OCCURRENCE OF PROTOZOAN PARASITES IN SOUTH AFRICAN SOURCE AND TREATED WATER

Report to the
WATER RESEARCH COMMISSION

by

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EXECUTIVE SUMMARY

Background

Giardia and *Cryptosporidium* are waterborne parasites which have been identified in many parts of the world as the most frequently occurring intestinal parasites and as the most common causes of water-related diarrhoea. These parasites cause acute, sporadic gastroenteritis in otherwise healthy people, particularly children, in both developed and under-developed countries, and in travellers, most of whom are adults. They can cause potentially fatal infections in the immunocompromised.

Contaminated water supplies have been shown to be a route for such infections and most of the reported outbreaks have been as a result of a breakdown in water treatment processes. *Giardia* cysts and *Cryptosporidium* oocysts are known to be highly resistant to environmental stress and can withstand extreme environmental conditions. Cysts and oocysts were also found to be more resistant to certain water purification processes than other bacterial indicators.

In recent years urban and rural settlements along waterways have become common in South Africa. Such settlements are often informal and have no infrastructure, resulting in increased levels of pollution of drinking water sources. Studies in other parts of the world have indicated that contamination of surface water by domestic sewage coincides with increased levels of parasites. In South Africa, very little information is available on the prevalence of these parasites in the population and in water sources.

Objectives

The major aims of this study were:

a. to develop, evaluate and apply concentration and detection methods for waterborne protozoan parasites *i.e., Giardia* and *Cryptosporidium.*

b. to study the occurrence of protozoan parasites in South African source and drinking waters, and to evaluate various treatment processes to determine their efficacy in the removal of protozoan parasites.

c. to evaluate commonly used indicator organisms for their ability to indicate possible contamination by protozoan parasites.

Results and conclusions

The applicability of various enumeration techniques for protozoan parasites in water was evaluated. Different systems for the concentration of 10ℓ and 100ℓ samples were compared, using water samples seeded with *Giardia muris* cysts. All experiments were carried out using *Giardia muris* cysts isolated and purified in our laboratory. The

cyst concentration in the seeded samples was kept very low to allow for the simulation of levels which may occur in polluted surface or drinking water.

Initial experiments compared the recovery of *Giardia* cysts after concentration of 10ℓ samples by either membrane filtration or ultrafiltration. Similar recoveries were obtained after the processing of seeded water samples using ultrafiltration (recoveries ranged between 2.7% and 25.5%) and membrane filtration (recoveries between 4.5% and 23%). The ultrafiltration method has the advantage of co-processing the same water sample for the analysis of enteric viruses and protozoan parasites, thus reducing the cost of analysis significantly.

It is, however, very important to concentrate large volume water samples, especially for drinking water, where low numbers of parasites are expected. Risk assessment studies, assessing fitness for use of both drinking water and recreational water, have also indicated the need for evaluating large volume water samples for the detection of protozoan parasites.

Cuno wynd cartridge filters are the most commonly used for concentration of large volume samples for the enumeration of protozoan parasites. Cuno wynd filters were evaluated and recoveries ranging from 1.6% to 46% were obtained. The recovery range found for the Cuno wynd filters in this study was similar to that reported in the literature. Erratic recoveries, as found in this study, have previously been reported for *Cryptosporidium* oocysts using Cuno wynd filters.

Due to a shortage in the supply of Cuno wynd filters in South Africa, a comparative study evaluating the Cuno wynd filter against the more readily available Cuno wound cartridge filter was initiated. A set of experiments was done in which *Giardia* cysts were seeded into 100ℓ of PBS and filtered using both the Cuno wynd and Cuno wound filters. Results showed recoveries ranging from 31% to 43%, with an average recovery of 37% for the Cuno wynd filters. Recoveries ranging between 33% and 67% with an average recovery of 55% were recorded for the Cuno wound filters. The concentration procedure using AMF Cuno wound cartridge filters proved effective and practical for the enumeration of protozoan parasites as indicated by the higher recovery rates recorded. The recovery range found for the Cuno wound filters was relatively narrow and consistent recovery rates were obtained. The wound filters did not clog as rapidly as the wynd filters, which is an advantage when surface water samples are processed. It should also be noted that the Cuno wound filters are cheaper which leads to a significant cut in the cost per analysis. Based on these results the Cuno wound filters were used in all further studies.

Results have indicated that where the presence of both viruses and protozoan parasites has to be investigated, Filterite cartridge filters (100ℓ samples) can be used, thus shortening the time of sample preparation and reducing the cost per analysis. The efficacy of utilising the same cartridge filter for the enumeration of both enteric viruses and protozoan parasites from large volume water samples was done by concentrating seeded 100ℓ water samples and recoveries ranging between 5% and 24.2% were obtained.

The occurrence of *Giardia* cysts and *Cryptosporidium* oocysts in South African raw and treated water was investigated by studying source and treated water obtained from the following water purification works: Potchefstroom, Parys, Goldfields Water (Balkfontein), Wallmannstal, Western Transvaal, Rietvlei, Schoemansville, Temba, Rand Water and Umgeni Water. Samples were collected throughout the year at all the plants to allow for seasonal variation.

Large volumes (100ℓ) of both raw and treated water samples were concentrated using Cuno wound cartridge filters. Samples were taken at the same location at each of the sampling points, in order to give accurate and representative counts for comparison of results. A Hydrofluor Combo kit from Meridian Diagnostics was used for identifying presumptive *Giardia* cysts and *Cryptosporidium* oocysts. This kit provides for the simultaneous detection of *Giardia* cysts and *Cryptosporidium* oocysts from environmental sources. The kit contains monoclonal antibodies which target the cysts and oocysts of the two protozoan parasites.

Many South African untreated source waters were found to be contaminated with both *Giardia* cysts and *Cryptosporidium* oocysts. Raw water samples taken at all the water purification works tested positive for cysts and oocysts and only a small percentage of raw water samples were free of protozoan parasites. The average levels of *Giardia* cysts detected in surface water samples varied between 0 and 197 cysts/100ℓ and the average *Cryptosporidium* oocyst levels varied between 10 and 198 oocysts/100ℓ.

All the final treated water samples evaluated in the study were free of *Cryptosporidium* oocysts, while only one treated water sample showed the presence of *Giardia* cysts (4 cysts/100ℓ).

The efficacy of water treatment plants in removing protozoan parasites from source water was evaluated by studying water from two water purification works at different stages of treatment with special emphasis on flocculation, sand filtration and final chlorination. The presence of *Giardia* cysts and *Cryptosporidium* oocysts was investigated at various stages of the treatment at the Temba and Schoemansville water purification works. Samples (100ℓ) of the raw water, water collected after flocculation and sand filtration and the final treated water was concentrated using Cuno wound cartridge filters.

At Shoemansville 100% of the raw water samples tested positive for the presence of *Giardia* cysts, with concentrations ranging between 10 and 400 cysts/100ℓ. The cysts were effectively removed by flocculation and sand filtration and no *Giardia* cysts were detected in any of the treated water samples. *Cryptosporidium* oocysts were observed in 41% of the raw water samples, but were removed effectively and none of the treated water samples contained oocysts.

Seventy percent of the raw water samples collected at Temba water works contained *Giardia* cysts, which were effectively removed during treatment. *Cryptosporidium* oocysts were found in 67% of the raw water samples. All the final water samples tested negative for the presence of both *Giardia* cysts and *Cryptosporidium* oocysts.

As a result of the high cyst and oocyst concentrations detected in South African source waters, care should be taken to ensure that treatment plants are functioning effectively. *Cryptosporidium* outbreaks have been reported where water has undergone treatment, including coagulation, sedimentation, sand filtration and chlorination, but due to poor operational practices oocysts were not inactivated or removed. It is also important to test the final treated water from small plants and facilities where sand filtration is not used for the presence of protozoan parasites.

At present no simple test can be used routinely to evaluate the occurrence of protozoan parasites in water. It was therefore decided to determine whether current microbial indicators of water quality, such as total and faecal coliforms, can be used to give an indication of the presence of protozoan parasites in water. Water samples were collected at the Schoemansville and Temba water purification works and tested for the presence of routinely used indicators *i.e.* total coliforms and faecal coliforms, including standard plate counts. Raw water, water after flocculation and sand filtration as well as the final treated water were evaluated for the presence of indicator organisms. All stages of treatment were included to compare the survival of the indicator organisms throughout the process with the behaviour of the protozoan parasites.

In this study the parasites were completely removed, while the coliforms were still present after treatment. In most cases the bacterial indicators were present in high numbers in the raw water samples, while no *Giardia* or *Cryptosporidium* could be detected. From the results obtained it can be seen that total and faecal coliforms are not good indicators of the presence of protozoan parasites in water. These findings are in agreement with results obtained by other researchers, who also demonstrated that coliforms are inadequate indicators of the presence of pathogens, especially viruses and parasites.

The suitability of *Candida albicans* and *Clostridium perfringens* as indicators was also investigated. Water samples at the four different stages of treatment were collected at the Schoemansville and Temba water works. From the results obtained in the study it is clear that these organisms are inadequate indicators of the presence of protozoan parasites in water, as both *Candida* and *Clostridium* survive the treatment process better than the parasites. Therefore, only direct monitoring can be relied on for determining the presence of *Giardia* and *Cryptosporidium* in water.

Recommendations

Current South African water quality guidelines do not recommend limits for *Giardia* cysts and *Cryptosporidium* oocysts in water. As conventionally used microbial indicators can not be used to give an indication of the presence of protozoan parasites in water, it is of great importance to develop water quality guidelines regarding the presence of these parasites in water, particularly drinking water. We recommend that drinking water should contain no *Giardia* cysts/100ℓ and no *Cryptosporidium* oocysts/100ℓ. It is also recommended that water samples should be evaluated on a monthly basis.

Methods for the detection of *Giardia* and *Cryptosporidium* can be improved. Several reports indicate that alternate enumeration and detection techniques yield higher recoveries. These methods should be evaluated and incorporated in current methods if found to be applicable. A disadvantage of the methods currently being used for the detection of protozoan parasites in water, is that one can not distinguish between viable and non-viable cysts and oocysts. Attention should be given to the development of assays to determine the viability of protozoan parasites in water supplies.

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This project, entitled **The occurrence of protozoan parasites in South African drinking water**, was funded by the Water Research Commission.

The Steering Committee responsible for this project consisted of the following members:

Mrs APM Oelofse	Water Research Commission
Mr G Offringa	Water Research Commission
Dr MJ Pieterse	Water Research Commission
Mr FP Marais	Water Research Commission (Secretary)
Prof WOK Grabow	University of Pretoria
Dr J Frean	SA Institute for Medical Research
Mr W v d Merwe	Department of Health
Mr FS Vivier	Department of Health
Mr IW Bailey	Umgeni Water
Ms CME de Wet	Rand Water
Dr R Kfir	Division of Water Technology, CSIR

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Thanks are also due to the staff of the water purification plants who granted permission for sampling and kindly assisted with sample collection.

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2. PRESENTATIONS AND VISITS

1. Contact with international experts working in the field of detection of protozoan parasites in water were established. These include:

a. Dr Walter Jakuboswki
Dr Frank Schaefer
EPA, USA

b. Dr Mark LeChevallier
Dr Morteza Abbaszadegan
American Water Works Services
USA

c. Prof Joan Rose
University of South Florida
USA

d. Prof Charles Gerba
University of Arizona
USA

e. Dr David Casemore
Public Health Laboratory
UK

f. Dr Eric Carrington
WRC
UK

2. The following papers and posters were presented:

a. A paper *The occurrence of protozoan parasites in South African water* was presented by Dr Kfir at the WISA Conference, Durban, May 1993.

b. Dr Kfir presented a paper *" Studies evaluating the applicability of utilising the same concentration techniques for the detection of protozoan parasites and viruses in water"* and a poster *" Studies on the prevalence of Giardia cysts and Cryptosporidium oocysts in South African water"* at the IAWQ Conference in Budapest, Hungary, during July 1994.

3. International laboratories visited:

a. During May 1993 Dr Kfir visited the laboratories of Prof Joan Rose at the University of Southern Florida, Tampa, Florida to discuss detection methods for protozoan parasites. The methodology utilized in this laboratory is similar to the large volume concentration method utilized in our laboratory. The

1. INTRODUCTION

Giardia and *Cryptosporidium* are waterborne parasites which have been identified in many parts of the world as the most frequently occurring intestinal parasites and as the most common causes of water-related diarrhoea. These parasites cause acute, sporadic gastroenteritis in otherwise healthy people, particularly children, in both developed and under-developed countries, and in travellers, most of whom are adults. They can cause potentially fatal infections in the immunocompromised (Casemore, 1990).

Contaminated water supplies have been shown to be a route for such infection and most of the reported outbreaks have been as a result of a breakdown in water treatment processes. *Giardia* cysts and *Cryptosporidium* oocysts are known to be highly resistant to environmental stress and can withstand extreme environmental conditions. Cysts and oocysts were also found to be more resistant to certain water purification processes than other bacterial indicators (Rose *et al.*, 1990).

In recent years urban and rural settlements along water courses (rivers and dams) have become common in South Africa. Such settlements are often informal and have no infrastructure, resulting in increased levels of pollution of drinking water sources. Studies in other parts of the world have indicated that contamination of surface water by domestic sewage coincides with increased levels of parasites.

In South Africa very little information is available on the prevalence of these parasites in the population and in any water sources or potable water supplies. Initial studies indicated that both *Giardia* cysts and *Cryptosporidium* oocysts occur in South African raw and treated effluents as well as in surface water utilized as drinking water. The Department of National Health and Population Development has been supporting studies in this field as part of a study evaluating sewage purification processes. The presence of the protozoan parasites in drinking water samples has been detected by the Health Programme in isolated investigations where diarrhoeal outbreaks were reported in South Africa.

The major aims of this study are:

a. to evaluate, develop and apply concentration and detection methods for waterborne protozoan parasites *i.e., Giardia* and *Cryptosporidium.*

b. to study the occurrence of the protozoan parasites in South African source and drinking waters and to evaluate various treatment processes to determine their efficacy in the removal of protozoan parasites.

c. to evaluate commonly used indicator organisms for their ability to indicate possible contamination by protozoan parasites.

enumeration of the parasites is done using Meridian antibodies and no phase contrast microscopy is carried out on a routine basis.

b. Dr Kfir attended the Annual Meeting of the American Society for Microbiology, Atlanta, Georgia (May 1993) and discussed the subject of protozoan parasite detection with a number of researchers. There are several laboratories investigating the use of gene probe and polymerase chain reaction (PCR) technology for the detection of both cysts and oocysts in water.

c. During May 1994, Ms Gericke visited the laboratories of Dr CP Gerba at the University of Arizona, Tucson, Arizona; Dr W Jakubowski at the EPA, Cincinnati, Ohio; Dr Morteza Abbaszadegan at the American Water Works Services, Belleville, Illinois and Dr Eric Carrington at the WRc, Medmenham, UK to discuss the detection of protozoan parasites.

