pathogens that is capable of infection (viability)
Vc = Daily individual consumption of unboiled drinking water in Litres

**Table 5. Probability of infection, morbidity and mortality constituted by coxsackie B viruses in drinking water supplies as determined by a deterministic model**

| Supply | Infection | | Morbidity | | Mortality | |
|---|---|---|---|---|---|---|
| | per Day | per Year | per Day | per Year | per Day | per Year |
| Treated | | | | | | |
| Gauteng | 6,36E-06 | 2,32E-03 | 4,77E-06 | 1,74E-03 | 2,81E-08 | 1,03E-05 |
| Northwest | 1,85E-05 | 6,73E-03 | 1,39E-05 | 5,05E-03 | 8,19E-08 | 2,98E-05 |
| Free State | 1,64E-05 | 5,89E-03 | 1,23E-05 | 4,48E-03 | 7,27E-08 | 2,64E-05 |
| Reclaim | 3,81E-04 | 1,30E-01 | 2,86E-04 | 9,74E-02 | 1,69E-06 | 5,75E-04 |
| Untreated | | | | | | |
| B River | 2,47E-04 | 8,63E-02 | 1,85E-04 | 6,47E-02 | 1,09E-06 | 3,82E-04 |
| B Borehole | 1,85E-04 | 6,55E-02 | 1,39E-04 | 4,91E-02 | 8,21E-07 | 2,90E-04 |
| FH River | 5,50E-04 | 1,82E-01 | 4,12E-04 | 1,36E-01 | 2,43E-06 | 8,05E-04 |
| FH Borehole | 2,93E-04 | 1,01E-01 | 2,19E-04 | 7,60E-02 | 1,30E-06 | 4,48E-04 |
| U Spring | 4,12E-04 | 1,40E-01 | 3,09E-04 | 1,05E-01 | 1,83E-06 | 6,19E-04 |
| U Borehole | 4,12E-04 | 1,40E-01 | 3,09E-04 | 1,05E-01 | 1,83E-06 | 6,19E-04 |
| V River | 2,47E-04 | 8,63E-02 | 1,85E-04 | 6,47E-02 | 1,09E-06 | 3,82E-04 |
| V Borehole | 2,47E-04 | 8,63E-02 | 1,85E-04 | 6,47E-02 | 1,09E-06 | 3,82E-04 |

### 5.6.4. Implications of risk assessment data

The intention of this study was not to quantitatively compare the risk of infection constituted by viruses in different treated or untreated drinking water supplies to the consumers concerned. Such a quantitative comparison of risk was not possible for a number of reasons, including the following:

5.6.4.1. The efficiency of the glass wool adsorption-elution technique used for the recovery of viruses is higher for low turbidity water than for high turbidity water for at least the following two reasons:

5.6.4.1.1. Glass wool adsorption-elution procedures based on electrostatic charges on the viruses and on the glass wool have been used to recover viruses from water. Viruses likewise adsorb to turbidity material in untreated water. This implies that viruses adsorbed to material in turbid waters fail to adsorb to the glass wool and will not be recovered by the technique used. In addition, the nature and type of material responsible for turbidity (for instance organic material or clay particles) determine the strength of the adsorption of viruses to the particles. Depending on the strength of adsorption to turbidity material, viruses may not be released to adsorb to the glass wool instead, and run through the filters adsorbed to the turbidity material during filtration of the water under investigation. Although sound evidence has been presented that the efficiency of recovery (EOR) of glass wool adsorption-elution is substantially higher for low turbidity than for high turbidity water, there are no quantitative data on the difference in EOR (Villaginès et al, 1997).

5.6.4.1.2. Turbid waters clog the glass wool filters quite rapidly and often it is not possible to conveniently pass more than 10 litres of turbid waters through the filters. Consequently the volume of treated water from which viruses were recovered was 100 litres (as specified by SABS-241:2001) or more, while the volume of untreated water from which viruses were recovered was limited to 10 litres. Generally 10 litres did not clog the glass wool filters and contained sufficient viruses for the purposes of the study. For practical and logistic reasons the volume of reclaimed drinking water was also restricted to 10 litres.

This implies that due to the difference in the volume of water tested and the effect of turbidity on the efficiency of virus recovery, the underestimate of the incidence of viruses reported here is higher for the untreated water than for the conventional drinking water supplies. It should be noted that SABS-241:2001 is intended for conventionally treated drinking water and not for untreated water used for drinking purposes.

5.6.4.2. The EOR of the glass wool adsorption-elution technique is not directly related to the volume of water passed through the filter. In other words, the number of viruses recovered from 100 litres will not necessarily be ten times more than the number of viruses recovered from 10 litres.

5.6.4.3. Exposure parameters for the consumer communities of various drinking water supplies can be expected to differ. Factors likely to play a role in this regard include immunity to waterborne pathogens, the incidence of highly susceptible individuals such as the very young, elderly and immunocompromised people, the state of nourishment, and general condition of health.

In this study every drinking water supply was analysed in its own merit, using the most practical techniques available for the recovery of viruses and for calculating the risk of infection constituted by the viruses detected in that particular drinking water supply. The different volumes used in the analysis of the various drinking water supplies had the benefit of casting some light on optimal methods for the virological analysis of various types of drinking waters.

The estimated risk of infection by CBVs in the drinking water supplies under investigation (Table 5) was higher than the acceptable risk of one infection per 10 000 consumers per year proposed by the US Environmental Protection Agency (EPA) (Macler and Regli, 1993). Risks of infection

constituted by enteroviruses which exceed this acceptable risk, have also been reported for drinking water supplies elsewhere. Crabtree et al (1995) calculated that CBVs in drinking water supplies in the US and Canada constituted a risk of infection of $2{,}55 \times 10^{-4}$ (CBV count of $5 \times 10^{-5}$ PFU/litre) and $7{,}30 \times 10^{-1}$ (CBV count of 0,31 PFU/litre) per year. Previously reported data on counts of CBVs in drinking water (Hejkal et al, 1982; Payment et al, 1985) were used in the assessment. The results of risk assessment carried out on a drinking water supply in South Africa other than those investigated here, 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).

The reliability of the calculations of the risk of infection constituted by the drinking water supplies depends on the accuracy of the input parameters, notably efficiency of recovery (R), drinking water consumption (Vc) and the dose response parameter for CBVs. Uncertainty analysis of these variables confirmed that the calculated risk of infection may be affected substantially by variation in these parameters (Vivier et al, 2002b). However, there seems to be no reason to believe that the values assumed for these variables in this study may be incorrect to the extent that the calculated risk of infection constituted by the drinking water supplies under investigation may be incorrect to meaningful extent. More accurate calculation of the risk of infection constituted by the drinking water supplies, would require more accurate data on the input parameters concerned. These uncertainties duly warrant more detailed research.

Factors which will have to be taken into account in research along these lines, include the fact that 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. The latter would include a rapidly growing spectrum of people with deficient immune systems due to organ transplantation, various forms of cancer, acquired immunodeficiency syndrome (AIDS), other diseases which affect the immune system, malnourishment, etc (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 certainly includes South Africa, where an exceptionally high incidence of AIDS in many communities is likely to 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 values used for the above variables in this study were chosen not to overestimate the risk of infection of CBVs in the drinking water supplies under investigation. On the other hand, the results of the risk assessment data reported in this study using CBVs as model viruses may underestimate the risk of infection constituted by the drinking water supplies concerned. Reasons include:

(a) CBVs are not typically associated with waterborne transmission. CBVs were used as model because they generally occur in substantial numbers in drinking water and because they have a number of features which render them convenient for risk assessment. Unfortunately suitable techniques were not available for the detection of viruses most commonly associated with drinking water transmission such as the noroviruses. The numbers of these viruses in the drinking water supplies may exceed those of CBVs to the extent that they constitute a higher risk of infection. Available information suggests that other viruses in the drinking water supplies which may constitute a high risk of infection include adeno, hepatitis A and rota viruses.

(b) The natural host is generally more susceptible to virus infection than cell cultures, to the extent that many viruses infect only the natural host and no cell cultures presently available at all. Examples include the entire family *Caliciviridae* (which includes the important waterborne group of noroviruses), hepatitis E virus, and for all practical purposes also HAV which reluctantly multiplies under special conditions in a restricted range of available cell cultures. This implies that the number of viruses capable of infecting consumers may be higher than the number of viruses detected by the cell culture plus molecular detection procedures used in this study.
(c) The EOR of the recovery procedure may be lower for some viruses than for the polioviruses used in assessment of the EOR. This implies an underestimate of the numbers of these viruses in the drinking water supplies.

Taking into consideration all the variables and shortcomings of the techniques used for the detection of viruses and the calculation of the risk of infection constituted by the CBVs in the drinking water supplies, the results indicate that all of the drinking water supplies constitute a risk of infection that exceeded the risk of one infection per 10 000 consumers per year proposed by the EPA as an acceptable risk for drinking water. These findings justify further investigation. This would include more 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 the revision of quality guidelines for viruses in drinking water supplies, and, if necessary, upgrading treatment and disinfection processes. Accurate data on the risk of infection constituted by viruses in drinking water supplies are also essential for assessment of the burden of disease associated with the water supplies, and for the definition of an acceptable risk for drinking water.

The main risk assessment study of this project discussed in detail above, was supplemented by two additional risk assessment studies on drinking water supplies treated by conventional methods. One of these also used CBVs as model virus (Vivier et al, 2002b) and the other used human adenoviruses as model viruses (Van Heerden and co-workers, 2004) (see 5.6.5). In these two studies the results of virological analyses on smaller sets of samples were used for risk assessment, samples with different volumes were included, and other values were assumed for some of the variables. Results obtained were in agreement with those of the main study in so far as that the risk of viral infection constituted by all of the water supplies analysed also exceeded the acceptable risk proposed by the EPA.

### 5.6.5. Risk assessment for adenoviruses

In view of the relatively high counts of adenoviruses obtained in most drinking water supplies, risk assessment has also been carried out for a selected set of treated drinking water supplies using these viruses (Van Heerden et al, 2004). The high counts of adenoviruses in drinking water supplies is not unusual and has been reported for raw and treated drinking waters in other parts of the world. In view of their high incidence in water environments, association with a wide spectrum of diseases, and exceptional resistance to at least some treatment and disinfection processes, the EPA has included adenoviruses in a priority list of contaminants in water which require special attention.

The results presented by Van Heerden et al (2004) show that the drinking water supplies investigated exceed the acceptable risk of infection for drinking water proposed by the EPA.