These laboratories use similar concentration techniques to those used in our laboratory. The enumeration of cysts and oocysts is done using fluorescent antibodies. New methods for detection, e.g. PCR and gene probes are being investigated. Cyst and oocyst viability is also being studied and the American Water Works Sevices is investigating new staining methods to test for viability.

d. During the same visit Ms Gericke attended the 94th Annual Meeting of the American Society of Microbiology in Las Vegas, Nevada. Contact was made with researchers working on protozoan parasites.

4. A literature study on available concentration and detection methodologies for *Giardia* cysts and *Cryptosporidium* oocysts in water was updated and summarised in Appendix A.

3. MATERIALS AND METHODS

3.1. Inoculation and Purification of *Giardia* Cysts

Giardia muris cyst stock was kindly provided by Dr Schaefer, American Environmental Protection Agency, Cincinnati, USA. Mice were obtained from the South African Institute for Medical Research.

3.1.1. Inoculation of Cysts

Two to three week old female C3H/HE mice were inoculated by intubation of the inoculum (1000 cysts/0.1mℓ) into the lower oesophagus or stomach. The mice were housed in groups of ten in mesh bottomed, suspended cages with transfer pans. Twenty mice were inoculated weekly to maintain a continuous cyst cycle.

3.1.2. Faecal Matter Collection

The cysts were harvested on the seventh day post inoculation, when cyst shedding is at its maximum. The day before faecal matter collection, the inoculated mice (20 per cage) were placed in clean, wire mesh bottomed suspension cages with transfer pans. Paper towelling was put at the bottom of the transfer pans, dampened with water and placed under the cages to collect the mice faeces. The faeces was collected no more than 24 h after the transfer pans have been placed under the cages. Using a spatula, all faecal matter was transferred from the paper towel to a sieve.

3.1.3. Purification of Cysts

The faeces was sieved to get rid of as much of the fine, unwanted food particles as possible. The contents of the sieve was transferred onto a flat surface and the faeces transferred to a beaker containing 50 mℓ of 0.01 % Tween-20 solution. The faeces was homogenized by mixing with a glass rod or spatula until the mixture resembled a thick paste. This was placed into a 150 mesh sieve, filtered into a container and washed through a sieve with 500 mℓ 0.01% Tween-20 solution. All faecal slurry was collected in a container. The sieved faecal slurry was further concentrated by centrifugation at 500g for 6 minutes in 250 mℓ round bottomed centrifugation bottles. Pellets were resuspended and pooled by resuspending in 420 mℓ 0,01% Tween-20 solution. The cysts were separated from other faecal debris using sucrose flotation. Aliquots (20 mℓ) of the faecal pellet suspension were carefully overlaid onto 20 mℓ of 1.0 M sucrose solution in 50 mℓ centrifuge bottles and centrifuged at 800g for 10 minutes in a swinging bucket rotor. The layer above the Tween/ sucrose interface was discarded and the interface and about 20 mℓ of the Tween/sucrose layer from each bottle was collected in a large beaker. *Giardia* cysts are concentrated at the interface. This mixture was diluted with fresh 0.01% Tween-20 solution to 1 000 mℓ and the *Giardia* cysts were re-concentrated by centrifugation at 500g for 10 minutes. This procedure was repeated twice in order to wash the concentrated cysts.

In cases where the pellets contained an undesirable density of contaminants in addition to the desired *Giardia* cysts, further purification was carried out using an additional sucrose gradient step. In these cases the *Giardia* cysts were re-concentrated over a layer of 20 mℓ 1.0 M sucrose in 50 mℓ bottles and centrifuged at 800g for 10 minutes. The interface cyst layer was collected, centrifuged at 800g for 10 minutes and then washed twice in 0.01% Tween- 20 solution.

3.1.4. Cyst Yield

On average 10^7-10^8 cysts were harvested from 20 infected mice in one day. Cyst viability upon harvesting was very high as observed under a phase contrast microscope and using fluorogenic dyes. Fresh cysts (not older than a week) were reinoculated into the mice on a weekly basis.

3.1.5. Cyst storage

Clean cyst suspensions were stored at 4°C, supplemented with a solution of

antibiotics, containing both penicillin and streptomycin, to inhibit grazing of the cysts by faecal bacteria which are attached to some of the cyst walls.

3.2. Concentration Systems for the Concentration of Large Volumes of Water Samples

Concentration facilities for 100ℓ of water, using cartridge filters were established in our laboratory. These facilities can be used on-line directly from a tap or for field applications. The apparatus layout for all cartridge filters in use is basically identical except for additional proportioners which are used for increased absorption when using cartridge filters for the enumeration of both protozoan parasites and enteric viruses. The cartridge filters are set into specially designed holders and are attached to an inert system of Teflon pipes which is combined through Snaptite connectors made of stainless steel. The filter holders are made of blue polypropylene caps and acrylic bowls and the top cap is screwed into the bottom part of the holder (holder made by Opella Ltd). The system uses an o-ring seal and can be sterilised by chlorination. For field application a power supply is attached to the unit. After filtration the cartridges are transferred to the laboratory and backwashed for the removal of the test organisms i.e protozoan parasite cysts and oocysts or/and enteric viruses.

Various cartridge filters and elution equipment were acquired. Cartridge filters that are commonly used world-wide for concentration of water samples for the enumeration of protozoan parasites were tested. In addition methods for the enumeration of protozoan parasites and enteric viruses were set up and tested, in order to evaluate the possible use of the same concentrate for the enumeration of both protozoan parasites and enteric viruses.

The AMF Cuno wynd (DPPPY1) and wound (P10P1) cartridge filters were tested for the enumeration of protozoan parasites. The DPPYI filter has a core of a polypropylene support and a covering blanket of finely woven polypropylene with a nominal pore size of 1μm. The blanket is supported with wynd strings which do not affect the flow rate. The filter dimensions are 25.4 cm in length and 6.5 cm in width. The P10P1 filter has similar dimensions and an identical core but the filter is not made as a blanket, but from separately wound strings that cover the core. It resembles a cylindrical ball of string. The cut-off point is also identical to the wynd (DPPPY1) filter i.e. 1μm. The difference between the two filters is their clogging potential and possible breakthrough. According to the manufacturer the P10P1 filter has a larger breakthrough potential and might clog sooner than the DPPPY1 filter. The P10P1 filter is cheaper and more readily available.

Filterite DNF, 0,45-10 μm cartridge filters were used for the concentration of both enteric viruses and protozoan parasites. The filter is made of pleated micro-fibreglass and its core material is made of polypropylene. The filter pore size is 0.45 μm and the filter dimensions are 25.4 cm in length and 6.6 cm wide. The area of filtration available is about 0.75 m^2.

3.3. Evaluation of the Various Concentration Methods Using Seeded Water Samples

The applicability of various enumeration techniques for protozoan parasites in water was evaluated. The concentration of seeded water samples was carried out using different water sample volumes and filtration methods.

All experiments were carried out using *Giardia muris* cysts isolated and purified in our laboratory. The age of the cysts used for each experiment varied, but in general, cysts not older than two and a half weeks were used. The cyst stock was counted before every experiment in order to calculate the number of cysts to be seeded.

The cyst concentration in the seeded samples was kept very low to allow for the simulation of levels which may occur in polluted surface or drinking water. This resulted in a final concentration of between 3-30 cysts per mℓ of water. Cysts were counted before and after concentration using a haemacytometer and viability was observed under phase contrast. Percentage recoveries were calculated as the number of cysts recovered after concentration, divided by the original number of cysts seeded, multiplied by 100.

3.3.1. Membrane filtration

Ten litres of seeded water sample were put into an Amicon 40M stainless steel tank and passed through a flat membrane (Milipore pure size 1.2μm) using a Sartorius filter holder and air pressure. Filtration was carried out within approximately 3 minutes per 10ℓ of water sample. After filtration all residual liquid in the tank was collected, the filter was removed and placed in the collected liquid and sonicated for 10 minutes in a water bath. The filter was cleaned further with the help of a rubber policeman and the deposit and the sonicated solution were collected and further concentrated by centrifugation at 2 100 rpm for 6 minutes. The pellet was investigated for the presence of *Giardia* cysts and the percentage recovery of seeded cysts was calculated.

3.3.2. Amicon flat membrane ultrafiltration

Ten litres of the seeded water sample were passed through an Amicon XM50, 150mm diameter, flat membrane using positive pressure. The membranes were obtained from Amicon Ltd. and have a pore size of 46-50 Å and a molecular cut-off of 50 000 daltons. The water sample was put into a 20ℓ stainless steel reservoir and passed through inert polypropylene tubing into a cell using 1.3 bar positive nitrogen pressure. In the cell (still under pressure) the sample was passed through the Amicon membrane filter with constant stirring. Fluid remaining in the reservoir was collected by means of a syringe and centrifuged at 2 000 rpm for 1 minute after which the supernatant was discarded and the pellet retained. The membrane was then eluted with 20mℓ Eagle's minimum essential medium (Earle's salts), using a latex rubber policeman, and the resultant fluid added to the centrifuge tube containing the former pellet. The latter was mixed well and centrifuged for 1 minute at 2 000 rpm. The supernatant was again discarded and the pellet was used to study the percentage

recovery of the seeded cysts. In studies where both protozoan parasites and enteric viruses are investigated the supernatant is used for the enumeration of viruses.

3.3.3. AMF Cuno wynd (DPPPY1) and wound (P10P1) cartridge filters

The method of filtration is identical for both the wynd and the wound filters. One hundred litres of seeded water sample were passed through the filter at an approximate rate of 3 litres per minute. After filtration, the filters (either wynd or wound) were removed from their housings and all residual liquid left in the holder was collected. The filters were cut longitudinally. Cut segments varied in size between 5 and 7 cm. The wynd filter was teased apart, while the cut segments of the wound filter were vigorously shaken loose.

The cut segments were thereafter placed in a Sputnik MKII pressure washing machine along with the residual sample from the housing. An aliquot of 10 mℓ of Tween-80 (1%)/sodium dodecyl sulphate (1%) solution was added. This was followed by rotation of the machine by hand for 30 seconds. The entire mass of cut filter segments and residual water was removed and placed into a Zyliss Salad Spinner (Switzerland) and spun dry. The residual water collected at the bottom of the Zyliss was transferred to centrifuge canisters (250 mℓ) and further concentrated by centrifugation at 2 400 rpm for 10 minutes. The whole wash process was repeated several times if the cut filter segments were not considered clean enough. The washed filter segments were then discarded. After centrifugation the supernatant was discarded and the final pellet volume was recorded and further evaluated for cyst recovery. The number of cysts per equivalent volume was calculated.

Alternatively, instead of using the washing machine, the filter segments plus the residual water sample collected from the filter holder were placed in a special freezer bag (pre-sterilised) which could hold up to 3ℓ of liquid. The bag was placed in a stomacher (Jencons, Lab Blender 3 500) and was processed for 3 minute periods over a period of 15 minutes. The bag was removed from the stomacher and the liquid drained into a sterile beaker. The cysts were then concentrated by centrifugation at 2 400 rpm for 10 minutes. The pellet containing the cysts was used for microscopical evaluation of cyst recovery.

3.3.4. Filterite DFN 0,45-10µm cartridge filter

One hundred litres of the seeded water sample were passed through the cartridge filter at a rate of approximately 3 litres per minute, while introducing a solution containing 6,66 g of aluminium chloride ($AlCl_3$), 7,9 g of sodium thiosulphate ($Na_2S_2O_3$) and 20 mℓ hydrochloric acid (HCl) per hundred litres. This is mainly for viruses. In order to remove all concentrated cysts a backwashing procedure was carried out. The filter was washed through with 3ℓ of glycine eluent (11,36 g/3ℓ), with a pH of 9,5 (adjusted with sodium hydroxide), in the opposite direction to the normal flow while occasionally shaking the cartridge filter vigorously. The resulting fluid was subsequently passed through an Amicon XM 50 ultrafilter which was eluted with 20mℓ

Eagle's minimum essential medium (Earle's salts), using a latex rubber policeman, and centrifuged for 1 minute at 2 000 rpm. The procedure described is used for the concentration of water samples for viruses and was followed in order to study the use of the same filter for the enumeration of both enteric viruses and protozoan parasites. The supernatant was discarded (can be used for viral analysis) and the pellet was studied for the recovery of *Giardia* cysts.

3.4. Evaluation of the Applicability of Using AMF Cuno Wound Cartridge Filters instead of AMF Cuno Wynd Filters

Due to problems in obtaining wynd filters in South Africa, a study evaluating the Cuno wynd filters against the more readily available wound filters was initiated.

The recovery of *Giardia* cysts after concentration of 100ℓ seeded samples was evaluated. *Giardia* cysts (10^6) were seeded into 100ℓ phosphate buffered saline (PBS). The method of concentration described in paragraph 3.3.3 was followed.

3.5. Identification of *Giardia* Cysts and *Cryptosporidium* Oocysts from Environmental Samples

A Hydrofluor Combo kit from Meridian Diagnostics (Cincinnati OH, USA) was used for identifying presumptive *Giardia* cysts and *Cryptosporidium* oocysts. This kit provides for the simultaneous detection of *Giardia* cysts and *Cryptosporidium* oocysts from environmental sources. The kit contains monoclonal antibodies which target the cysts and oocysts of the two protozoan parasites. The reaction is made visible by the addition of a FITC-conjugated, fluorescent anti-immunoglobulin. This kit utilizes the principle of indirect immunofluorescence whereby the prepared pellet is incubated with murine monoclonal antibodies directed against surface antigen components of *Giardia* cysts and *Cryptosporidium* oocysts and labelled with a FITC-conjugate. Unbound conjugate is rinsed away and the preparation is counterstained with Evans Blue in order to study morphological details. The filters are mounted with glycerol phosphate buffered saline or commercially available glycerol, and the entire filter is examined under an epifluorescent microscope. *Cryptosporidium* oocysts and *Giardia* cysts are identified by the bright, apple-green fluorescence on their outside walls and their typical size and characteristic shape.

3.6. The Occurence of Protozoan Parasites in South African Source and Treated Water

The following water purification works were selected for evaluating the occurrence of protozoan parasites in their raw and treated water:

(i) Potchefstroom
(ii) Parys
(iii) Goldfields Water-Balkfontein

(iv) Wallmannstal
(v) Western Transvaal
(vi) Rietvlei
(vii) Schoemansville
(viii) Temba
(ix) Rand Water
(x) Umgeni Water

Large volumes (100ℓ) of both raw and treated water samples were concentrated using Cuno wound cartridge filters. Enumeration and detection of both *Giardia* cysts and *Cryptosporidium* oocysts were carried out as described in paragraphs 3.3.3. and 3.5. All water purification works, unless otherwise stated, were sampled and analysed at monthly intervals to allow for possible seasonal variation. Samples were taken at the same location at each of the sampling points, in order to give accurate and representative counts for comparison of results.

3.7. Evaluation of the Efficacy of Treatment Processes for the Removal and Elimination of Protozoan Parasites

The presence of *Giardia* cysts and *Cryptosporidium* oocysts was investigated at various stages of the treatment at the Temba and Schoemansville water purification works. Samples (100ℓ) of the raw water, water collected after flocculation and sand filtration and the final treated water were concentrated using Cuno wound cartridge filters as previously described (Paragraph 3.3.3.). The cysts and oocysts were detected using the Hydrofluor Combo kit (Paragraph 3.5).

3.8. Evaluation of the Efficacy of Commonly Used Indicator Microorganisms for the Indication of the Presence of Protozoan Parasites in Water

The applicability of using an indicator organism to give an indication of the presence of *Giardia* cysts and *Cryptosporidium* oocysts in water was investigated. Water samples were collected at the Schoemansville and Temba water purification works and tested for the presence of routinely used indicators i.e. total coliforms and faecal coliforms, including standard plate counts. Raw water, water after flocculation and sand filtration as well as the final treated water were evaluated for the presence of indicator organisms. All stages of treatment were determined to compare the survival of the indicator organisms throughout the process with the behaviour of the protozoan parasites.

Standard plate counts were carried out using the pour plate method (APHA, 1989). The plates were incubated at 37°C for 2 days. The samples were examined for the presence of the indicator bacteria using membrane filtration (APHA, 1989). Total coliforms were enumerated on m-Endo agar Les (37°, 24h) and faecal coliforms on m-FC agar (44.5°C, 24h),(Grabow, 1990).

3.9. Evaluation of the Applicability of Using *Candida albicans* and *Clostridium perfringens* as Indicators of the Presence of Protozoan Parasites in Water

The applicability of using *C. perfringens* and *C. albicans* as indicators of the presence of protozoan parasites was studied. Water samples at the four different stages of treatment, as mentioned in paragraph 3.8, were collected at the Schoemansville and Temba water works.

Following membrane filtration, *C. perfringens* was enumerated on Perfringens agar base supplemented with TSC selective supplement (Oxoid) (Burger *et al.*, 1984). The plates were incubated at 42° C, 24h in anaerobic jars containing gas generating kits. *C. albicans* was incubated on potato dextrose agar at 37° C for 2 days (Oxoid).

3.10. Statistical Analysis

The results were analysed using the statistical analysis programme, Statgraphic version 7.1. A non-parametric statistical analysis was used to evaluate the correlation between the protozoan parasites and the indicator organisms (Spearman rank correlation).

4. RESULTS

4.1. Evaluation of Different Enumeration Methods for Protozoan Parasites

A number of comparative studies were carried out. Initial experiments compared the recovery of *Giardia* cysts after concentration of 10ℓ samples by either membrane filtration or ultrafiltration.

Table 1 summarises the results of a set of experiments in which *Giardia* cysts were seeded into 10ℓ of water and concentrated through membrane filtration. The percentage recovery obtained ranged between 4.5% and 23%.

TABLE 1: Recovery of *Giardia* cysts using membrane filtration

Age of cysts	Concentration cysts/mℓ	Total sample volume (ℓ)	% Recovery
2½ weeks	28	10	4.5
2½ weeks	28	10	4.5
2½ weeks	28	10	6.3
8 days	29	10	20.4
8 days	29	10	23.0
8 days	29	10	8.2

To evaluate the efficacy of concentration by ultrafiltration, the same volume of water (10ℓ) was seeded with the same number of cysts/mℓ. As shown in Table 2 the recoveries utilizing ultrafiltration ranged between 2.7% and 25.5%.

To assess not only the efficacy of the recovery of cysts after concentration of water samples using cartridge filters, but also the advantage of concentrating water samples which are 10 times larger in volume, the same concentration of cysts as used for the 10ℓ studies was maintained when evaluating the use of cartridge filters unless otherwise indicated.

TABLE 2: Recovery of *Giardia* cysts using ultrafiltration

Age of cysts	Concentration cysts/mℓ	Total sample volume (ℓ)	% Recovery
2½ weeks	28	10	8.2
2½ weeks	28	10	2.7
2½ weeks	28	10	8.0
8 days	29	10	25.5
8 days	29	10	17.0
8 days	29	10	8.5

The first set of experiments evaluated the use of Cuno wynd filters. These are the most commonly used filters for the concentration of large volume water samples for the enumeration of protozoan parasites worldwide. The results showed recoveries from 1.6% to 46% (Table 3). The low recoveries could only partially be explained by the initial age of the seeded cysts. When only a few cysts per mℓ were seeded an overall reduction in recovery rate was observed. This may have been due to the counting accuracy and efficacy which might have been affected by the low concentration of cysts recovered.

TABLE 3: Recovery of *Giardia* cysts using Cuno Wynd cartridge filters and the Sputnik washing machine

Age of cysts	Concentration cysts/mℓ	Total sample volume (ℓ)	% Recovery
2½ weeks	28	100	20.9
2½ weeks	28	100	21.4
2½ weeks	28	100	15.71
8 days	29	100	5.1
8 days	29	100	5.1
8 days	29	100	6.4
5 days	19	100	36.4
5 days	19	100	27.4
5 days	19	100	10.9
13 days	3	100	4.4
13 days	3	100	8.0
14 days	5	100	2.9
14 days	5	100	3.9
15 days	7	100	46.0
15 days	7	100	3.8
16 days	4	100	1.6
16 days	4	100	1.6
17 days	4	100	2.3
17 days	4	100	2.8

As elution of cysts from the Cuno cartridge filters can be done by different washing procedures which may affect cyst recoveries, a comparison between our method in which a manually driven washing machine was used, with the method described in the literature where a stomacher was used, was carried out. The results of processing the sample by a stomacher indicated a similar range of recoveries (Table 4) to that found for the samples processed by the washing machine (Table 3).

TABLE 4: Recovery of *Giardia* cysts using Cuno Wynd cartridge filters and a stomacher

Age of cysts	Concentration cyst/mℓ	Total sample volume (ℓ)	% Recovery
2½ weeks	28	100	32.4
2½ weeks	28	100	11.1
2½ weeks	28	100	0

The applicability of using Cuno wound filters for the enumeration of protozoan parasites was evaluated. Table 5 summarizes the results of a set of experiments in which 100 *Giardia* cysts/mℓ were seeded into 100ℓ of PBS and filtered using both the Cuno wynd and Cuno wound filters. The results showed recoveries ranging from 31% to 43% with an average recovery of 37% for the Cuno wynd filters. Recoveries ranging between 33% and 67% with an average recovery of 55% were shown for the Cuno wound filter. The Cuno wound filters were therefore used for the concentration of parasites in all further experiments.

TABLE 5: Percentage recovery of *Giardia* cysts utilizing Cuno Wynd and Wound cartridge filters

Sample*	Cuno Wynd Filter	Cuno Wound Filter
1	37	67
2	43	50
3	31	63
4	33	33
5	41	60

* 100ℓ PBS seeded with 100 cysts/mℓ

The efficacy of utilising the same cartridge filter for the enumeration of both enteric viruses and protozoan parasites from large volume water samples was done by concentrating seeded 100ℓ water samples. The results are summarised in Table 6. Recoveries ranged between 5% and 24.2%. The age of the seeded cysts did not affect the recovery significantly.

TABLE 6: Recovery of *Giardia* Cysts Using the Filterite Cartridge Filter

Age of cysts	Concentration cysts/mℓ	Total sample volume (ℓ)	% Recovery
5 days	19	100	16.1
5 days	19	100	11.1
13 days	3	100	24.2
13 days	3	100	16.1
14 days	5	100	11.1
14 days	5	100	10.2
15 days	7	100	5.0
15 days	7	100	7.9
16 days	4	100	9.9
16 days	4	100	21.3
17 days	4	100	13.0
17 days	4	100	15.7

4.2. The Occurrence of *Giardia* Cysts and *Cryptosporidium* Oocysts in South African Raw and Treated Water

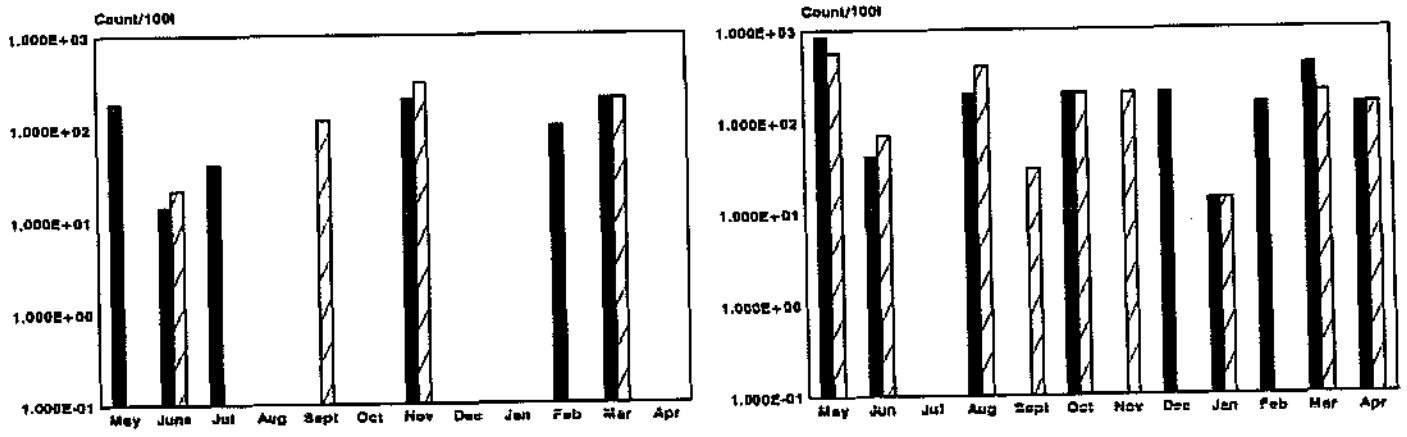
The occurrence of *Giardia* cysts and *Cryptosporidium* oocysts in South African raw and treated water was investigated by studying source and treated water obtained from ten water purification works. Samples were collected throughout the year at all the plants to allow for seasonal variations, unless otherwise stated.

No *Cryptosporidium* oocysts were found in any of the final treated water samples tested. *Giardia* cysts (4 cysts/100ℓ) were found in only one final treated water sample from Wallmannstal water purification works. Final treated samples collected at all the other water works tested negative for the presence of *Giardia* cysts.

Figure 1 (A-J) summarizes the occurrence of *Giardia* cysts and *Cryptosporidium* oocysts in raw water sources from the different water purification works studied.

Giardia cysts were present in the raw water obtained from all the water purification works except at Umgeni (Fig 1J). Umgeni, however was only sampled 5 times during the year. *Giardia* cysts were present throughout the year in raw water from Schoemansville, Wallmannstal, Parys and Potchefstroom (Figs 1G,E,B,C). At Rietvlei, *Giardia* cysts were detected from May to August (Fig 1D).

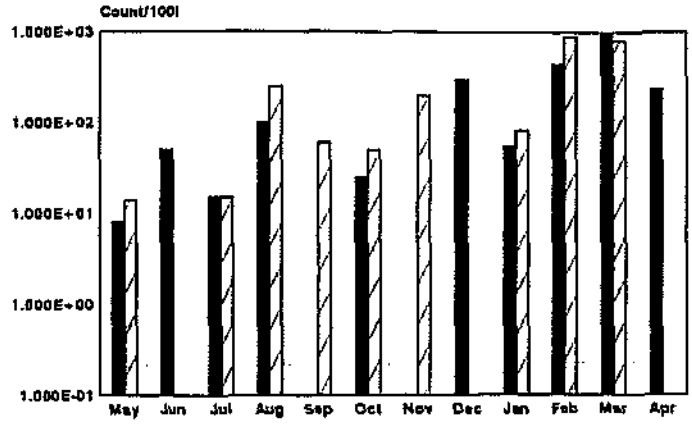
Cryptosporidium oocysts were only observed during March at Umgeni (Fig 1J) and during March and May in water from Western Transvaal (Fig 1F). Oocysts were also present in raw water from the other water works.