This is in agreement with the results of risk assessment for CBVs in the main study (see 5.6.).

The implications and significance of the risk assessment data for adenoviruses have been discussed by Van Heerden et al (2004). Risks of infection constituted by adenoviruses in treated drinking water and recreational water have been assessed in a previous study (Crabtree et al, 1997). Advantages of using adenoviruses for this purpose include their presence in relatively high numbers in raw and treated drinking water supplies. Disadvantages include the wide spectrum of clinical manifestation with which the 51 strains of adenoviruses are associated. This complicates meaningful hazard identification and risk assessment. In addition, there is not sufficient information on the 51 strains for reliable exposure and dose-response assessment. However, using reasonable assumptions the results by Van Heerden et al (2004) indicate reasons for concern about adenoviruses in treated drinking water supplies. Further investigation is duly warranted. Typing of the adenoviruses detected is currently in progress. Details on the strains involved may cast more light on a number of the variables in the risk assessment model.

## 5.7. Assessment of Indicator Organisms

The absence of indicator organisms detectable by conventional techniques from drinking water in which enteric viruses were detected (Tables 1 to 3), confirms shortcomings of these indicator tests to confirm the absence of viruses from treated drinking water supplies. These shortcomings of conventional indicator organisms have been addressed in detail by Grabow et al (2001). The WHO is concerned about the shortcomings of conventional indicator organism tests for the end-point analysis of treated drinking water supplies to the extent that a new strategy for water quality control is recommended. Conventional indicator tests do not only have shortcomings for viruses, but also for other pathogens, notably protozoan parasites. The WHO therefore recommends that end-point analysis of treated drinking water supplies is abandoned and replaced by a new strategy for water quality control based on the principles of HACCP (Hazard Assessment and Critical Control Points). Details on the new strategy have been described by Fewtrell and Bartram (2001) and the strategy is recommended in detail in WHO (2004).

Research on the application of F-RNA coliphages for distinction between the human and animal origin of faecal pollution in water confirmed the benefits of the phages for the purpose, but also shortcomings. Among other things, there are exceptions to the specificity of F-RNA serotypes for humans and animals, and there are differences in the survival of serogroups in water environments (Schaper et al, 2002). These limitations imply that F-RNA coliphages have to be applied with caution for the purpose, and that confirmation by other indicators, such as faecal sterols, may be important (Sundram et al, 2003).

## 5.8. Assessment of quality guidelines and specifications

The following discussion about quality guidelines and specifications focusses on enteric viruses because this is what the project was about. However, it should be kept in mind that very much the same arguments also apply to other pathogens in drinking water, notably highly resistant cysts and oocysts of protozoan parasites.

Most current water quality guidelines world-wide recommend an unspecified absence of viruses, and other pathogens, from drinking water. One exception is the South African Bureau of Standards which more specifically specifies the absence of enteric viruses from 100 litres of treated drinking water (Grabow et al, 2001; SABS, 2001).

The results obtained in this project show that the expected absence of viruses from drinking water is unrealistic in terms of current specifications for indicator organisms in drinking water, as well as recommendations for the treatment and disinfection of drinking water supplies. In other words, current technology and expertise for the detection of enteric viruses show that treated drinking water which conforms to current specifications for indicator organisms such as coliform and heterotrophic bacteria, as well as enterococci, and even more resistant indicators such as coliphages (SABS, 2001), may contain viable viruses. Likewise, drinking water supplies treated and disinfected according to internationally recognised specifications (WHO, 1996), may also contain viable viruses. This implies that current specifications for both indicator organisms and treatment and disinfection, fail to ensure the absence of viruses from drinking water.

The results of risk assessment using both CBVs and HAds carried out in this project reveal another question about current specifications. The results show that drinking water treated and disinfected according to current specifications, and which meets current specifications for indicator organisms, constitutes a risk of viral infection which exceeds the acceptable risk for treated drinking water of one infection per 10 000 consumers per year proposed by the EPA (Grabow et al, 2001). In South Africa an acceptable risk of infection has not yet been defined. However, it may be assumed that until an official acceptable risk of infection has been defined for the country, the EPA recommendation may be accepted at least as a guideline for practical purposes as is being done in most of the rest of the world.

The discrepancies in current water quality guidelines and specifications may be resolved by a number of options, including revision of the following:

### 5.8.1. Recommendations for viruses in drinking water

Recommendations for an undefined absence of viruses from drinking water should be abandoned because it is unrealistic and not feasible. Quality guidelines and specifications for drinking water should clearly specify the viruses which should be absent from a specified volume of water. This is illustrated by SABS-241:2001which specifies the "absence of enteric viruses from 100 litres of treated drinking water". Similar specifications were recommended earlier (Department of Water Affairs, 1996).

At the time when SABS-241:2001 was formulated, this definition was acceptable because at that time the term "enteric viruses" was for all practical purposes understood to mean "cytopathogenic enteric viruses". However, in terms of more recent technology and knowledge, there is a major difference between "enteric viruses" and "cytopathogenic enteric viruses".

As a first step forward in endeavours to resolve controversies about specifications for viruses in drinking water, it is recommended that in SABS-241:2001 the term "enteric viruses" is replaced by "cytopathogenic enteric viruses". This would be a feasible and realistic definition because this is what actually the original intention of the specification was, and data in this project show that the great majority of treated drinking water supplies conformed to this specification.

Although this recommended revision of SABS-241:2001 would not be the final answer to questions about the virological quality of drinking water, it would at least for the time being put to rest concerns of water supply utilities who have their drinking water supplies analysed for enteric viruses.

What remains to be resolved is the viable non-cytopathogenic enteric viruses in drinking water, which represent the great majority of viruses detectable by current technology. This includes the CBVs and HAds used in risk assessment carried out in this project which revealed that all drinking water supplies investigated exceeded the EPA recommendation for an acceptable risk of infection for drinking water. This controversy may be resolved by revision of the acceptable risk of infection discussed in 5.8.3, or by upgrading the treatment and disinfection of drinking water supplies to eliminate these viruses, discussed in 5.8.2.

### 5.8.2. Specifications for treatment and disinfection

Recommendations such as the absence of viruses, and proposed acceptable risks for viruses in drinking water, may be met by upgrading treatment and disinfection processes to levels where viruses are eliminated to the extent that the recommendations are met. Although no calculation of the implications has yet been made, there is little doubt that this will have major technological and economical consequences for water supply utilities. Expectations in this regard should, therefore, be avoided until there is absolute clarity about specifications for viruses in drinking water, and a clearly defined acceptable risk for infections constituted by drinking water.

### 5.8.3. Acceptable risk of infection

As far as can be established the EPA was the first, and to date the only, water quality authority in the world that had the courage to propose an acceptable risk for infections constituted by micro-organisms in drinking water. Other water quality authorities refer to the EPA proposal as an example, without specifying their own acceptable limits. A few authorities and countries have adopted the EPA proposal as the official acceptable risk for themselves.

However, it must be kept in mind that the definition of an official acceptable risk for infections associated with drinking water has major implications and must be considered with great care. Decisions about acceptable risks for drinking water is basically a political decision which must be formulated by national public health authorities. In South Africa, as in most other countries, this would be the Department of Health, which would consult authorities such as the Department of Water affairs, Medical Research Council, the SABS, and relevant experts from the water industry and academic establishments. The WHO does not make any recommendations about acceptable risks other than emphasising that decisions in this regard must be taken by countries themselves.

The EPA recommendation has the benefit of serving as an example of what an acceptable risk might look like. However, it possibly has the disadvantage of creating false expectations in the mind of many people in other parts of the world. The EPA recommendation may be feasible in a highly developed country such as the USA. However, in many parts of the world without the financial resources and infrastructure of the USA, this may be unrealistic. The definition of a realistic acceptable risk for individual countries must take into consideration variables such as public health priorities, available infrastructure, financial resources on the part of relevant authorities, water supply utilities and consumers, as well as other variables such as the susceptibility of consumers to infection, and drinking water consumption patterns.

At this stage there is no indication of endeavours in South Africa to initiate a process that may lead to the definition of an acceptable risk of infection for drinking water in the country. Since this is a long and complex task, it will probably take some time before an official acceptable risk

is available.

Australia is one of a number of countries that is well advanced on new technology and policies for water quality control. The country has an optimally functioning infrastructure of close collaboration between all role players concerned, and offers an excellent example of what should be done.

As a matter of interest it should be noted that the acceptable risk proposed by the EPA specifies "infections", without reference to disease. In the case of enteric viruses as a rule the great majority of infections do not result in clinical disease. One possible variation on the EPA proposed acceptable risk may, therefore, be an acceptable risk based on cases of clinically diagnosable disease. Among other things this would solve the almost impossible task of monitoring infections which do not result in clinical disease. Monitoring infections without disease is difficult because there is no reliable relation between infections with or without clinical disease, which varies for different communities and situations, and different viruses.

### 5.8.4. Indicator organisms

It would not appear possible to revise guidelines and specifications for indicator organisms to the extent that they may reliably indicate the presence of viruses in treated drinking water. The reason is basically that viruses are more resistant to treatment and disinfection than indicators commonly used for water quality assessment. Consequently indicators outnumber viruses in raw water sources, but during treatment and disinfection the indicators are eliminated more efficiently than the viruses and eventually viruses outnumber indicators.

The shortcomings of indicators should certainly not be interpreted to discredit the most valuable role of indicators in water quality control and water quality assessment. Indicators remain of fundamental importance for the rapid, simple and economic assessment of faecal pollution, and for monitoring the efficiency of water treatment and disinfection processes.

### 5.8.5. New strategy for water quality control

The end-point monitoring of treated drinking water supplies as has been customary in the drinking water industry in the past, has major disadvantages, some of which are evident from the discussion above. A particularly important disadvantage of end-point monitoring is that by the time the results are available, which in particular refers to virological analyses which take a relatively long time, the water is in the distribution system and most of it has probably been consumed by customers. Therefore, if the results of end-point monitoring reveal a problem it is too late to introduce any remedial action or to prevent relevant consequences.

For this reason the WHO recommends the new approach to water quality monitoring which is based on the principles of HACCP (Hazard Assessment and Critical Control Points). Basically this implies that the quality of water is monitored at a selected set of critical control points in the treatment train of a drinking water production system. The first critical control point is the raw water source. Hazard assessment is used to determine the quality of the final product, as well as the efficiency of each critical control point to accomplish the desired final quality. The efficiency of critical control points may be monitored by physico-chemical parameters and practical indicator systems. In the case of virological quality this implies that once the requirements for

viruses have been established and the efficiency of critical control points has been defined accordingly, routine virological monitoring is no longer required. One important advantage of this approach is that water quality problems can be discovered before the water is released into the distribution system and consumers can be protected from problems.

Details on the strategy has been described in detail by Fewtrell and Bartram (2001) and is recommended in WHO (2004). The WHO feels so strong about this approach that no further attention is being given to the problems and challenges of end-point monitoring, including the controversies about viruses, and other pathogens such as protozoan parasites, in treated drinking water.

A number of countries, including Australia, are making sound progress on the introduction of HACCP strategies in their policies for water quality control and safety.

### 5.9. Future research

This project has laid the foundations for most important future research. Skilled manpower has been trained to take the work forward. Technology and expertise has been established for refining risk assessment for viruses in water. Refinement includes aspects such as more reliable details for variables like hazard identification and exposure, dose-response and risk assessment. One important variable for which more reliable details should be obtainable easily, is the average drinking water consumption patterns by consumer communities concerned.

The know-how and experience in virus recovery, detection and typing can be improved further to obtain more accurate data on the quantitative numbers of viruses in drinking water. The current statistical calculation of viral counts based on qualitative presence-absence data for viruses is not optimal. The assessment of accurate counts of viruses heavily depends upon reliable data on the sensitivity of recovery and detection techniques for various viruses, which currently is a major shortcoming.
For instance, more reliable recovery and detection techniques may promote the hepatitis A virus to its full potential of virus of choice for risk assessment.

More accurate typing of viral isolates will improve the reliability of risk assessment, and may lead to the emergence of more reliable model viruses for purposes of risk assessment. This refers to viruses such as the large group of adenoviruses, and the important waterborne members of the family *Caliciviridae*.

Reliable data on risks constituted by viruses in drinking water supplies should be followed up by calculation of the related burden of disease. The skills for doing this are now available. Burden of disease data make it possible to quantitatively assign public health priorities to waterborne diseases. This has been done by the WHO for waterborne diseases in the global context of public health priorities (Prüss and Havelaar, 2001). A public health priority for waterborne diseases in South Africa, will enable national health authorities to take decisions on fundamentally important issues such as an acceptable risk of infection for viruses and other pathogens in drinking water. On its part an acceptable risk of infection will enable the water industry to define goals for the quality of treated drinking water supplies. On their part these goals will make it possible to define specifications for critical control points in drinking water treatment trains, which are required for the implementation of water quality control strategies based on HACCP principles.

Progress in research, technology development and the training of manpower in this field is essential for obvious reasons. Water is the single most important natural resource limiting growth and development in South Africa. As the population grows, the demands for safe water derived from constant natural sources escalates. At the same time the production of safe water from these sources becomes more difficult because of escalating pollution with pathogenic micro-organisms and hazardous chemical compounds from increasing populations of humans and domestic animals, and industrial and agricultural activities. The challenge to supply safe water to the growing population of the country does, therefore, increase rapidly in complexity, which requires new technology and expertise to keep up with.

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## 7. PROJECT OUTPUTS

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Vivier JC, Clay CG, Grabow WOK (2001a) Detection and rapid differentiation of human enteroviruses in water sources by restriction enzyme analysis. Water Science and Technology 43, 209-212.

Vivier JC, Ehlers MM, Grabow WOK (2002a) Detection of enteroviruses in treated drinking water. Water Research (in press).

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Vivier JC, Ehlers MM, van Heerden J, Venter JME, Taylor MB, Grabow WOK (2002c) An assessment of candidate viruses for harmonised risk assessment of water related microbiological hazards. Oral paper: Symposium on Health-Related Water Microbiology, World Water Congress, Convention Centre, Melbourne, 7-12 April.

## 7.2. Conference Presentations

Ehlers MM, Grabow WOK, Müller EE (2003a) Biolog identification of *Escherichia coli* O157:H7 and other sorbitol-negative bacteria isolated from river water and sewage samples. Poster: Symposium on Health-Related Water Microbiology of the International Water Association, Cape Town Convention Centre, 14-19 September.

Ehlers MM, Grabow WOK, Pavlov DN (2003b) Detection of enteroviruses in raw and treated drinking water supplies in South Africa. Poster: Symposium on Health-Related Water Microbiology of the International Water Association, Cape Town Convention Centre, 14-19 September.

Grabow WOK (2000) Viruses in drinking water. Invited Lecture: Institute of Hygiene, University of Tübingen, Germany, 14 July.

Grabow WOK (2001) Specifications for enteric viruses in drinking water. Seminar on Effective Approaches to Managing Microbial Water Quality, Adelaide, Australia, 9 May. See: Health Stream (Public Health Newsletter of the Australian Cooperative Research Centre for Water Quality and Treatment) 22, 5 (2001).

Grabow WOK (2002a) Indicators and tests in the water safety paradigm of water management. Invited oral paper: World Health Organization Seminar: Development of WHO Guidelines for Drinking-Water, Wastewater Reuse and Recreational Waters, Convention Centre, Melbourne, 12 April.

Grabow W O K (2002b) Monitoring the virological safety of water: from coliforms to HACCP. Invited oral paper: Symposium on Water Virology, Science Park, Barcelona, Spain, 26 September.

Grabow WOK (2003a) Assessing microbial quality and treatment efficiency. Oral paper: Symposium on Health-Related Water Microbiology of the International Water Association, Cape Town Convention Centre, 14-19 September.

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Microbiology, World Water Congress of the International Water Association, Paris, France, 4-7 July 2000.

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Müller EE, Ehlers MM, Grabow WOK (2003) Development of a multiplex-PCR assay for the detection of enterohaemorrhagic *E coli* and *E coli* O157:H7 virulence factors from water sources and faecal samples. Oral paper: Symposium on Health-Related Water Microbiology of the International Water Association, Cape Town Convention Centre, 14-19 September.

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Potgieter N, Ehlers MM, Grabow WOK (2003) Inactivation of pathogens and indicator organisms in drinking water stored in rural household containers. Oral paper: Symposium on Health-Related Water Microbiology of the International Water Association, Cape Town Convention Centre, 14-19 September.

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Sundram A, Jumanlal N, John W, Bailey IW, Ehlers MM, Grabow WOK (2003) F-RNA coliphages and faecal sterols used as indicators in tracing the source of faecal pollution to either animal or human origin in the Umgeni Water catchment area. Poster: Symposium on Health-Related Water Microbiology of the International Water Association, Cape Town Convention Centre, 14-19 September.

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Van der Linde M, Taylor MB, Grabow WOK, van Zyl WB (2001) Occurrence of rotavirus in selected water samples. Poster: Faculty Day, Faculty of Health Sciences, University of Pretoria, 22 August.

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Van Zyl WB, Williams PJ, Grabow WOK, Taylor MB (2003) Application of a molecular method for the detection of group A rotaviruses in raw and treated water. Oral paper: Symposium on Health-Related Water Microbiology of the International Water Association, Cape Town Convention Centre, 14-19 September.

Venter JME, Grabow WOK, Taylor MB (2001) Comparison of techniques for the isolation and detection of hepatitis A virus in water samples. Poster: Faculty Day, Faculty of Health Sciences, University of Pretoria, 22 August.

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Venter JME, Vivier CJ, Grabow WOK, Taylor MB (2002b) Is hepatitis A virus in surface recreational water a cause for concern? Faculty Day, Faculty of Health Sciences, University of Pretoria, 21 August.

Venter JME, Vivier JC, Grabow WOK, Taylor MB (2003) Hepatitis A virus in surface recreational water in South Africa: what is the risk? Oral paper: Symposium on Health-Related Water Microbiology of the International Water Association, Cape Town Convention Centre, 14-19 September.

Vivier JC, Clay CG, Grabow WOK (2000) Detection and molecular typing of enteroviruses in water sources. Oral paper: Biennial Conference of the Water Institute of Southern Africa, Sun City, 28 May to 1 June. Proceedings in press.

Vivier JC, Ehlers MM, van Heerden J, Venter JME, Taylor MB, Grabow WOK (2002b) An assessment of candidate viruses for harmonised risk assessment of water related microbiological hazards. Oral paper: Symposium on Health-Related Water Microbiology, World Water Congress, Convention Centre, Melbourne, 7-12 April.

Vivier JC, Grabow WOK, Ehlers MM, Havelaar AH (2001) Assessment of the risk of infection associated with coxsackie B viruses in drinking water. Oral paper: World Water Congress of the International Water Association, Berlin, Germany, 15-19 October.

Vrey MA, Ehlers MM, van Zyl WB, Taylor MB, Pavlov DN, van Heerden J, Venter JME, Vivier JC, de Villiers JC, van Zyl E, Grabow WOK (2003) Microbiological quality of ground and

surface water used for domestic purposes in rural areas of South Africa. Poster: Symposium on Health-Related Water Microbiology of the International Water Association, Cape Town Convention Centre, 14-19 September.

Webber LM, Taylor MB (2003) HIV/AIDS and groundwater: a grave concern for South Africa? Poster: Symposium on Health-Related Water Microbiology of the International Water Association, Cape Town Convention Centre, 14-19 September.

Williams PJ, Ehlers MM, Grabow WOK, van Zyl WB (2002) Application of a novel method for the molecular detection of rotavirus in raw and treated water. Faculty Day, Faculty of Health Sciences, University of Pretoria, 21 August.

Williams PJ, Müller EE, Pavlov DN, Grabow WOK (2003) Point-of-use water treatment units: new procedure for assessment of the elimination of microorganisms Poster: Symposium on Health-Related Water Microbiology of the International Water Association, Cape Town Convention Centre, 14-19 September.

## 8. TECHNOLOGY TRANSFER AND CAPACITY BUILDING

### 8.1. Involvement of collaborators and students

At the Department of Medical Virology, University of Pretoria, ten post-graduate students were directly involved in various aspects of the project. Some of these students have already qualified to BSc(Hons), MSc and PhD degrees, and others are in the final stages of obtaining these qualifications (see 8.2). The research projects of the students formed part of this WRC project. The students produced a substantial number of publications and conference presentations on various aspects of the project. These students have, therefore gained first hand training and experience on the work involved in the project.

Students at the Technikon Free State were involved in certain aspects of the project such as the selection of sampling sites, the collection and transport of samples, and the analysis of the water samples for various indicator organisms. In most cases this work formed part of their training. Students directly involved were: Ms Leatile (Lea) Koekoe, Ms Nkope Jemina Ntsherwa, and Ms Maronel Steyn.