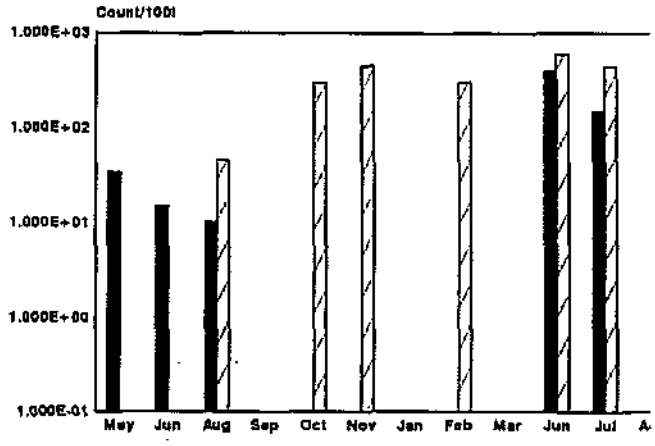
1A: Balkfontein 1B: Parys

Fig.1. The occurrence of protozoan parasites in South African raw waters

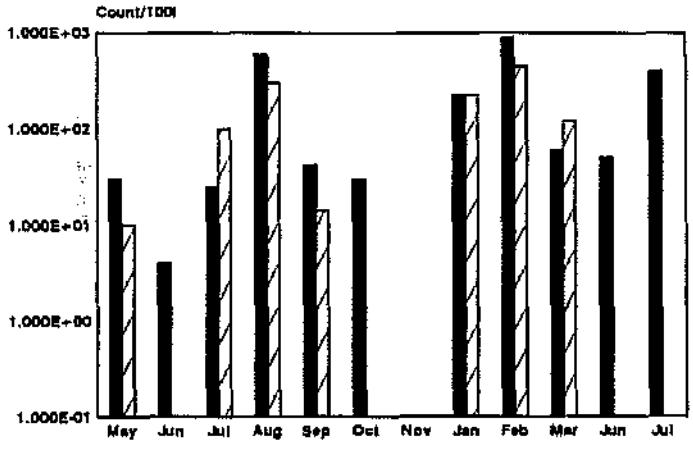
■ *Giardia* ▨ *Cryptosporidium*



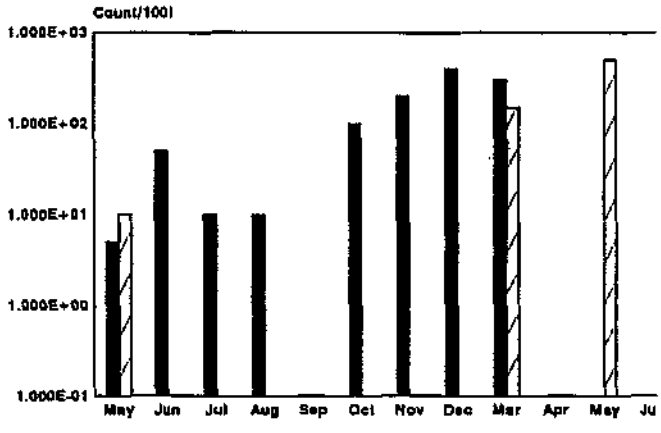
1C: Potchefstroom



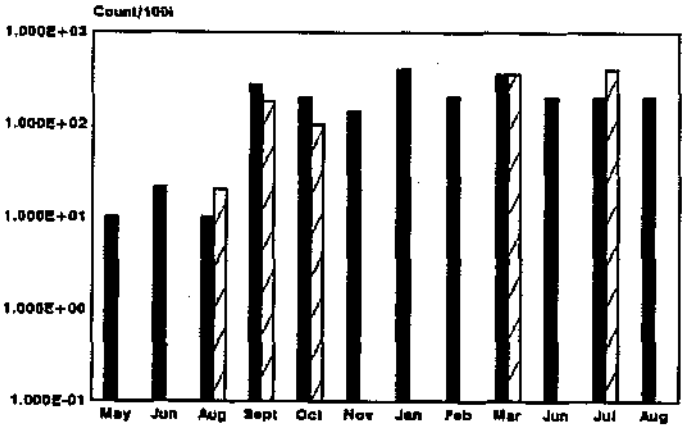
1D: Rietvlei



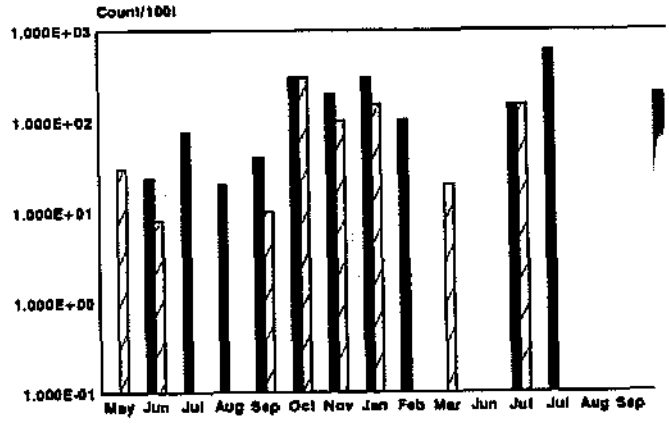
1E: Wallmannstal



1F: Western Transvaal



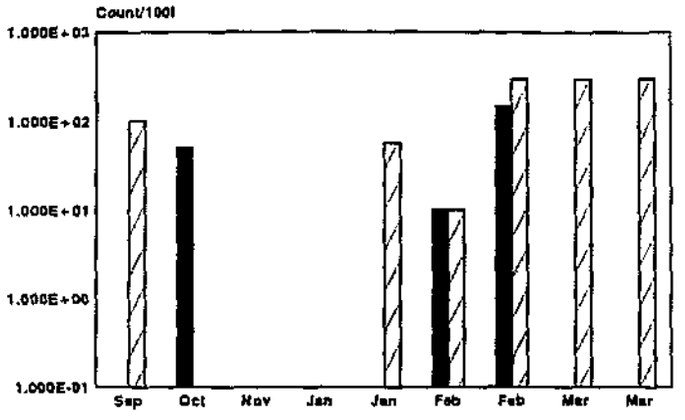
1G: Schoemansville



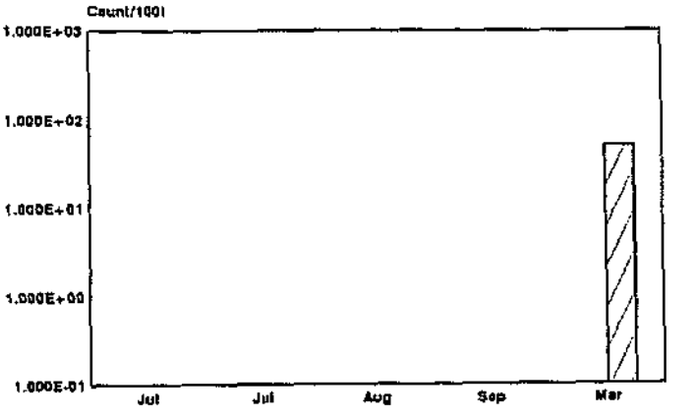
1H: Temba

Fig.1 (continues) The occurrence of protozoan parasites in South African raw waters

■ *Giardia* ▨ *Cryptosporidium*



1I: Rand Water



1J: Umgeni Water

Fig.1 (continues) The occurrence of protozoan parasites in South African raw waters

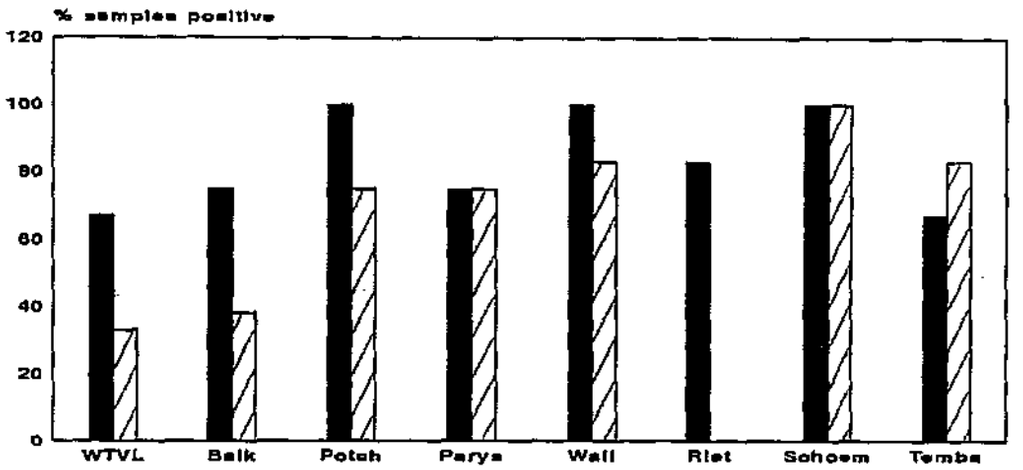
■ *Giardia* ▨ *Cryptosporidium*

Surface water samples were sorted by season (May to August as winter and September to April as summer). The percentage of raw water samples positive for the presence of *Giardia* and *Cryptosporidium*, both during winter and summer, was determined for each of the water works (Fig 2A, B). Rand Water and Umgeni were not included in this evaluation, because samples were taken only during summer at Rand Water, while Umgeni was only sampled five times.

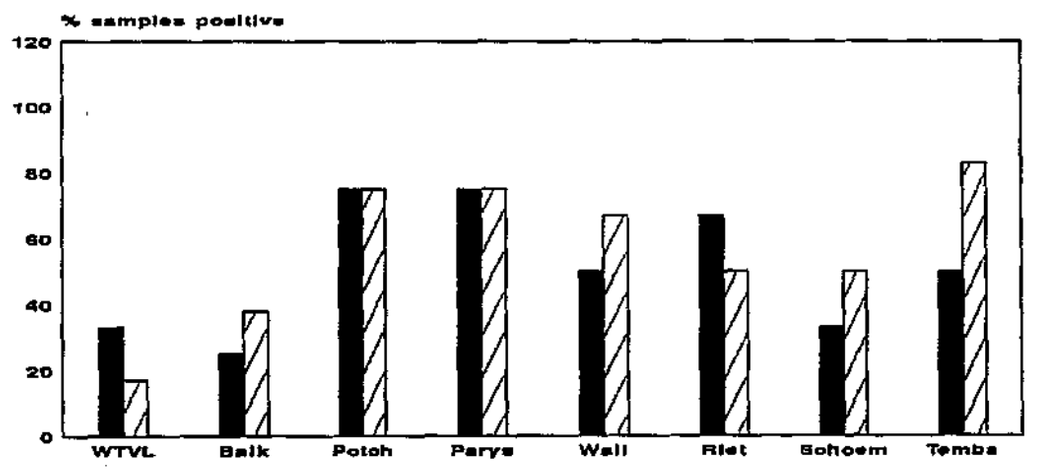
At five of the water works evaluated, the percentage of samples positive for the presence of *Giardia* was higher during winter than in summer. However more raw water samples contained cysts during summer than winter at Temba and at Rietvlei, no *Giardia* cysts could be detected during summer (Fig 2A). At four of the water purification works, the percentage of samples positive for *Cryptosporidium* was higher during summer than winter, while 75% of all samples (both during winter and summer) tested at Parys and Potchefstroom contained oocysts (Fig 2B).

Fig 3A and B give an indication of the average counts for cysts and oocysts recorded at the different water purification works during winter and summer. The average number of cysts obtained was higher during winter in only two cases, namely at Parys and Rietvlei. At all the other water works higher cyst counts were recorded during summer (Fig 3A). Average counts for *Cryptosporidium* oocysts were higher during winter at Western Transvaal, Parys and Rietvlei. At the other five water works higher counts were observed during summer (Fig 3B). From the results it seems that at the majority of water works, higher average counts for both cysts and oocysts are recorded during the summer period.

Due to the limited number of observations and spread of counts it was not possible to conduct statistical studies to determine seasonal variations. Different seasonal variations were also shown at different water purification plants.



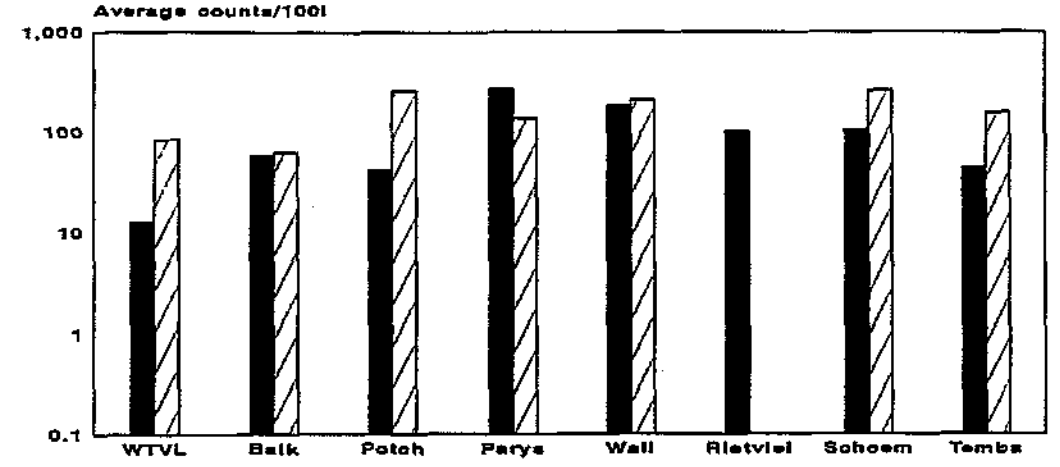
A: *Giardia*



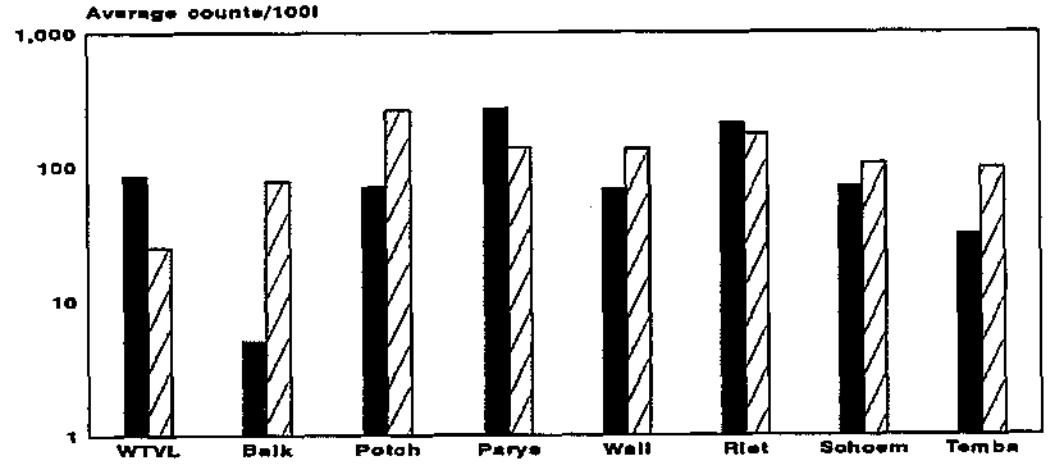
B: *Cryptosporidium*

Fig.2. Percentage samples positive for *Giardia* and *Cryptosporidium* during winter and summer

■ Winter ▨ Summer



A: *Giardia*



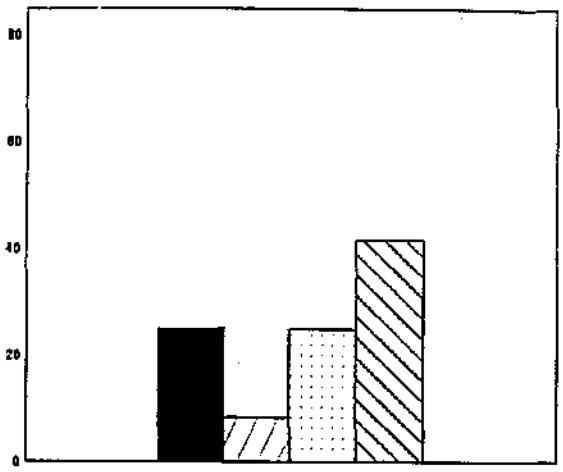
B: *Cryptosporidium*

Fig.3. Average counts for *Giardia* and *Cryptosporidium* at different water works during winter and summer

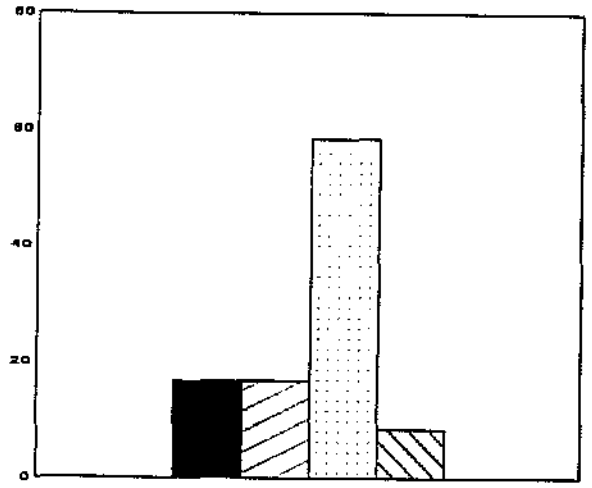
■ Winter ▨ Summer

Fig 4 (A-J) gives an indication of the percentage samples positive for *Giardia* only, *Cryptosporidium* only, as well as samples containing both parasites and samples where no parasites were detected. Both protozoan parasites, i.e. *Giardia* cysts and *Cryptosporidium* oocysts, occurred simultaneously in 58% of the raw water samples collected at Parys, Wallmannstal and Potchefstroom (Figs 4B,E,C). The highest percentage of raw water samples free from both cysts and oocysts were recorded at Balkfontein and Umgeni (Fig 4A, 41%, 12 samples; Fig 4J, 80%, 5 samples, respectively).