A number of students at the University of Venda was likewise directly involved in the project. One of these was Ms M G Florah Mashau, a MSc student who specialised in the analysis of water supplies for coliphages with special reference to the application of F-RNA coliphage serotypes to distinguish between faecal pollution of human and animal origin. Other students at the University of Venda directly or indirectly engaged in the project included Ms AL Nemurade and Mr HG Ngoveni. Students from the University of Venda visited the Department of Medical Virology at the University of Pretoria on a number of occasions for training in various aspects of their work.

Two students at the University of Fort Hare were directly involved in the project. They used the analysis of drinking water samples as part of a comprehensive survey of the quality of drinking water supplies in selected rural areas. At least one publication is in preparation on the findings.

Staff at laboratories of participating water supply utilities were also directly engaged in the project. Mr A Sundram at the Umgeni Water laboratory is using the results of a study he is carrying out as part of the project for purposes of an MSc degree.

A special highlight in the training of the students was the International Symposium on Health-Related Water Microbiology held on 14-19 September 2003 in Cape Town. This Symposium offered students the opportunity to gain first-hand experience on an international symposium in their field of interest. They personally met world leaders in the field, and heard these people present their work. All students from the Department of Medical Virology, University of Pretoria, presented at least one oral paper or poster, and were directly involved in others. Presentations were also made by students involved in the project at collaborating education establishments and water utilities.

Technology transfer included information on the microbiological quality of water, as well as technology and expertise on the microbiological analysis of drinking water. The latter refers in particular to techniques for somatic and F-RNA coliphages, and the application of F-RNA serotypes for distinguishing between faecal pollution of human and animal origin.

The information obtained by this project should be of special interest to water quality authorities such as the South African Bureau of Standards, the Department of Water Affairs and Forestry, and the Department of Health, since it contains details of fundamental importance to the formulation of water quality guidelines and specifications, as well as the formulation of strategies for the control of waterborne diseases.

## 8.2. Degrees awarded by the University of Pretoria

**BSc(Hons)**

Jongman, L (2002) Evaluation of methods for the recovery of polioviruses from water.

Naus, TM (2002) The recovery and detection of hepatitis A virus and human astrovirus from irrigation waters and associated minimally processed foods.

Van der Linde, M (2002) Rotavirus A occurrence in different selected water samples and its survival in the environment. BSc(Hons).

Williams, PJ (2002) Assessment of the anti-viral activity of ammonium oxifulvate (cum laude).

**MSc**

Nadan, S (2002) Characterisation of astroviruses from selected clinical and environmental settings.

Sundram A (2004) Assessment of F-RNA coliphage genotypes and faecal sterols to distinguish between faecal pollution of human and of animal origin in water from the Umgeni catchment area (Dissertation in preparation).

Venter JME (2004) Hepatitis A and astroviruses in raw and treated water supplies (Dissertation in preparation).

Vivier, JC (2001) Molecular typing of enteroviruses (cum laude).

Williams, PJ (2004) Hepatitis E virus in South Africa: Seroprevalence in swine and detection of the virus in swine stool specimens and waste water (Dissertation in preparation).

**PhD**

Müller, EE (2004) Prevalence of *Escherichia coli* O157:H7 in South Africa (Dissertation in preparation).

Pavlov, DN (2004) Genomic mutations in oral poliovirus vaccine strains: Implications for the eradication of poliovirus (Dissertation in preparation).

Potgieter, N (2004) Water storage in rural households: Intervention strategies to prevent waterborne diseases (Dissertation in preparation).

# ASSESSMENT OF THE RISK OF INFECTION ASSOCIATED WITH VIRUSES IN SOUTH AFRICAN DRINKING WATER SUPPLIES

Report to the Water Research Commission
Project K5/1164/0/1

by

W O K Grabow MM Ehlers, MB Taylor and Co-Workers
Department of Medical Virology
University of Pretoria

March 2004

# 1. PROJECT OUTPUTS

## 1.1 REFEREED PUBLICATIONS


1 Ashbolt NJ, Grabow WOK, Snozzi M (2001) Chapter 13: Indicators of microbial water quality. In: Water Quality Guidelines: Guidelines, Standards and Health. Editors Fewtrell L and Bartram J. World Health Organization Water Series. IWA Publishing, London. Pp 289-315.

2 Grabow WOK (2001) Bacteriophages: Update on application as models for viruses in water. Water SA 27, 251-268.

3 Grabow WOK (2002) Enteric hepatitis viruses. In: Guidelines for Drinking Water Quality, Second Edition, Addendum: Microbiological agents in drinking water, 18-39. World Health Organization, Geneva. 142 pp.

4 Grabow WOK, Taylor MB, Clay CG, De Villiers JC (2001) Molecular detection of viruses in drinking water: implications for safety and disinfection. In: Craun GF, Hauchman FS, Robinson DE (eds). Microbial Pathogens and Disinfection By-products in Drinking Water: Health Effects and Management of Risks. ILSI Press, Washington DC, pp 607-610.

5 Grabow WOK, Taylor MB, de Villiers JC (2001). New methods for the detection of viruses: call for review of drinking water quality guidelines. Water Science and Technology 43,1-8.

6 Grabow WOK, Taylor MB, Vivier JC, Potgieter N, Gaobepe MG (2002) The Health Impact of Waterborne Viruses and Methods of Control in High Risk Communities. WRC Report No 743/1/02. Water Research Commission, Pretoria.

7 Jagals P, Grabow WOK, Griesel M, Jagals C (2000) Evaluation of selected membrane filtration and most probable number methods for the enumeration of faecal coliforms, *Escherichia coli* and enterococci in environmental waters. Quantitative Microbiology 2,129-140.

8 Müller EE, Ehlers MM, Grabow WOK (2001) The occurrence of *E coli* O157:H7 in South African water sources intended for direct and indirect human consumption. Water Research 35, 3085-3088.

9 Müller EE, Grabow WOK, Ehlers MM (2003) Immunomagnetic separation of *Escherichia coli* O157:H7 from environmental and wastewater in South Africa. Water SA 29, 427-432.

10 Müller EE, Taylor MB, Grabow WOK, Ehlers MM (2002) Isolation and characterization of *Escherichia coli* O157:H7 and shiga toxin-converting bacteriophages from strains of human, bovine and porcine origin. Water Science and Technology: Water Supply 2, 29-38.

11 Nadan S, Walter JE, Grabow WOK, Mitchell DK, Taylor MB (2003). Molecular characterization of astroviruses by reverse transcriptase PCR and sequence analysis: comparison of clinical and environmental isolates from South Africa. Applied and Environmental Microbiology 69, 747-753.

12 Pavlov D, de Wet CME, Grabow WOK, Ehlers MM (2002). Determination of cytotoxicity and invasiveness of heterotrophic plate count bacteria isolated from drinking water. Water Science and Technology: Water Supply 2, 115-122.

13 Pavlov D, de Wet CME, Grabow WOK, Ehlers MM (2003). Potentially pathogenic features of heterotrophic plate count bacteria isolated from treated and untreated drinking water. Special Issue - HPC Bacteria in Drinking Water. International Journal of Food Microbiology (in press).

14 Schaper M, Jofre J, Uys M, Grabow WOK. Distribution of genotypes of F-specific RNA bacteriophages in human and non-human sources of faecal pollution in South Africa and Spain. Journal of Applied Microbiology 2002, 92, 657-667.

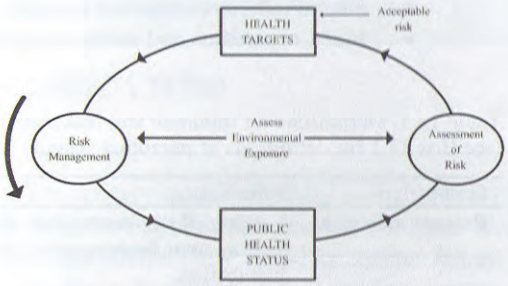
15 Sundram A, Donnelly L, Ehlers MM, Vrey A, Grabow WOK, Bailey IW (2002). Evaluation of F-RNA coliphages as indicators of viruses and the source of faecal pollution. Water SA Special Edition WISA Proceedings 2002, 86-91.

16 Taylor MB, Cox N, Vrey MA, Grabow WOK (2001) The occurrence of hepatitis A and astroviruses in selected river and dam waters in South Africa. Water Research 35, 2653-2660.

17 Van Heerden J, Ehlers MM, Van Zyl WB and Grabow WOK (2003). Incidence of adenoviruses in raw and treated water. Water Research 37, 3704-3708.

18 Vivier JC, Clay CG, Grabow WOK (2001). Detection and rapid differentiation of human enteroviruses in water sources by restriction enzyme analysis. Water Science and Technology 43, 209-212.

19 Vivier JC, Ehlers MM, van Heerden J, Venter JME, Taylor MB, Grabow WOK (2002). An assessment of candidate viruses for harmonised risk assessment of water related microbiological hazards. Oral paper: Symposium on Health-Related Water Microbiology, World Water Congress, Convention Centre, Melbourne, 7-12 April.




# 13

# Indicators of microbial water quality

*Nicholas J. Ashbolt, Willie O.K. Grabow and Mario Snozzi*

Current guidelines in the three water-related areas (drinking water, wastewater and recreational water) assess quality, in microbiological terms, by measuring indicator organisms. This chapter looks at the history and examines some of the methods used to assess the microbiological quality of water, highlighting the current limitations and also possible future developments.

# Bacteriophages: Update on application as models for viruses in water

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## Abstract

Phages are valuable models or surrogates for enteric viruses because they share many fundamental properties and features. Among these are structure, composition, morphology, size and site of replication. Even though they use different host cells, coliphages and *Bacteroides fragilis* phages predominantly replicate in the gastro-intestinal tract of humans and warm-blooded animals where enteric viruses also replicate. A major advantage of phages is that, compared to viruses, they are detectable by simple, inexpensive and rapid techniques. In view of these features, phages are particularly useful as models to assess the behaviour and survival of enteric viruses in the environment, and as surrogates to assess the resistance of human viruses to water treatment and disinfection processes. Since there is no direct correlation between numbers of phages and viruses, phages cannot to a meaningful extent be used to indicate numbers of viruses in polluted water. The presence of phages typically associated with human and animal excreta indicates the potential presence of enteric viruses. However, the absence of these phages from water environments is generally a meaningful indication of the absence of enteric viruses. This is because phages such as somatic coliphages, F-RNA coliphages and *B. fragilis* phages generally outnumber enteric viruses in water environments, and they are at least as resistant to unfavourable conditions including those in water treatment and disinfection processes. However, using highly sensitive molecular techniques viruses have been detected in drinking water supplies which yielded negative results in conventional tests for phages. Initially, data on phages were rather confusing because a wide variety of techniques was used. However, techniques for the detection of phages are being standardised internationally. This applies in particular to somatic and F-RNA coliphages, and *B. fragilis* phages, which are most commonly used in water quality assessment. Reliable and practical techniques now available include direct quantitative plaque assays on samples of water up to 100 mℓ, and qualitative tests on 500 mℓ or more using highly sensitive enrichment procedures.

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# Guidelines for drinking-water quality

SECOND EDITION

## *Addendum*
## *Microbiological agents in drinking water*

# Enteric hepatitis viruses[1]

## Description

The term "hepatitis viruses" refers to a diverse group of viruses all of which have the human liver as the primary target of replication and give rise to hepatitis or inflammation of the liver. Their replication may result in mass destruction of liver cells. Consequences include failure of the liver to fulfil basic functions such as removal of bilirubin from the circulatory blood system. Bilirubin is a red pigment released from red blood cells as they break down and are replaced by new cells. Excessive accumulation of the pigment in the bloodstream is manifest as yellow coloration of sites such as the eyes and the palms of the hands. This condition is known as jaundice and is also marked by dark urine and stools resulting from the excretion of bilirubin. Another typical consequence of massive liver cell damage is release into the bloodstream of liver enzymes, including alanine transaminase (ALT) and aspartate transaminase (AST). Serum levels of these enzymes are used to diagnose hepatitis (Zuckerman & Thomas, 1993).

Hepatitis may also be caused by other systemic pathogens such as cytomegalovirus, yellow fever virus, and *Leptospira* bacteria, although the liver is not the primary or only target of these organisms. Liver cell damage and jaundice may also be caused by toxic compounds, including alcohol.

Since the clinical symptoms caused by hepatitis viruses are very similar, and some of the viruses only emerged on a large scale in recent years, distinction of different aetiological agents has been progressively accomplished only since the 1960s. The first two hepatitis viruses that were distinguished were simply

# Molecular Detection of Viruses in Drinking Water: Implications for Safety and Disinfection

*W.O.K. Grabow, M.B. Taylor, C.G. Clay, and J.C. de Villiers*
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Drinking water supplies have a long history of association with a wide spectrum of viral infections. Evidence of waterborne transmission is predominantly based on epidemiological data. Waterborne transmission has only in exceptional cases been confirmed by direct detection of viruses in drinking water supplies. This is because most viruses typically transmitted by water (enteric viruses) are not detectable by conventional methods. However, molecular techniques based on the reverse-transcription polymerase chain reaction (RT-PCR) have made it possible to detect low levels of a wide variety of enteric viruses (Grabow 1996).

# New methods for the detection of viruses: call for review of drinking water quality guidelines

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**Abstract** Drinking water supplies which meet international recommendations for source, treatment and disinfection were analysed. Viruses recovered from 100 L–1,000 L volumes by in-line glass wool filters were inoculated in parallel into four cell culture systems. Cell culture inoculation was used to isolate cytopathogenic viruses, amplify the nucleic acid of non-cytopathogenic viruses and confirm viability of viruses. Over a period of two years, viruses were detected in 23% of 413 drinking water samples and 73% of 224 raw water samples. Cytopathogenic viruses were detected in 6% raw water samples but not in any treated drinking water supplies. Enteroviruses were detected in 17% drinking water samples, adenoviruses in 4% and hepatitis A virus in 3%. In addition to these viruses, astro- and rotaviruses were detected in raw water. All drinking water supplies had heterotrophic plate counts of <100/mL, total and faecal coliform counts of 0/100 mL and negative results in qualitative presence-absence tests for somatic and F-RNA coliphages (500 mL samples). These results call for a revision of water quality guidelines based on indicator organisms and vague reference to the absence of viruses.

**Keywords** Viruses; drinking water; quality; guidelines; cell culture; polymerase chain reaction

## Introduction

Drinking water supplies have a long history of association with a wide spectrum of viral

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# EXECUTIVE SUMMARY OF RESEARCH ON

# THE HEALTH IMPACT OF WATERBORNE VIRUSES AND METHODS OF CONTROL IN HIGH RISK COMMUNITIES

Report to the Water Research Commission

by

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# THE OCCURRENCE OF *E. COLI* O157 : H7 IN SOUTH AFRICAN WATER SOURCES INTENDED FOR DIRECT AND INDIRECT HUMAN CONSUMPTION

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**Abstract**—The occurrence of *Escherichia coli* O157:H7 in selected water samples in South Africa was investigated. The chromogenic Rainbow™ agar O157 medium designed for the rapid identification of *E. coli* O157:H7 was used for the detection of these organisms in various river-water samples in the Vaal Barrage Reservoir drainage basin of South Africa. A total of 204 samples were obtained from 15 sites where water was used for direct and indirect human consumption. Samples were filtered through Gelman filter-units and incubated on Rainbow™ agar O157 which produced different colours according to the bacterial chromogenic properties. Six hundred and sixty-three suspected *E. coli* O157:H7 colonies, with colours ranging between dark blue, grey and black, were subcultured onto sorbitol-MacConkey agar and screened for different virulence factors specific for *E. coli* O157:H7 and agglutination with anti-*E. coli* O157 antiserum. The results indicated that none of the suspected colonies contained all of the virulence factors necessary to classify them as *E. coli* O157:H7. None of these organisms agglutinated with antisera against *E. coli* O157. The probability of being infected with *E. coli* O157:H7 from direct or indirect consumption of these river water sources is therefore low. Some samples did, however, contain enterohaemorrhagic *E. coli* virulence properties, such as Stx1, Stx2 and enterohaemolysin, which might impose a health risk if ingested. 

*Key words*—*Escherichia coli* O157:H7, rainbow™ agar O157, virulence factors, agglutination, river water

*Abbreviations:* EHEC, Enterohaemorrhagic *Escherichia coli*; Stx, Shiga toxin; HC, Haemorrhagic colitis; HUS, Haemolytic uraemic syndrome; A/E, Attaching and effacing; kb, kilo base pairs; LEE, Locus for enterocyte effacement; EPEC, Enteropathogenic *Escherichia coli*; kDa, kilo Dalton; OMP; Outer membrane protein; EspA, *Escherichia coli* secretory protein A; EspB, *Escherichia coli* secretory protein B; EspD *Escherichia coli* secretory protein D; Tir, Translocated intimin receptor; *eaeA*, *Escherichia coli* attaching and effacing gene, VT, Vero cytotoxin; EHEC-Hly, Enterohaemorrhagic *Escherichia coli* haemolysin; Mda, Mega Dalton; P, Plasmid

# Immunomagnetic separation of *Escherichia coli* O157:H7 from environmental and wastewater in South Africa

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## Abstract

Recreational and drinking water supplies polluted with sewage have become an important source of *E. coli* O157:H7 infection. Immunomagnetic separation (IMS) has been extensively used for the isolation of *E. coli* O157:H7 from food and stool samples but not for samples such as wastewater. In this study the IMS method was used in combination with the *E. coli* O157:H7 selective media, immunoassays, biochemical tests and PCR, to assess the prevalence of *E. coli* O157:H7 in selected sewage and environmental water in South Africa. Environmental and wastewater were seeded with *E. coli* O157:H7 to determine the sensitivity and selectivity of the enrichment-IMS-selective agar method. Naturally occurring *E. coli* O157:H7 organisms were recovered from selected samples by means of IMS. The IMS concentrates were plated on three selective *E. coli* O157:H7 media. *E. coli* O157:H7 was detected in seeded sewage and river water samples with numbers as low as 1.2 cfu·mℓ$^{-1}$. The IMS procedure was used to investigate the prevalence of *E. coli* O157:H7 in randomly selected sewage and river water samples in South Africa. A total of 91 sewage- and 40 river water samples were tested and 17.6% and 20% yielded suspected *E. coli* O157:H7 colonies on CT-SMAC agar medium respectively. PCR was used to confirm the presence of genes coding for Shiga toxin-1 (Stx1), Shiga toxin-2 (Stx2), enterocyte attaching and effacing genes (*eae*A) and enterohaemolysin (*hly*). Standard immunoassay kits specific for the O157 and H7 antigen and a biochemical indole test were used for further *E. coli* O157:H7 confirmation. Three colonies from one sewage sample (1.1 % of all sewage samples) agglutinated with anti-*E. coli* O157 and H7 antiserum and contained the genes coding for Stx2, *eae*A and *hly*. None of the colonies isolated from the river water samples were positive for *E. coli* O157:H7. CT-SMAC proved to have limited *E. coli* O157:H7 selective capabilities from samples such as sewage with high bacterial counts. Seeded sample experiments indicated that IMS is a suitable method for isolating *E. coli* O157:H7 from samples with high bacterial interference and low numbers of *E. coli* O157:H7. Evidence has been presented that the enrichment-IMS-selective agar procedure substantially increased the sensitivity of *E. coli* O157:H7 isolation compared to direct plating of test samples onto selective agar generally practised in the past.

**Keywords:** *Escherichia coli* O157:H7, immunomagnetic separation, river water, sewage

# Isolation and characterization of *Escherichia coli* O157:H7 and shiga toxin-converting bacteriophages from strains of human, bovine and porcine origin

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**Abstract** Toxin-converting bacteriophages encoding the Stx2 gene were induced from strains of *Escherichia coli* O157:H7 isolated from sewage, bovine and porcine faeces. Toxin synthesis can be stimulated by the induction of integrated toxin-converting phages from the host *E. coli* O157:H7 organism by ultra-violet (UV) exposure. The UV-mediated DNA damage of *E. coli* O157:H7 triggers a bacterial SOS response resulting in phage release. Free ranging phages outside their *E. coli* O157:H7 hosts were detected but could not be isolated directly from environmental samples such as sewage and river water. *E. coli* O157:H7 colonies carrying the genes coding for Stx2 were isolated from 1 sewage sample (0.76% of positive samples), 8 cattle faecal samples (16.67% of positive samples) and 2 pig faecal samples (14.28% of positive samples). Characterization of *E. coli* O157:H7 was done by repetitive sequence analysis using ERIC-PCR to determine the relationships between the individual *E. coli* O157:H7 strains. The ERIC-PCR analysis revealed distinct patterns for all *E. coli* O157:H7 strains with some small differences between the strains. DNA sequencing of some of the *E. coli* O157:H7 positive isolates carrying the Stx2 genes were performed confirming the amplified DNA nucleotide sequences of Stx2. Electron microscopic analysis revealed, for the first time in South Africa, that Stx2-converting phages induced from *E. coli* O157:H7 have different morphologies to that of phage lambda which was previously described. The role of the induced integrated Stx2 phages in natural environments such as river and dam water remains unclear. With the induction of Stx2-converting phages from environmental *E. coli* O157:H7 isolates, it is now possible to determine the potential of these phages to convert non-pathogenic *E. coli* strains and other enterobacteriaciae into pathogenic strains.