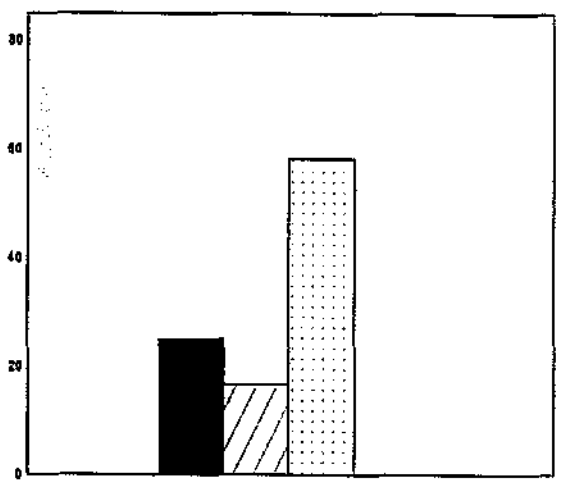
With the exception of Western Transvaal (Fig 4F, 50%), Schoemansville (Fig 4G, 58%) and Wallmanstal (Fig 4E, 33%), the percentage of samples positive for only *Giardia* cysts never exceeded 25%. Rietvlei (Fig 4D, 33%) and Rand Water (Fig 4I, 40%) showed the highest percentage of samples containing only *Cryptosporidium* oocysts and the percentage of samples positive for only *Cryptosporidium* oocysts varied between 0 and 20% for all other water purification works evaluated.



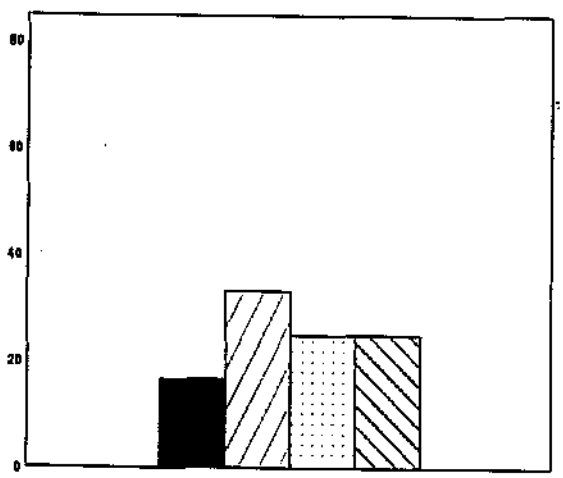
4A: Balkfontein



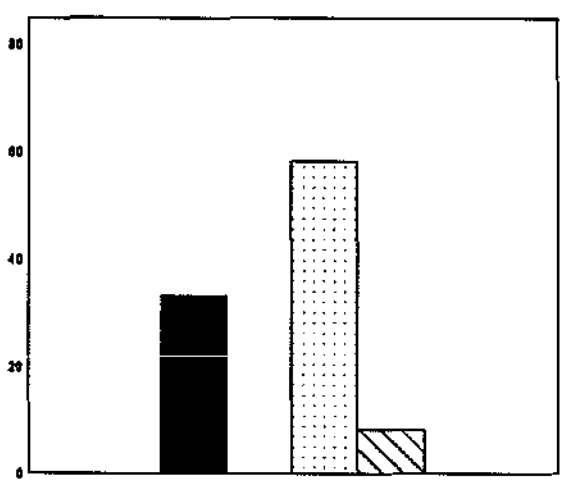
4B: Parys



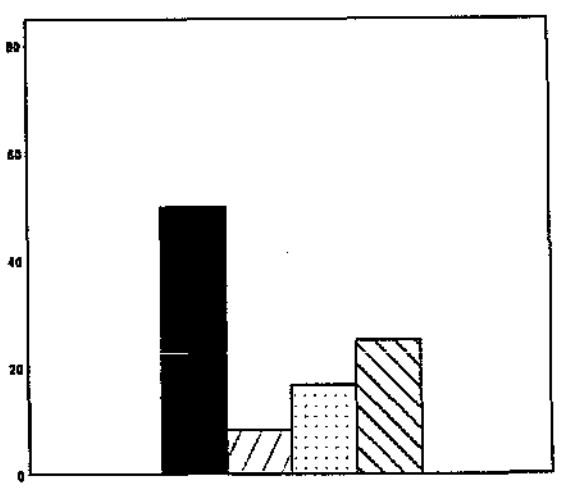
4C: Potchefstroom



4D: Rietvlei



4E: Wallmannstal



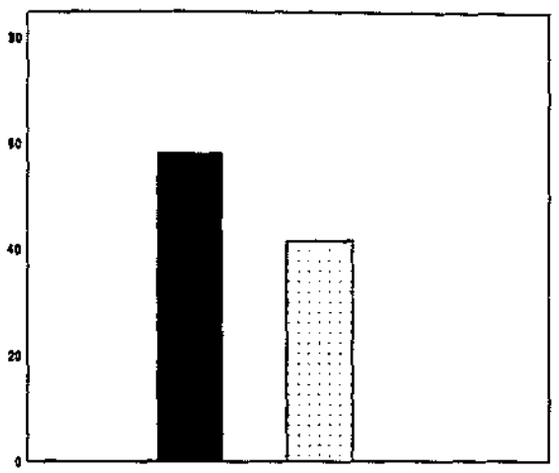
4F: Western Transvaal

Fig.4. The occurrence of protozoan parasite cysts and oocysts in raw water

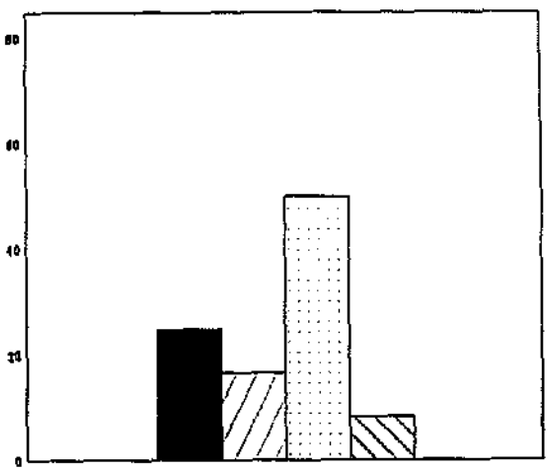
■ G only ▨ C only ⊞ G+C ▧ No G or C

G= *Giardia*

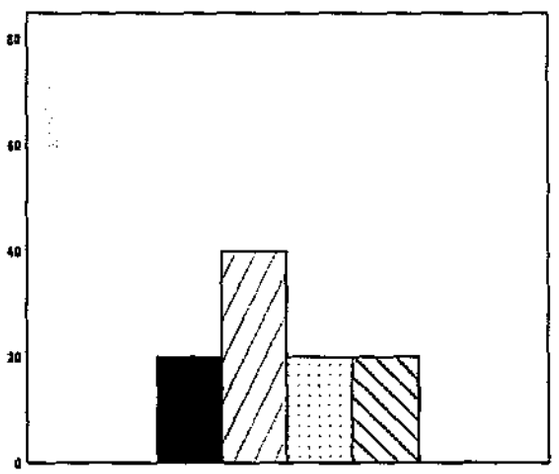
C= *Cryptosporidium*



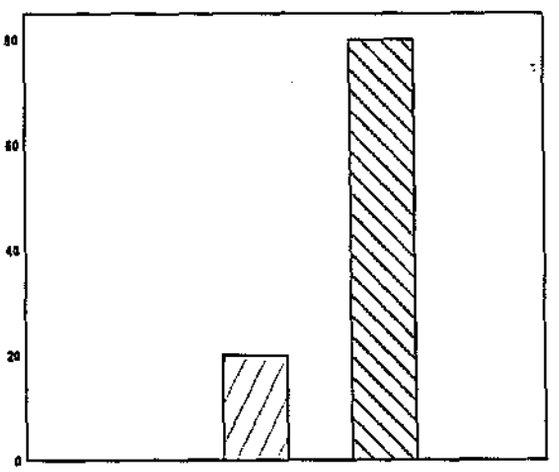
4G: Schoemansville



4H: Temba



4I: Rand Water



4J: Umgeni Water

Fig.4 (continues) The occurrence of protozoan parasite cysts and oocysts in raw water

■ G only ▨ C only ⊡ G+C ⊠ No G or C

G=*Giardia*
C=*Cryptosporidium*

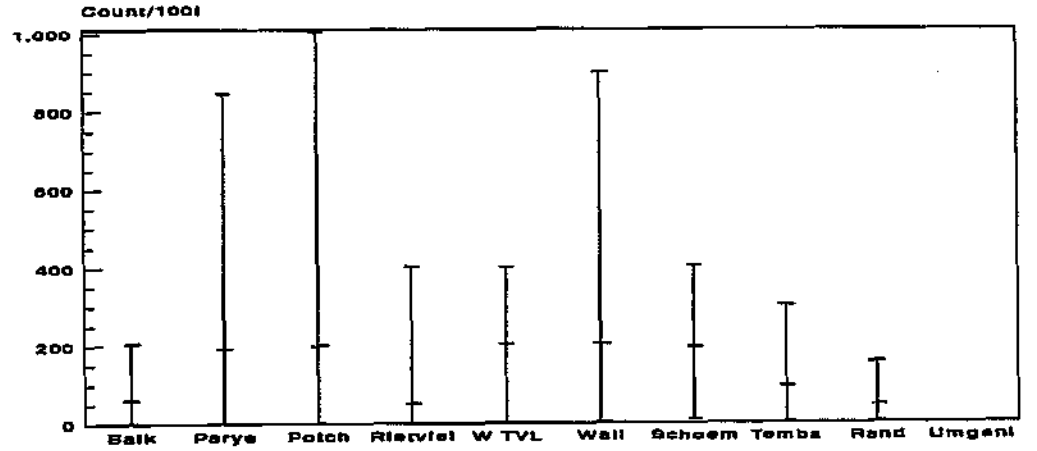
Table 7 and Fig 5A and B are summarizing the ranges of *Giardia* cysts and *Cryptosporidium* oocysts in South African raw waters. Minimum, maximum and average counts per 100ℓ samples are given.

The average levels of *Giardia* cysts detected in surface water samples varied between 0 and 197 cysts/100ℓ and *Cryptosporidium* oocyst levels between 10 and 198 oocysts/100ℓ were recorded. High maximum counts for both cysts and oocysts were shown at Parys (840 and 560, respectively), Potchefstroom (1000 and 900, respectively) and Wallmanstal (900 and 450, respectively). At Rietvlei, a maximum count of 600 oocysts/100ℓ was recorded (Table 7, Fig 5A,B).

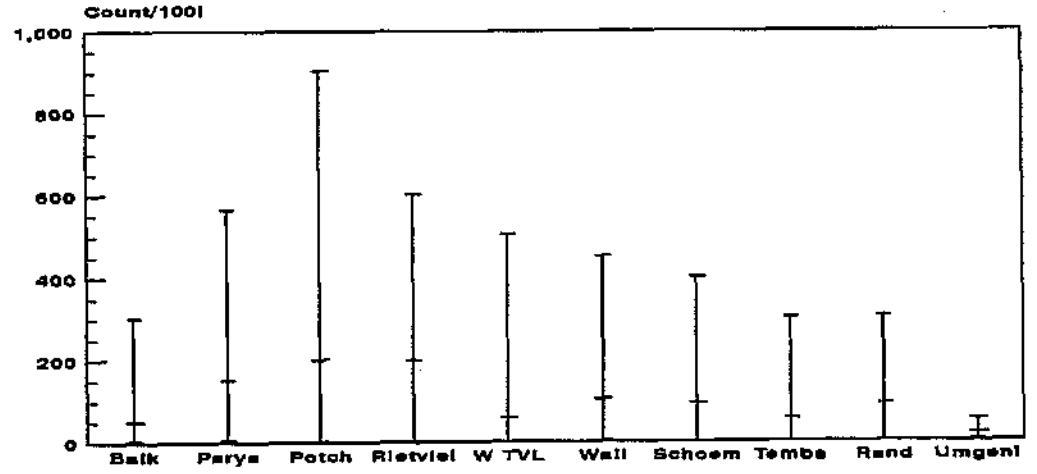
At Balkfontein, Parys and Potchefstroom the average numbers of *Giardia* cysts and *Cryptosporidium* oocysts detected were similar, whereas the average oocyst counts exceeded the cyst counts at Rietvlei, Rand Water and Umgeni Water. Higher average cyst levels than oocyst levels were recorded at Western Transvaal, Wallmannstal, Schoemansville and Temba (Table 7, Fig 5A,B).

The levels of *Giardia* cysts detected at Potchefstroom varied between 0 and 1000 cysts/100ℓ (average: 187 cysts/100ℓ) and at Wallmannstal between 0 and 900 cysts/100ℓ with an average of 197 cysts/100ℓ. A much narrower range was detected at Rand Water (0-150 cysts/100ℓ; average: 31 cysts/100ℓ) and at Balkfontein (0-200 cysts/100ℓ; average: 61 cysts/100ℓ) (Table 7, Fig 5A,B).

The widest range for oocyst levels was observed at Potchefstroom (0-900 oocysts/100ℓ) with an average of 198 oocysts/100ℓ. At Umgeni the levels ranged between 0 and 50 oocysts/100ℓ (only 5 samples tested) (Table 7, Fig 5A,B).



5A: *Giardia*



5B: *Cryptosporidium*

Fig.5. Minimum, maximum and average counts of cysts and oocysts per 100ℓ water

TABLE 7: The ranges of *Giardia* cyst and *Cryptosporidium* oocyst counts in South African raw waters

Water sample		*Giardia* cysts/100ℓ	*Cryptosporidium* oocysts/100ℓ
Balkfontein	Minimum	0	0
	Maximum	200	300
	Average	61	53
Parys	Minimum	0	0
	Maximum	840	560
	Average	183	152
Potchef-stroom	Minimum	0	0
	Maximum	1000	900
	Average	187	198
Rietvlei	Minimum	0	0
	Maximum	400	600
	Average	51	191
Western Transvaal	Minimum	0	0
	Maximum	400	500
	Average	90	55
Wallmannstal	Minimum	0	0
	Maximum	900	450
	Average	197	102
Schoemansville	Minimum	10	0
	Maximum	400	400
	Average	183	88
Temba	Minimum	0	0
	Maximum	300	300
	Average	101	64

Table 7 (continued)

Water sample		*Giardia* cysts/100ℓ	*Cryptosporidium* oocysts/100ℓ
Rand Water	Minimum	0	0
	Maximum	150	300
	Average	31	107
Umgeni Water	Minimum	0	0
	Maximum	0	50
	Average	0	10

4.3. Evaluation of the Efficacy of Treatment Processes for the Removal and Elimination of the Protozoan Parasites.

The efficacy of parasite removal at various stages of the treatment with special emphasis on flocculation, sand filtration and final chlorination was evaluated at two water purification works, *i.e.* Schoemansville and Temba.

4.3.1. *Schoemansville*

Fig 6 is a schematic presentation of the different stages of treatment at the Schoemansville water works. The raw water is flocculated using $FeCl_3$, followed by sand filtration and chlorination (gas).

Fig 7A and B represents the results of an evaluation of the removal of *Giardia* cysts and *Cryptosporidium* oocysts at different stages of treatment at Schoemansville water purification works. One hundred percent of the raw water samples tested positive for the presence of *Giardia* cysts, with concentrations ranging between 10 and 400 cysts/100ℓ. The cysts were effectively removed by flocculation and sand filtration and only two samples, containing 6 and 150 cysts/100ℓ respectively, were detected after the flocculation step during May and July (Fig. 7A). No *Giardia* cysts were detected in any of the treated water samples.

The *Cryptosporidium* oocysts were also removed effectively and none of the treated water samples contained oocysts. Only one sample, also collected after flocculation during May, contained 6 oocysts/100ℓ (Fig. 7B). Oocysts were observed in 41,7% of the raw water samples, but were absent after treatment.

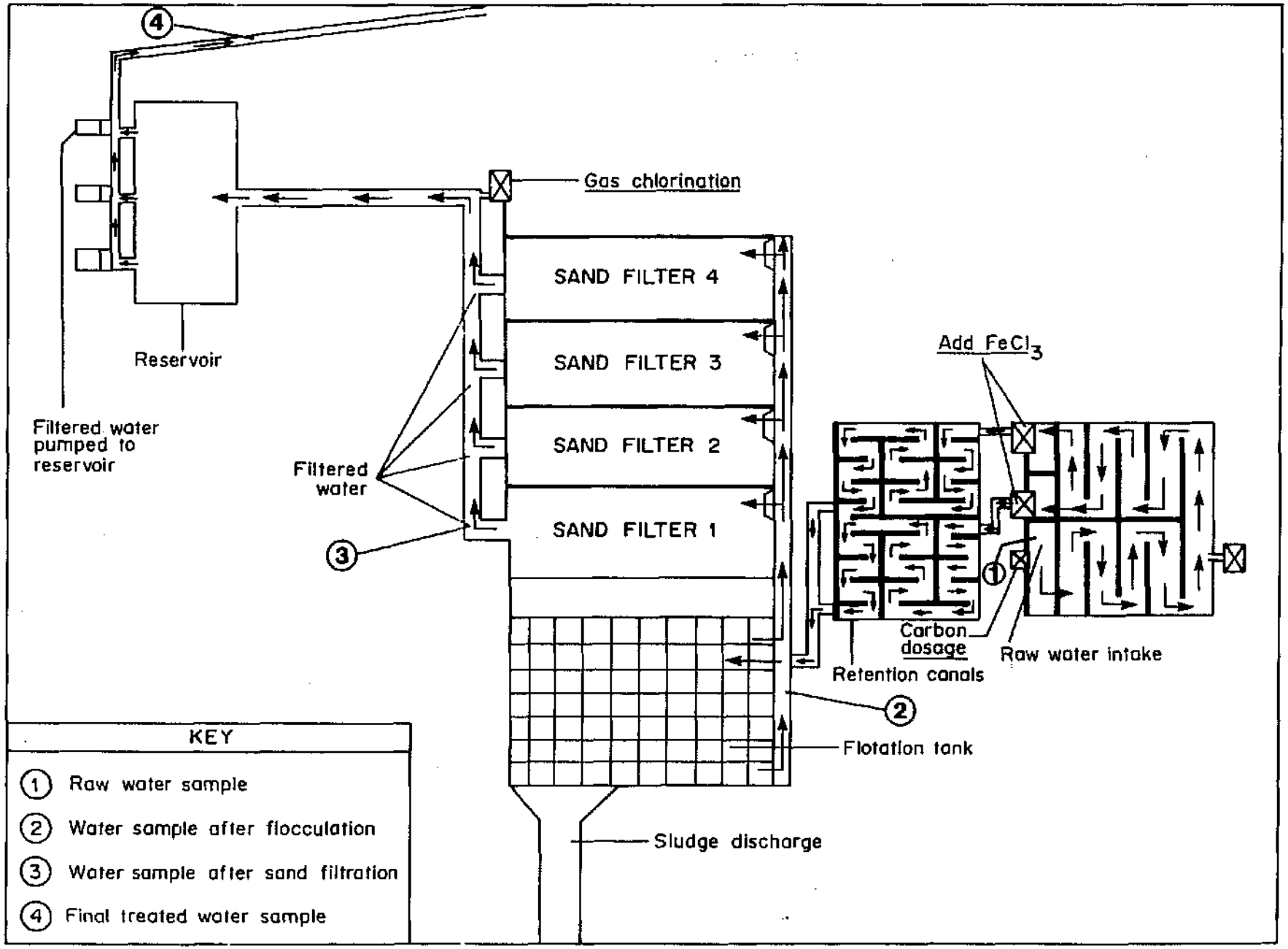
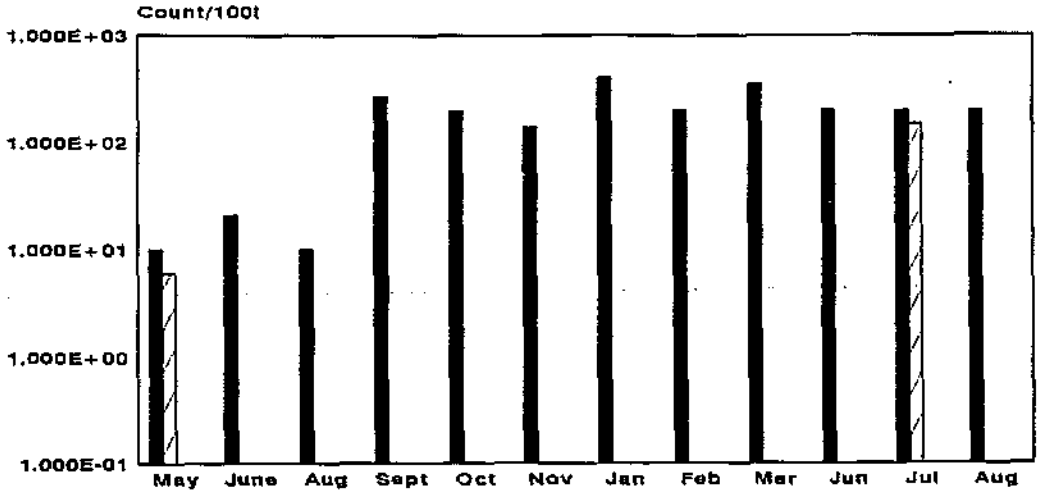
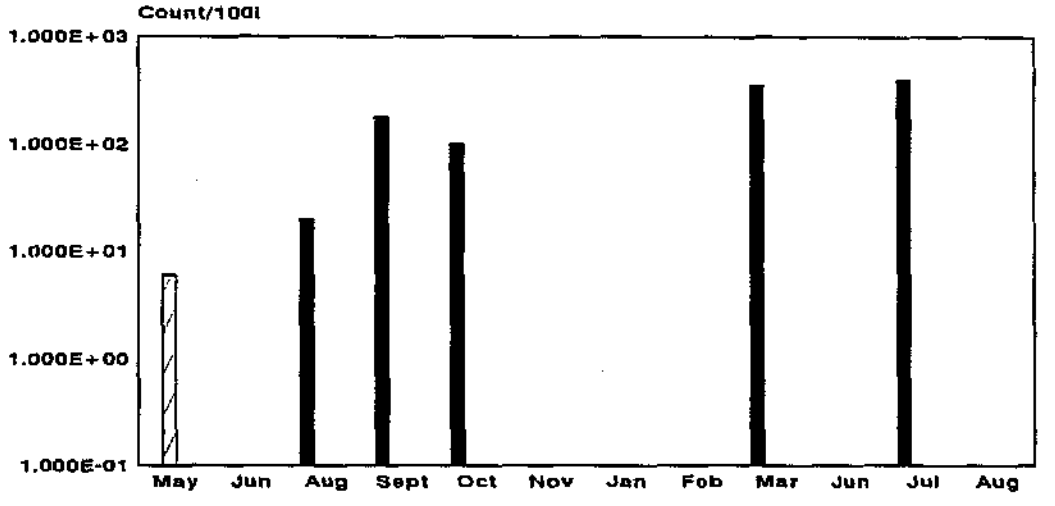


Fig.8. Schematic plan of the various stages of treatment at the Schoemansville water works



7A: *Giardia*



7B: *Cryptosporidium*

Fig.7. The removal of protozoan parasites during treatment at Schoemansville

Raw water After flocculation After sand filtration Final water

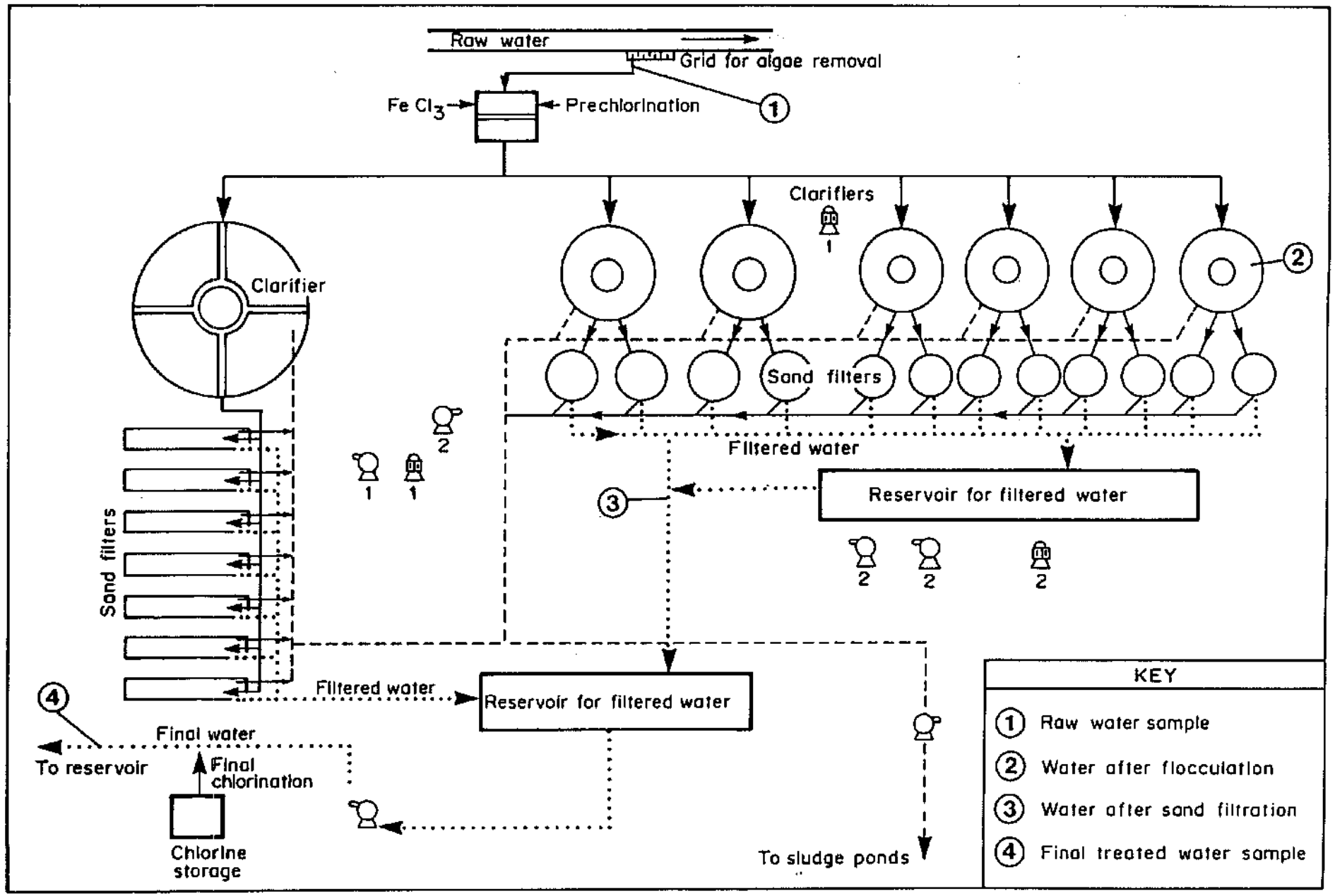
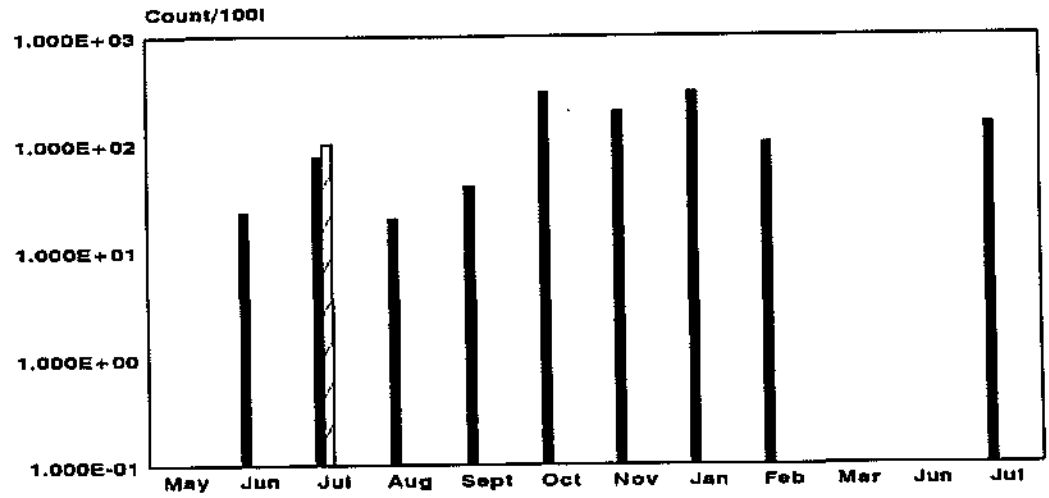


Fig.8. Schematic plan of the water works at Temba

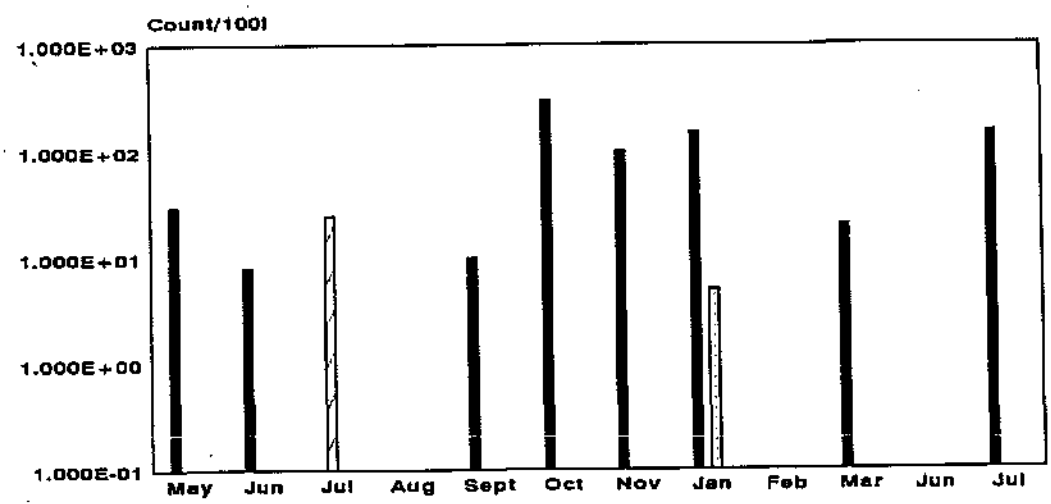
4.3.2. *Temba*

Fig 8 is a schematic presentation of the stages of treatment at the Temba water purification works. Raw water is flocculated using ferric chloride. Rapid gravity sand filtration is followed by chlorination.