**Keywords** *E. coli* O157:H7; enterobacterial repetitive intergenic consensus; immunomagnetic separation; toxin-converting bacteriophages

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# Molecular Characterization of Astroviruses by Reverse Transcriptase PCR and Sequence Analysis: Comparison of Clinical and Environmental Isolates from South Africa

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**A comparative analysis was performed with 25 isolates of astroviruses (AstVs) detected in sewage sources and 22 concurrently identified clinical AstV isolates from the Tshwane (Pretoria) Metropolitan Area in South Africa. The samples and specimens were screened for AstVs by using an enzyme immunoassay and/or a reverse transcriptase PCR (RT-PCR) for the highly conserved untranslated region (3′ end) of the genome. The RT-PCR results were confirmed by oligonucleotide probe dot blot hybridization. Viable viruses were propagated in cell cultures for amplification when a minimal specimen was available or indeterminate sequences were obtained. AstV strains were characterized by RT-PCR and partial sequence analysis of the capsid region. The presence of multiple human AstV (HAstV) types in a single sewage sample complicated identification of individual strains, and additional type-specific RT-PCR and sequence analyses of the capsid region were required for characterization. Amplification and characterization of one genotype from a sample, therefore, did not preclude the possibility that a sample harbored additional different genotypes. Genotype and sequence information obtained from AstVs in wastewater samples were compared to information obtained from AstV strains from human stools. HAstV type 1 (HAstV-1), as well as HAstV-3, -5, -6, and -8, were identified among the clinical isolates, and HAstV-1, -2, -3, -4, -5, -7, and -8 were identified among the environmental samples. Phylogenetic analysis demonstrated that HAstV-1, -3, -5, and -8, which were present in human stool and sewage samples, clustered together, indicating that these viruses are closely related. The concurrent presence of identical HAstV strains in wastewater samples and in hospitalized patients suggests that AstVs present in the environment pose a potential risk to communities in which fecally contaminated water is used for recreational and domestic purposes.**

# Determination of cytotoxicity and invasiveness of heterotrophic plate count bacteria isolated from drinking water

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**Abstract** Evidence has been presented that some heterotrophic bacteria often detected in drinking water supplies possess features associated with pathogenicity. This suggests that even the low numbers of heterotrophic bacteria considered acceptable by drinking water specifications may constitute a health risk, particularly to immunocompromised consumers. In this study, 339 bacteria were isolated at random from routine heterotrophic plate count (HPC) tests on selected drinking water supplies in South Africa. In a first screen for potentially pathogenic properties, 188 of the isolates (55.5%) displayed α- or β-haemolysis on blood agar. Further analysis of the haemolytic isolates for enzymes associated with virulence revealed the presence of chondroitinase (5.3%), coagulase (16.0%), DNase (60.6%), elastase (33.0%), fibrinolysin (53.7%), gelatinase (62.2%), hyaluronidase (21.3%), lecithinase (47.9%), lipase (54.8%) and proteinase (64.4%) of the isolates. No fluorescein or pyocyanin was detected in any of the isolates. Among the haemolytic isolates 68.6% were resistant to oxacillin (1 µg), 59.6% to penicillin G (2 units), 47.3% to penicillin G (10 units), 53.7% to ampicillin (10 µg) and 42.6% to ampicillin (25 µg). Cytotoxicity, invasiveness and adherence properties of the haemolytic isolates was determined on HEp-2 and $Caco_2$ cell lines. Among the haemolytic isolates 96% were cytolytic on the HEp-2 cell line. All the haemolytic isolates adhered to HEp-2 and $Caco_2$ cells but gram-negative isolates tended to adhere in larger numbers than gram-positive isolates. HEp-2 cells were invaded by 42% of the haemolytic isolates. Heterotrophic bacteria, which most frequently revealed the above features associated with pathogenicity included species of the following genera: *Aeromonas, Acinetobacter, Aureobacterium, Bacillus, Klebsiella, Moraxella, Pseudomonas, Staphylococcus, Tsukamurella* and *Vibrio*. The results obtained in this study support earlier indications that bacteria detected by routine heterotrophic plate counts on drinking water supplies may include bacteria associated with potentially pathogenic properties. The extent to which these bacteria in drinking water supplies may constitute a health risk remains to be investigated.

**Keywords** Adherence; cytotoxicity; drinking water; health risk; heterotrophic plate count bacteria; invasiveness; virulence factors

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# Potentially pathogenic features of heterotrophic plate count bacteria isolated from treated and untreated drinking water

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**Abstract**

Heterotrophic plate counts (HPCs) are commonly used to assess the general microbiological quality of drinking water. Drinking water quality specifications worldwide recommend HPC limits from 100 to 500 cfu $ml^{-1}$. A number of recent studies revealed evidence that these bacteria may not be as harmless as generally accepted. It appears that immuno-compromised individuals are particularly at risk. This would include the very young and very old patients with diseases such as AIDS and patients on therapy for purposes such as organ transplantation and cancer treatment. In this study, 339 bacterial colonies were isolated at random from selected treated and untreated drinking water in South Africa using routine heterotrophic plate count tests. In a first step to screen for potentially pathogenic properties, 188 (55.5%) of the isolates showed α- or β-haemolysis on human- and horse-blood agar media. Subsequent analysis of the haemolytic isolates for enzymatic properties associated with pathogenicity revealed the presence of chondroitinase in 5.3% of the isolates, coagulase in 16.0%, DNase in 60.6%, elastase in 33.0%, fibrinolysin in 53.7%, gelatinase in 62.2%, hyaluronidase in 21.3%, lecithinase in 47.9%, lipase in 54.8% and proteinase in 64.4%. Fluorescein and pyocyanin were not produced by any of the isolates. Among the haemolytic isolates, 77.7% were resistant to oxacillin 1 μg, 59.6% to penicillin G 2 units, 47.3% to penicillin G 10 units, 54.3% to ampicillin 10 μg and 43.1% to ampicillin 25 μg. Cell culture studies revealed that 96% of haemolytic isolates were cytotoxic to HEp-2 cells, and 98.9% of the 181 cytotoxic isolates adhered to HEp-2 or Caco-2 cells. HEp-2 cells were invaded by 43.6%, and Caco-2 cells by 49.7%, of the 181 cytotoxic isolates. The invasion index on HEp-2 cells ranged from $1.9 \times 10^{-1}$ to $8.9 \times 10^{-6}$, whereas the invasion index on Caco-2 cells varied between $7.7 \times 10^{-2}$ and $8.3 \times 10^{-6}$. The most commonly isolated genera with these potentially pathogenic features were *Aeromonas*, *Acinetobacter*, *Aureobacterium*, *Bacillus*, *Chryseobacterium*, *Corynebacterium*, *Klebsiella*, *Moraxella*, *Pseudomonas*, *Staphylococcus*, *Tsukamurella* and *Vibrio*. The results obtained in this study support earlier findings on potentially pathogenic features of bacteria detected by routine HPCs on drinking water. These findings are in agreement with some epidemiological studies, which indicated an association between HPCs in drinking water and the incidence of gastroenteritis in consumers. However, the extent of the health risk concerned needs to be defined in more detail for meaningful revision of quality guidelines for HPCs in drinking water.


*Keywords:* Adherence; Cytotoxicity; Extracellular enzymes; HPCs; Invasiveness

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# Distribution of genotypes of F-specific RNA bacteriophages in human and non-human sources of faecal pollution in South Africa and Spain

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**Aims:** To assess whether the distribution of genotypes of F-specific RNA bacteriophages reflects faecal pollution of human and animal origin in water environments.

**Methods and Results:** Stool samples, animal feedlot waste slurries and a wide variety of faecally polluted waters were studied in South Africa and Spain. Genotyping was performed by plaque and spot hybridization with genotype-specific probes. Only genotypes II and III were detected in human stool. Animal faeces contained predominantly, but not exclusively, genotypes I and IV. Raw hospital and municipal sewage contained mostly genotypes II and III, whereas genotypes I and II prevailed in settled sewage, secondary treated sewage and non-point diffuse effluents from developing communities. Abattoir wastewaters contained mostly genotypes I and IV. No differences were observed between the distribution of genotypes in Spain and South Africa.

**Conclusions:** Although the association of genotypes II and III with human excreta and I and IV with animal excreta was statistically significant, the results suggest that the association cannot be used for absolute distinction between faecal pollution of human and animal origin.

**Significance and Impact of the Study:** This study contributes greatly to understanding the usefulness of genotypes of F-specific RNA bacteriophages in source tracking of faecal wastes.

# Evaluation of F-RNA coliphages as indicators of viruses and the source of faecal pollution

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## Abstract

It is a growing concern that analyses of true indicators of pathogenic viruses have not been properly established. Water treatment and catchment management strategies based on bacteriological indicators do not provide the necessary protection against viral infections because viruses are more persistent than bacteria in water environments. More than 100 types of human pathogenic viruses may be present in faecally polluted water, but only a small number can be detected by currently available methods. Analysing for enteric viruses such as the polio and hepatitis viruses is specialised and is neither practical nor commercially viable for a water utility. Human enteric viral disease is considered to be predominantly associated with human wastes, as opposed to animal wastes, and a distinction between these is desirable. Somatic coliphages are not always present whenever enteric viruses were detected and have been found to multiply in the environment outside the body. It has previously been shown that for monitoring purposes, F-specific RNA bacteriophages are model organisms and suitable indicators of the possible presence of human pathogenic enteric viruses as they behave like waterborne viruses. F-specific bacteriophages have been suggested as a useful alternative to the traditional bacterial indicators as their morphology and survival characteristics closely resemble those of some of the important human enteric viruses. F-specific RNA phages are becoming the indicators of choice for viruses in water and have been accepted as one of the rapid screening tests to determine the quality of water. Studies have shown that based on hybridisation tests, F-RNA coliphages could be associated with either a human or animal source. F-RNA coliphages have been grouped into four groups with Groups 2 and 3 being of human origin and Groups 1 and 4 originating from animals. This study aimed to fulfil the requirement of both developing a relatively simple method for F-RNA coliphage analyses in samples from the Umgeni Water catchment and further testing to elucidate whether the contamination was of animal or human origin using gene hybridisation techniques to type the F-RNA phages. The standard ISO method for the isolation of F-RNA coliphages was used and genotyping assays were performed according to methods adapted by the University of Pretoria's Medical Virology Department.