Fig 9A and B represents the results of an evaluation of the removal of *Giardia* cysts and *Cryptosporidium* oocysts at different stages of treatment at Temba water works. Seventy five percent of the raw water samples contained *Giardia* cysts, which were effectively removed during treatment. Only one sample, containing 100 cysts/100ℓ, was detected after flocculation during July (Fig. 9A). *Cryptosporidium* oocysts were found in 67% of the raw water samples and during July and January after sand filtration (Fig 9B). All the final water samples tested negative for the presence of both *Giardia* cysts and *Cryptosporidium* oocysts.



9A: *Giardia*



9B: *Cryptosporidium*

Fig. 9. The removal of protozoan parasites from surface water from Temba

Raw water After flocculation After sand filtration Final water

4.4. Evaluation of the Efficacy of Commonly Used Indicator Microorganisms for the Indication of the Presence of Protozoan Parasites in Water.

Schoemansville and Temba water purification works were evaluated at different stages of treatment for the presence of routinely used indicator organisms. Standard plate counts and total and faecal coliform counts are presented in Figs 10 A-C and 11 A-C

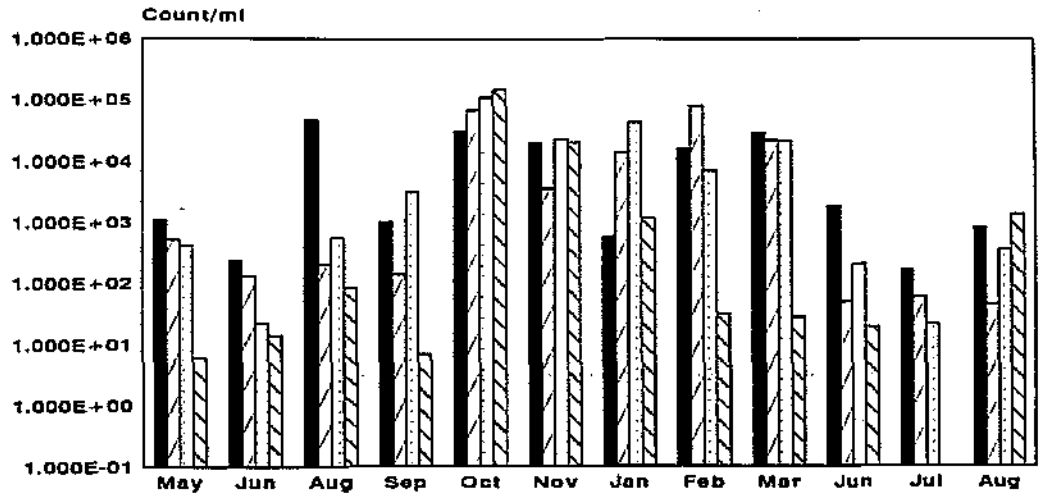
4.4.1. *Schoemansville*

A reduction in the standard plate counts after treatment was recorded at Schoemansville (Fig 10A). However, the treatment was not as effective from October to January (Fig 10A), as the total plate counts increased after flocculation and sand filtration. The counts recorded for the final water exceeded the counts obtained for the raw water samples. This is also reflected in the results obtained for the total coliforms (Fig 10B) and faecal coliforms (Fig 10C) from October to January, where no or very little removal was observed and relatively high counts for both total and faecal coliforms were obtained in the final treated water.

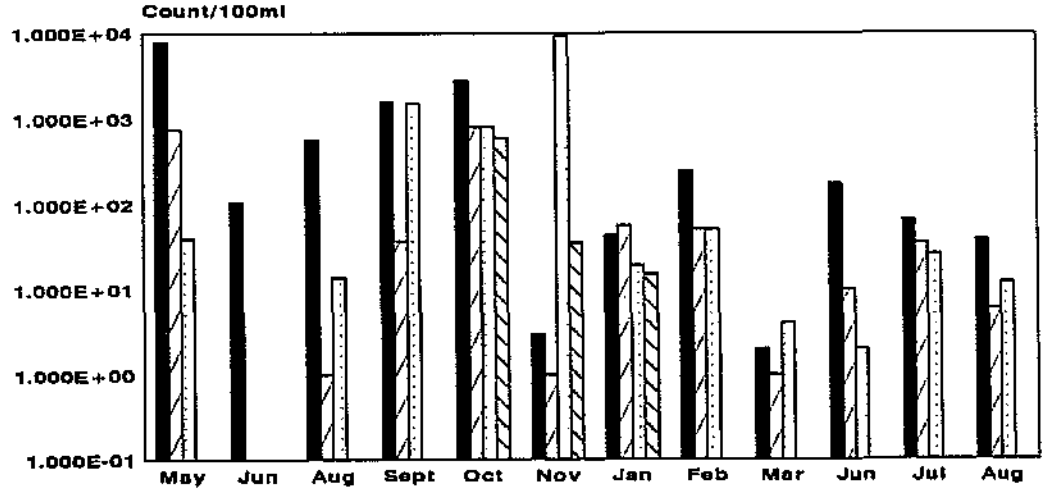
During the same period, *Giardia* cysts were detected in the raw water from Schoemansville, but no cysts were detected after treatment (Fig 7A), while *Cryptosporidium* oocysts were present in the raw water during October, but absent during November and January (Fig 7B).

4.4.2. *Temba*

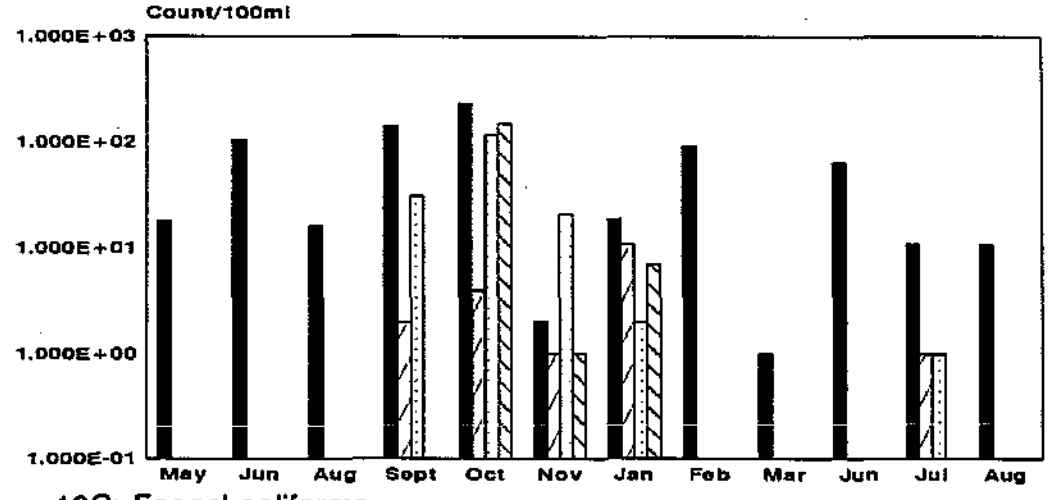
A reduction in the standard plate counts after treatment was recorded (Fig 11A). The removal of faecal coliforms was very effective and no faecal coliforms were detected in the treated water from Temba (Fig 11B). Total coliforms, however, were present in the final water during October, November and February (Fig 11A). *Giardia* and *Cryptosporidium* were found in the raw water, but never in the final drinking water (Fig 9A,B). During July, both *Giardia* and *Cryptosporidium*, were found after flocculation (Fig 9A,B), but no total or faecal coliforms could be detected after flocculation (Fig 11A,B).



10A: Standard plate counts



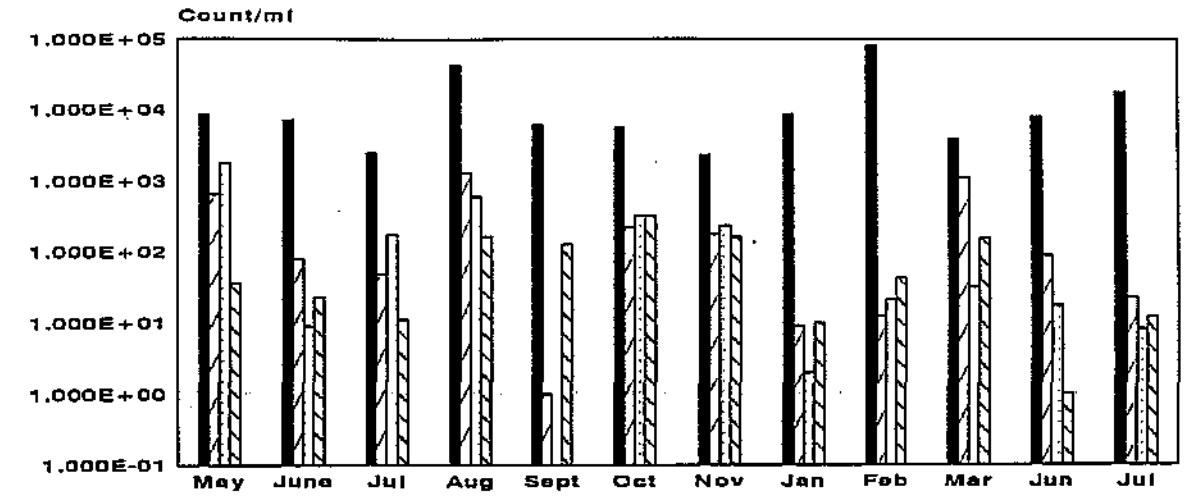
10B: Total coliforms



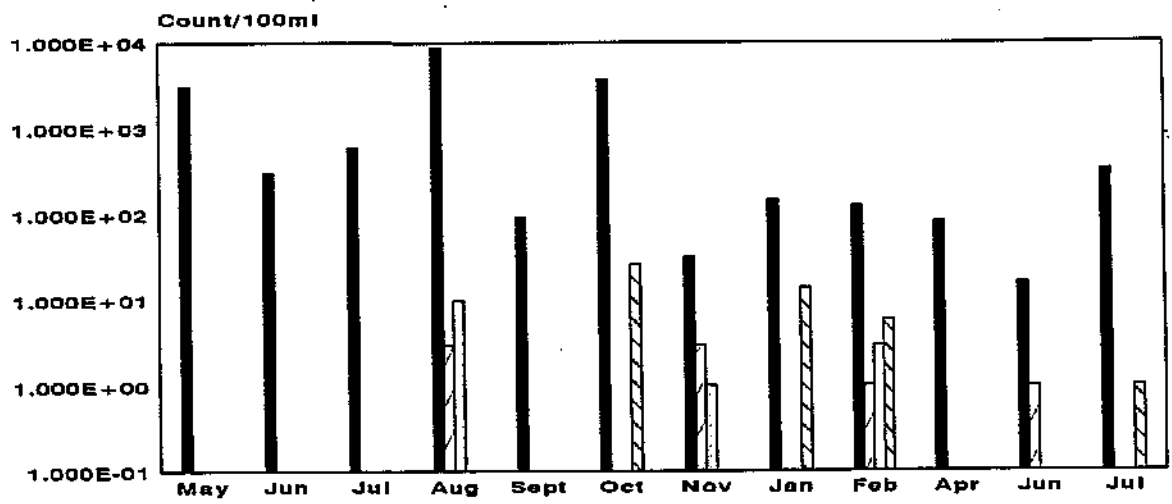
10C: Faecal coliforms

Fig. 10. Removal of routinely used indicator organisms during treatment at Schoemansville

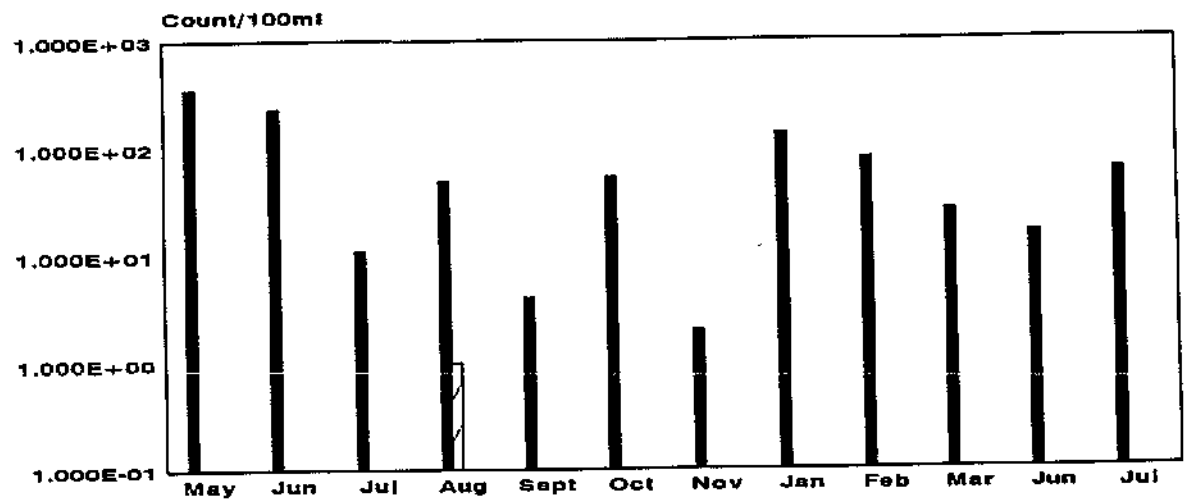
■ Raw water ▨ After flocculation ▦ After sand filtration ▧ Final water



11A: Standard plate counts



11B: Total coliforms



11C: Faecal coliforms

Fig. 11. Removal of routinely used indicators during treatment at Temba

Raw water | After flocculation | After sand filtration | Final water

4.5. Evaluation of the Applicability of Using *Candida albicans* and *Clostridium perfringens* as Indicators of the Presence of Protozoan Parasites

Schoemansville and Temba water works were evaluated at different stages of treatment for the presence of *C. perfringens* and *C. albicans.*

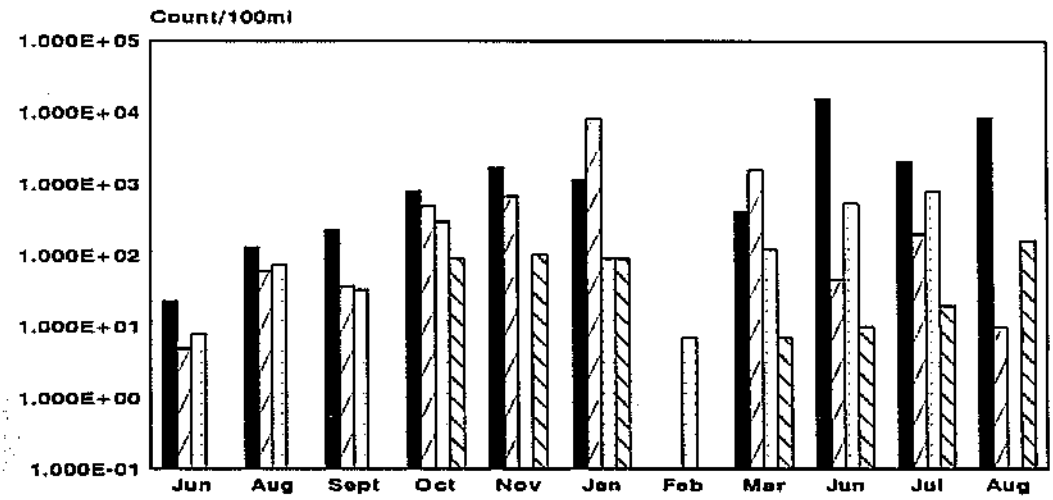
4.5.1. *Schoemansville*

As mentioned previously, both the *Giardia* cysts and *Cryptosporidium* oocysts were completely removed during the treatment process at the Schoemansville water works (Fig 7A,B). The removal of *Candida* and *Clostridium*, however, was not effective and both were still present in up to 42% of the final treated samples (Fig 12A,B). *Clostridium* and *Candida* were present in 75% and 83% respectively of the raw water samples tested, while *Giardia* cysts were present in 100% of the raw water samples. *Cryptosporidium* oocysts were detected in 40% of the raw water samples. The protozoan parasites and the indicators did not always present simultaneously in the raw water, e.g. during November and January *Clostridium* and *Candida* were detected at high concentrations in the raw water (Figs 12A,B), while no *Cryptosporidium* oocysts could be detected (Fig 7B).