Highly specific nucleic acid probes for the detection of F-specific RNA bacteriophages were used in this study to fingerprint the origin of faecal pollution. The *E. coli* data generated for the sample sites indicated that certain sites tended to have more faecal pollution problems than others. Somatic coliphages were isolated in numbers as high as 1 100 pfu.10 $m\ell^{-1}$ and were consistently isolated in higher numbers than F-RNA coliphages. The strongest positive correlation ($r = 0.842$) was between *E. coli* and F-RNA coliphage concentrations. The F-RNA coliphages isolated from one river sample and wastewater works effluent hybridised with the GA probe belonging to Group 2, which was of human origin.

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# THE OCCURRENCE OF HEPATITIS A AND ASTROVIRUSES IN SELECTED RIVER AND DAM WATERS IN SOUTH AFRICA

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*(First received 29 March 2000; accepted in revised form 1 November 2000)*

**Abstract**—Over a period of one year (June 1997–May 1998) samples of surface waters, used for domestic and recreational purposes, were collected weekly from the same sites on the Klip River and Vaal Dam, Gauteng, South Africa. Sensitive and specific reverse transcriptase–polymerase chain reaction–oligonucleotide probe assays were used to detect HAV and HAstV RNA in concentrates of the water and infectious virus in cell cultures infected with the water concentrates. HAV was detected in 18 (35.3%) of the river and 19 (37.3%) of the dam water samples, often in association with the RNA from other enteric viruses. HAstV was detected less frequently and was present in 11 (21.6%) of the river and 3 (5.9%) of the dam water samples. A seasonal pattern was noted for HAV but not for HAstV. Cell culture amplification in a variety of carefully selected cell culture systems enhanced the detection of both viruses. Infectious viruses were detected in dam water samples where microbiological indicators of faecal pollution were absent or within acceptable limits. The presence of these viruses in the dam and river water could pose a potential health risk for people using these waters for domestic or recreational purposes.


*Key words*—hepatitis A virus, astroviruses, cell culture, RT–PCR, environmental water

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# Incidence of adenoviruses in raw and treated water

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**Abstract**

Adenoviruses are of major public health importance and are associated with a variety of clinical manifestations, i.e. gastroenteritis, eye infections and respiratory infections. The importance of water in the epidemiology of adenoviruses and the potential health risks constituted by adenoviruses in water sources and supplies are widely recognised. This study was conducted to assess the incidence of human adenoviruses in raw and treated water systems. Various raw and treated water were routinely monitored for the presence of adenoviruses, over a 1-year period (July 2000–June 2001). The supplies were derived from acceptable quality surface water sources using treatment processes, which conform to international standards for the production of safe drinking water. Adenoviruses were detected by firstly amplifying the viruses in cell cultures and then amplifying the extracted nucleic acids of these viruses using molecular techniques (nested PCR). The results indicated human adenoviruses present in 13 (12.75%) of the raw and 9 (4.41%) of the treated water samples tested. The combination of cell culture and nested PCR has proved to be a quick and reliable method for the detection of adenoviruses in water environments.


*Keywords:* Human adenoviruses; Molecular techniques; Raw water; Treated water

# Detection and rapid differentiation of human enteroviruses in water sources by restriction enzyme analysis

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**Abstract** The objective of this study was to assess the application and efficiency of molecular techniques for the detection and serotyping of enteroviruses from environmental water samples. Samples of water were collected at regular intervals upstream and downstream of an informal settlement. Techniques for the detection of enteroviruses included a reverse transcription polymerase chain reaction (RT-PCR), nested PCR (n-PCR) and Sabin-specific triplex PCR. A specific 297 bp fragment was amplified by the n-PCR and subjected to restriction enzyme (RE) analysis to differentiate between various serotypes of prototypical enteroviruses. Enteroviruses that gave inconclusive restriction patterns were typed by partial sequencing of the VP1 region. Results indicated a high incidence of enteroviruses, predominantly coxsackie B viruses. The results on polioviruses, as well as other enteroviruses, contributed valuable information on enteroviruses circulating in the community. The molecular approach described here proved suitable for the rapid, sensitive, specific and cost effective, simultaneous detection and typing of enteroviruses in water.

**Keywords** Enteroviruses; restriction enzyme analysis; RT-PCR; virus differentiation

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3 Müller EE, Grabow WOK and Ehlers MM. Biolog identification of non-sorbitol fermenting bacteria isolated on *E. coli* O157 selective CT-SMAC agar. Water SA *(submitted)*

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**Detection of enteroviruses in untreated and treated drinking water supplies in South Africa**

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**Abstract**
Enteric viruses have been detected in many drinking water supplies all over the world. A meaningful number of these supplies were treated and disinfected according to internationally acceptable methods. In addition, counts of bacterial indicators (coliform bacteria and heterotrophic plate count organisms) in these water supplies were within limits generally recommended for treated drinking water and these findings have been supported by epidemiological data on infections associated with drinking water. The shortcomings of conventional treatment methods and indicator organisms to confirm the absence of enteric viruses from drinking water, was generally ascribed to the exceptional resistance of these viruses. In this study, the prevalence of enteroviruses detected from July 2000 to June 2002 in sewage, river-, borehole-, spring- and dam water as well as drinking water supplies treated and disinfected according to international specifications for the production of safe drinking water was analysed. A glass wool adsorption-elution technique was used to recover viruses from 10 – 20 L of sewage as well as environmental water samples, in the case of drinking water from more than 100 L. Recovered enteroviruses were inoculated onto two cell culture types (BGM and PLC/PRF/5 cells) for amplification of viral RNA with nested-PCR being used to detect the amplified viral RNA. Results from the study demonstrated the presence of enteroviruses in 42.5% of sewage and in 18.7% of treated drinking water samples. Furthermore, enteroviruses were detected in 28.5% of river water, in 26.7% of dam/spring water and in 25.3% of borehole water samples. The high prevalence of coxsackie B viruses found in this study suggested, that a potential health risk and a burden of disease constituted by these viruses might be meaningful. These findings indicated that strategies, other than end-point analysis of treated and disinfected drinking water supplies, may be required to ensure the production of drinking water that does not exceed acceptable health risks. More reliable approaches to ensure acceptable safety of drinking water supplies may be based on control by multiple-barrier principles from catchment to tap using Hazard Assessment and Critical Control Point (HACCP) principles.

## Development of a multiplex-PCR assay for the detection of

# enterohaemorrhagic *Escherichia coli* and *Escherichia coli* O157:H7 virulence factors isolated from environmental and animal faecal samples

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## Abstract

EHEC infections have been associated with transmission by water and food. The most common clinical manifestations include haemorrhagic colitis (HC) and haemolytic uraemic syndrome (HUS), the most familiar cause of acute renal failure in children. Some EHEC serotypes cause clinical disease indistinguishable from that caused by *E. coli* O157:H7 but they appear to cause less bloody diarrhoea and less severe HUS. Methods used in the past to identify pathogenic *E. coli* were labour intensive, expensive and time consuming. However, molecular techniques have been developed which offer more practical approaches for the identification of EHEC and other groups of *E. coli*. These techniques are based on the genetic identification of the genes, which code for the virulence factors responsible for the pathogenicity of *E. coli*. A multiplex PCR (m-PCR) system for the rapid identification of EHEC and *E. coli* O157 virulence factors was developed by employing primers specific for the conserved sequences of the phage-encoded Shiga toxins 1 and 2 (Stx1 and Stx2), the outer membrane protein intimin, which is encoded by the *eaeA* gene, the plasmid-encoded enterohaemolysin (*Hly*) and *rfb*O157, which encodes the *E. coli* somatic antigen O157. These virulence factors were chosen based on the role they play in the pathogenicity of EHEC and *E. coli* O157:H7 bacteria. This m-PCR yielded amplification products ranging between 130 bp and 840 bp. The m-PCR was tested on 14 of the most important and frequently isolated human EHEC serotypes (O2, O26, O48, O91, O103, O111, O113, O117, O118, O121, O128, O145, O146 and O153). The m-PCR confirmed the presence of Stx1/Stx2, *eaeA* and *Hly* in these strains. Sensitivity studies indicated a virulence detection limit of 1 x $10^3$ cfu for *E.* coli O157:H7 and 1 x $10^4$ cfu for *E. coli* O145 to detect all their respective virulence factors. A total of 139 sewage (198 sorbitol-negative and 72 sorbitol-positive isolates), 127 cattle (176 sorbitol-negative and 412 sorbitol-positive isolates) and 340 pig faecal samples (40 sorbitol-negative and 829 sorbitol-positive isolates) were analysed for EHEC using the m-PCR. A total of 30/606 (4.95%) samples, which included 3/139 (2.16%) of sewage samples, 14/340 (4.12%) of pig faecal samples and 13/127 (10.24%) of cattle faecal samples were positive for 1 or more EHEC virulence factors. From these positive samples 81 out of 1313 isolates (6.17%) were virulence positive. Two cattle faecal samples (15 isolates) contained bacteria, which possessed the *eaeA*, *Hly*, Stx2 and *rfb*O157 genes. These colonies were confirmed as *E. coli* O157 by using O157-antisera. These results indicated that the EHEC m-PCR assay was suitable for the detection of the virulence factors associated with EHEC bacteria and especially *E. coli* O157:H7 in environmental samples such as sewage and animal reservoirs.

**Biolog identification of non-sorbitol fermenting bacteria isolated on *E. coli* O157 selective CT-SMAC agar**

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## Abstract

*E. coli* O157:H7 is recognised as an important human pathogen worldwide and has been associated with diseases such as haemorrhagic colitis (HC), haemolytic uraemic syndrome (HUS) and thrombotic thrombocytopaenic purpura (TTP) (Nataro and Kaper, 1998). Accurate laboratory detection of *E. coli* O157:H7 is important for diagnostic purposes and to justify epidemiological data on *E. coli* O157:H7. A well-known phenotypic characteristic of *E. coli* O157:H7 bacteria is their inability to ferment sorbitol. The selective capability of a selective medium used for the isolation of *E. coli* O157:H7 from environmental sources was investigated. This agar medium consisted of Sorbitol MacConkey agar (SMAC) supplemented with Cefixime-Tellurite (CT). The high number of false positive results obtained by a number of researchers when selectively screening for *E. coli* O157:H7 on CT-SMAC has prompted an investigation into which other sorbitol-negative bacteria could grow on CT-SMAC. All sorbitol-negative colonies obtained from CT-SMAC, after selective enrichment and IMS were used for bacterial identification using the Biolog microbial identification system (Biolog Inc., Hayward, Calif.). The majority of sorbitol-negative isolates were identified with Biolog as *Burkholderia*, *Pseudomonas*, *Vibrio* and *Aeromonas* spp. Only two *E. coli* O157:H7 isolates were identified with Biolog and confirmed with a polymerase chain reaction (PCR) specific for the shiga toxin 1 (Stx1) genes and with O157 and H7 antisera. The shortcomings of CT-SMAC agar medium are confirmed by the results of this study. These observations call for further improvement of affordable methods for the selective isolation of *E. coli* O157:H7 in the presence of large numbers of interfering bacteria capable of growing on CT-SMAC.

**Poliovirus vaccine strains in sewage and river water in South Africa**

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## Abstract

Since the initiation of the global poliomyelitis eradication program in 1988, the number of wild-type polio cases decreased from 350 000 to less than 500 cases and the number of polio endemic countries declined from more than 125 to 10. The last case of polio, caused by a wild-type poliovirus in South Africa occurred in 1989. The live attenuated oral poliovirus vaccine (OPV) has been effectively used in the reduction and control of poliomyelitis. However, as OPV strains are excreted in stools after vaccination, this vaccine could become a source of dissemination of polioviruses in the environment and the potential cause of poliomyelitis. The aim of the study was, therefore, to determine the occurrence of OPV strains in sewage and river water. During the period between 2001 and 2003, 138 samples of river water and 213 samples of settled sewage were collected from selected areas of South Africa. A total of 860 pfu.ml$^{-1}$ were analysed, most of which were isolated from sewage samples (703 pfu.ml$^{-1}$). Using a RT-multiplex PCR, 50 PVs were successfully distinguished from 176 non-polio enteroviruses (NPEVs). The majority of enteroviruses (EVs) present were typed as coxsackievirus CBV2 (28.4%), followed by echovirus ECV11 (21.6%) and CBV5 (14.2%), which was in agreement with the prevalence of these EVs in other parts of the world. The Sabin-specific RT-triplex PCR revealed the presence of 29 Sabin poliovirus type 1 (58%), 8 Sabin poliovirus type 2 (16%) and 13 Sabin poliovirus type 3 (26%). BGM and PLC/PRF/5 cell cultures allowed the amplification of a broad spectrum of EVs, whereas HEp-2 cells were more selective for PVs. This study addressed some of the issues regarding the prevalence of OPV strains in the environment. The identification of fifty viable OPV strains in this study confirmed the presence and circulation of vaccine strains of polioviruses in sewage and river water. The extent of the potential health risk constituted by these OPV strains remains to be investigated.

**PREVALENCE OF HUMAN ADENOVIRUSES IN RAW AND TREATED WATER**

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## Abstract

Human adenoviruses (HAds), of which there are 51 antigenic types, are associated aetiologically with gastro-intestinal, respiratory, urinary tract and eye infections. The clinical importance of HAds and the potential health risks constituted by HAds in water environments is widely recognised. This study was conducted to assess the use of an optimised integrated cell culture molecular-based technique to determine the prevalence of HAds in raw and treated drinking water supplies in South Africa. Selected supplies were weekly monitored for the presence of adenoviruses, over a one-year period (July 2001 to June 2002). Drinking water supplies were derived from acceptable quality surface water sources using treatment processes, which conform to international standards for the production of safe drinking water. Adenoviruses were detected by firstly amplifying the viruses in cell cultures and then amplifying the extracted nucleic acids using molecular techniques (nested PCR). HAds were detected in 29.8% (59/198) of the treated drinking water, 16% (8/50) of dam water and 44% (22/50) of river water samples tested. The results of this study confirm the presence of HAds in some raw and treated drinking water supplies in South Africa.

## RISK ASSESSMENT OF ADENOVIRUSES DETECTED IN TREATED DRINKING WATER AND RECREATIONAL WATER

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ABSTRACT

Adenoviruses have previously been detected in sewage and polluted river and dam water, as well as treated drinking water. The 51 strains of adenoviruses cause a wide range of infections with clinical manifestations associated with the gastrointestinal, respiratory and urinary tracts, and the eyes. Water may play a meaningful role in the transmission of many of these adenovirus strains, specifically the enteric adenoviruses which are transmitted via the faecal-oral route. The presence of these viruses in water used for drinking and recreational purposes is considered to constitute a potential health risk. In this study the risk of infection by adenoviruses detected over a period of one year in selected drinking water supplies, and river and dam water used for recreational purposes, was assessed. Adenoviruses were detected in 9 of 204 (4.41%) samples of two drinking water supplies (A and B) treated and disinfected according to international specifications, in 4 of 51 (7.8%) samples of river water, and 9 of 51 (17.7%) samples of dam water. Application of these results in an exponential risk assessment model indicated an annual risk of infection of $5.86 \times 10^{-2}$ and $1.9 \times 10^{-1}$ for drinking water supplies A and B respectively, assuming a daily consumption of two litres per day. The daily risk of infection constituted by adenoviruses in the river water was calculated as $1.93 \times 10^{-4}$, and in the dam water as $3.53 \times 10^{-5}$, assuming a consumption of 30 ml of water per day. The risk of infection exceed the acceptable risk of one infection per 10 000 consumers per year proposed for drinking water. However, the results for river and dam water used for recreational purposes were within the acceptable risk of one infection per 1000 bathers per day proposed for environmental waters used for recreational purposes.

*Keywords:* Adenoviruses, Risk assessment, River water, Treated drinking water

## APPLICATION OF A MOLECULAR METHOD FOR THE DETECTION OF GROUP

## A ROTAVIRUSES IN RAW AND TREATED WATER

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## Abstract

Group A human rotaviruses (HRVs) are the most important aetiological agents of acute viral gastroenteritis in infants and young children in both developing and industrialized countries. Rotaviruses are resistant to many chemical disinfectants and reportedly survive well in treated tapwater and sewage. In this study a group A specific reverse transcriptase-polymerase chain reaction (RT-PCR) followed by a nested-PCR has been applied for the detection of HRVs in raw and treated drinking water samples drawn at a water reclamation plant. For a period of two years (July 2000 to June 2002), borehole, raw and treated drinking water samples were collected weekly. Viruses were recovered from the water samples using a glass wool adsorption-elution technique followed by secondary concentration using precipitation with polyethylene glycol. In the first year of the study group A HRVs were detected in 11% of sewage samples, 15% of partially treated waters and in 12% of final treated drinking waters. The results of the second year of the study showed the presence of group A HRVs in 11% of sewage and untreated surface water samples, 15% of partially treated water, in 6.5% of final treated drinking waters. No HRVs were detected in the water samples from the boreholes. The presence of group A HRVs in treated drinking water samples suggests that this water could be a potential source of infection to consumers. The data also implies that either the water treatment does not remove HRVs or that the treated water gets contaminated post-treatment.

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

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**Abstract**

Waterborne hepatitis A virus (HAV) infection and outbreaks are well documented worldwide. HAV is endemic in South Africa (SA) but immunity to the virus differs between the different socio-economic groups. In SA, HAV has been detected in dam and river water sources but there are no data on the potential health risk to persons using these water sources for domestic and recreational purposes. This novel investigation, with many variables and uncertainties, estimates the risk constituted by HAV in river and dam water in SA by using a simple exponential risk assessment model. This mathematical model works with mean values and conservative assumptions, which therefore gives an over estimate of the potential health risk. From June 1997-2000 HAV was detected in 17.53% of river and 15.58% of dam water samples. Results indicated that the probability of infection ($P_{inf}$) to the higher socio-economic, non-immune population using the river water for recreational purposes is $1.09 \times 10^{-3}$ per day and $3.28 \times 10^{-1}$ per annum if 100 ml is ingested per day. For recreation in the dam water the $P_{inf}$ value was $1.17 \times 10^{-4}$ per day and $4.18 \times 10^{-2}$ per annum. According to the US EPA standard of 1 infection per 1000 recreational users, the calculated risk values are acceptable for these surface water sources. Risk values for drinking purposes (2 L/day) by the lower socio-economic population were ten-fold greater. However since nearly 100% of these individuals acquire immunity by 10 years of age, the potential risk may only be significant for the very young.

## 1.4 PUBLICATIONS IN PREPARATION

1 Van Heerden J, Ehlers MM, Grabow WOK and Heim A. Typing of adenoviruses detected in river and treated drinking water in South Africa.

2 Van Heerden J, Ehlers MM and Grabow WOK. Detection and risk assessment of adenoviruses in swimming pool water.

## DISSERTATIONS

1 Jongman, L (2002) Evaluation of methods for the recovery of polioviruses from water. BSc(Hons).

*2* Müller, EE (2004) Prevalence of *Escherichia coli* O157:H7 in South Africa. PhD *(in preparation).*

3 Nadan, S (2002) Characterisation of astroviruses from selected clinical and environmental settings. MSc.

4 Naus, TM (2002) The recovery and detection of hepatitis A virus and human astrovirus from irrigation waters and associated minimally processed foods. BSc (Hons).

5 Pavlov, DN (2004) Genomic mutations in oral poliovirus vaccine strains: Implications for the eradication of poliovirus. PhD *(in preparation).*

6 Potgieter, N (2004) Water storage in rural households: Intervention strategies to prevent waterborne diseases. PhD (*in preparation*).

7 Sundram A (2004) Assessment of F-RNA coliphage genotypes and faecal sterols to distinguish between faecal pollution of human and of animal origin in water from the Umgeni catchment area. MSc *(in preparation).*

8 Van der Linde, Marianne (2002) Rotavirus A occurrence in different selected water samples and its survival in the environment. BSc (Hons).

9 Venter JME (2004) Incidence of hepatitis A virus in water sources and the associated risk of infection in South Arica. MSc *(in preparation)*

10 Vivier, JC (2001) Molecular typing of enteroviruses. MSc (cum laude).

11 Wiliams, PJ (2002) Assessment of the anti-viral activity of ammonium oxifulvate. BSc (Hons)(cum laude).

12 Williams, PJ (2004) Hepatitis E virus in South Africa: Seroprevalence in swine and detection of the virus in swine stool specimens and waste water. MSc *(in preparation).*