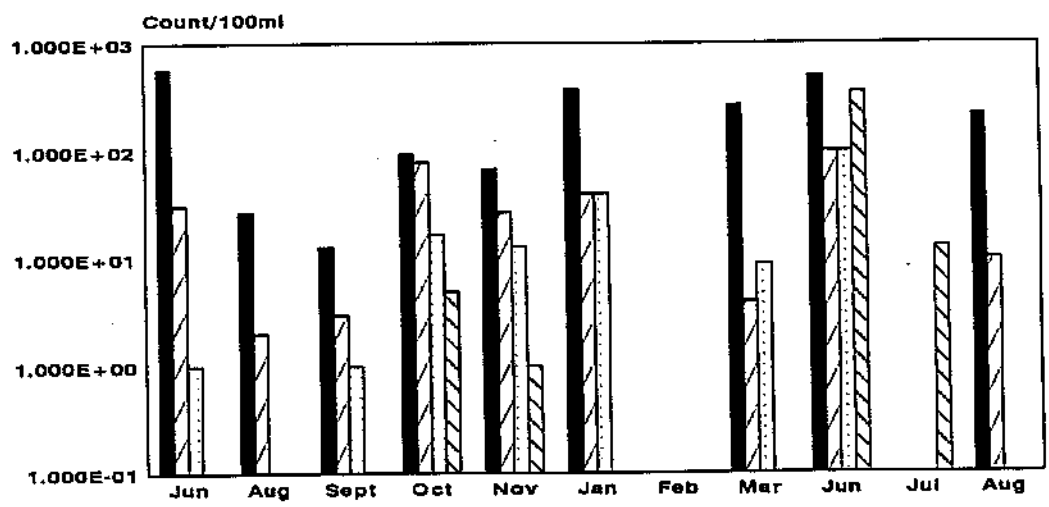
Spearman rank correlations were used to establish if correlation exist between the protozoan parasites and indicator organisms. Data from the two plants were grouped (raw water, after flocculation, sand filtration and treated water) and analyzed. No correlation could be shown between the parasites and indicators. The best correlation was found between *Giardia* and faecal coliforms (Table 8). However, at Temba no correlation could be found between *Giardia* and faecal coliforms (Table 9), showing that it is not a good indicator. Correlation coefficients among the parasites and indicator organisms in the raw water from Schoemansville are shown in Table 8.

TABLE 8: Correlation coefficients (r) among the protozoan parasites and microbial indicators in raw water from Schoemansville

	Total coliforms	Faecal coliforms	*C. perfringens*	*C. albicans*
Giardia	-0.3814	0.327	0.0669	0.1621
Cryptosporidium	0.234	-0.1094	-0.4397	0.0198



A: *Candida albicans*



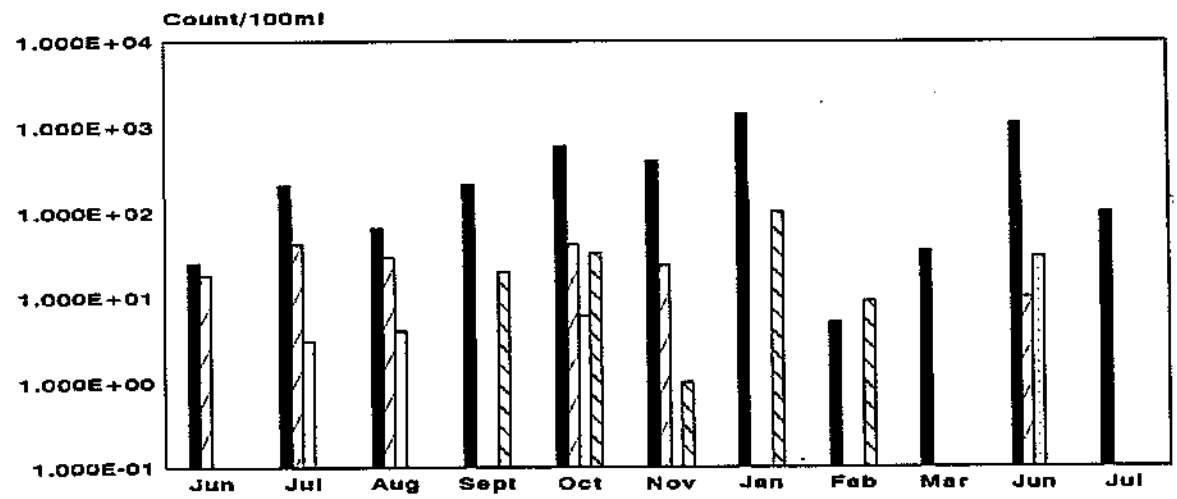
B: *Clostridium perfringens*

Fig.12. Removal of *Candida* and *Clostridium* during treatment at Schoemansville

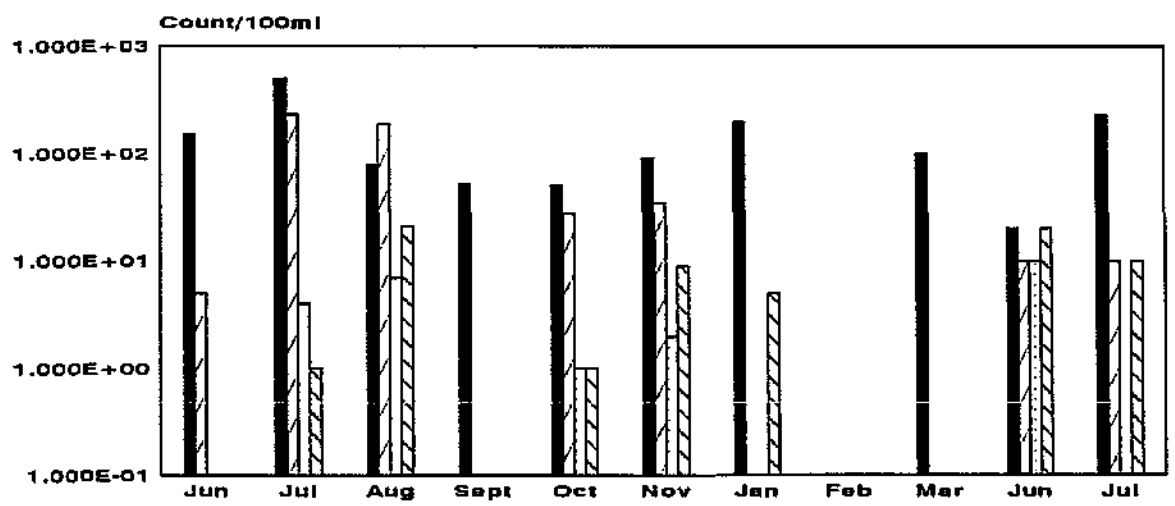
Raw water After flocculation After sand filtration Final water

4.5.2. *Temba*

Giardia cysts and *Cryptosporidium* oocysts were removed during the treatment process and no cysts or oocysts were detected in the final treated water (Fig 9A,B). However, 40% of the final treated samples contained *Candida* and 58% of the samples contained *Clostridium* (Fig 13A,B). Between 83% and 91% of the raw water samples tested positive for the presence of *Candida* and *Clostridium*, while 75% were positive for *Giardia* and 67% for *Cryptosporidium* (Fig 9A,B). During February high counts of *Giardia* cysts were recorded in the raw water (Fig 9A), but no *Clostridium* was detected (Fig 13B).



A: *Candida albicans*



B: *Clostridium perfringens*

Fig.13. Removal of *Candida* and *Clostridium* during treatment at Temba

Raw water | After flocculation | After sand filtration | Final water

TABLE 9: Correlation coefficients (r) among the protozoan parasites and microbial indicators in raw water from Temba

	Total coliforms	Faecal coliforms	*C. perfringens*	*C. albicans*
Giardia	0.1305	-0.0247	0.1598	0.3973
Cryptosporidium	0.1248	0.1997	0.2425	0.4151

Spearman rank correlation was used to determine the correlation coefficients between the parasites and microbial indicators. At Temba, better correlations were found between *Giardia* and *Cryptosporidium* and *C. albicans* (0.39 and 0.42 respectively) (Table 9). However, at Schoemansville no correlation could be found between the parasites and *C. albicans* (Table 8), showing that it is not a good indicator.

5. DISCUSSION

Similar recoveries were obtained after the processing of seeded water samples having a low concentration of *Giardia* cysts (water sample volumes of 10ℓ) using both ultrafiltration and membrane filtration techniques. In field studies in which both *Giardia* cysts and *Cryptosporidium* oocysts were enumerated, the membrane filtration technique was shown to be the better method for the concentration of surface water for the detection of *Giardia* cysts, although cysts were detected by both membrane filtration and ultrafiltration in most of the samples tested (Kfir *et al.*, 1993). Both membrane filtration and ultrafiltration were found to be equally efficient for the enumeration of *Cryptosporidium* oocysts from surface water samples (Kfir *et al.*, 1993). Flat-bed membrane filtration has also been used by other researchers (Wallis and Buchanan-Mappin, 1985; Ongerth and Stibbs, 1987). The ultrafiltration method has the advantage of co-processing the same water sample for the analysis of enteric viruses and protozoan parasites, thus reducing the cost of analysis significantly.

Cuno wynd cartridge filters are the most commonly used for the concentration of large volume samples for the enumeration of protozoan parasites (Whitmore and Carrington, 1993). The recovery range found for the Cuno wynd filter in this study was similar to that reported in the literature (Holman *et al.*, 1983; Rose *et al.*, 1991). It should, however, be noted that the number of reported laboratory seeding studies in which the recoveries of cysts using various concentration techniques are compared, is limited. Erratic recoveries, as found in this study, have previously been reported for *Cryptosporidium* oocysts using Cuno wynd filters (Ongerth and Stibbs, 1987; Musial *et al.*, 1987).

Due to a shortage in the supply of Cuno wynd filters in South Africa, a comparative study evaluating the Cuno wynd filter against the more readily available Cuno wound cartridge filter was initiated. The Cuno wynd and wound filters were evaluated for recoveries of cysts and filtration performance. The concentration procedure using AMF Cuno wound cartridge filters proved effective and practical for the enumeration of *Giardia* cysts and *Cryptosporidium* oocysts as indicated by the higher recovery

rates recorded. The recovery range found for the Cuno wound filters was relatively narrow and consistent recovery rates were obtained. These filters are also cheaper and more readily available in South Africa than the AMF Cuno wynd filters. The wound filters did not clog as rapidly as the wynd filters, which is an advantage when surface water samples are processed. This is contradictory to the manufacture's data which recommend the use of the wynd filter to avoid clogging and breakthrough. It should also be noted that the use of Cuno wound filters leads to a significant cut in the cost per analysis.

Results have indicated that where the presence of both viruses and protozoan parasites has to be investigated, Filterite cartridge filters can be used, thus shortening the time of sample preparation and reducing the cost per analysis (Kfir *et al.*, 1994). The use of the same cartridge filter and sample elution technique for the co-processing of the same large-volume sample for the enumeration of both protozoan parasites and enteric viruses, has thus far shown to give similar recoveries to those found for the Cuno wynd filter. The results to date have indicated that, where both viruses and protozoan parasites are to be investigated, the same cartridge filter can be used, thus shortening the time of sample preparation and reducing the cost per analysis. Payment *et al.* (1989) also reported on a single concentration technique for the enumeration of bacteria, coliphages, enteric viruses and *Giardia* cysts. However, there is a need for further studies in which a comparison of the efficacy of Filterite to that of Cuno wound, in their recoveries of both cysts and oocysts from surface and final water, should be carried out.

The use of the Sputnik washing machine indicated it to be as efficient for processing the filters as the commonly used stomacher. The use of the washing machine shortens the sample preparation time as well as the initial cost of building the necessary facilities for protozoan detection. The special freezer bags used in the stomacher are also relatively costly. The washing machine technique was also found to be very effective by Gilmour *et al.* (1991).

The results of this study indicated that concentration of 100ℓ of water has an advantage over concentration of 10ℓ samples. The recovery of protozoan parasites from water samples of 10ℓ, although similar to those obtained by the use of cartridge filters, only give recoveries representing a small fraction of the parasite cysts seeded in comparison to those recovered by the cartridge filters if 100ℓ of sample with the same concentration of cysts are tested. This shows the importance of investigating large sample volumes. If 20% of the seeded cysts are to be recovered from a concentrate of 10ℓ and 100ℓ respectively, the number of cysts recovered on concentrating 10ℓ will be 10 times lower that that recovered from 100ℓ. In cases where very low levels of cysts occur, which may still be a risk to human health, they will not be detected in cases where small sample volumes are tested. It should be noted that the actual methodology used for the concentration of 10ℓ of water shows relatively good recovery of cysts but one of the major disadvantages of these techniques is that they are limited to relatively small sample volumes due to rapid clogging of the systems. Risk assessment studies assessing fitness for use of both drinking water and recreational water have also indicated the need for increasing the volume of water samples tested for protozoan parasites and enteric viruses (Rodda,

1993).

The occurrence of *Giardia* cysts and *Cryptosporidium* oocysts in South African raw and treated waters was investigated by studying source and treated water obtained from ten different water works. Samples were collected throughout the year to allow observation of possible seasonal variation.

South African untreated source waters are polluted with both *Giardia* cysts and *Cryptosporidium* oocysts. Raw water samples taken at all the water purification works tested positive for both cysts and oocysts and only a small percentage of raw water samples were free of protozoan parasites. In a separate study, Rand Water tested raw water from the same source as described in this study, but the samples were not taken at the same time. They could not detect either *Giardia* or *Cryptosporidium*. Umgeni Water evaluated raw water samples and did find both *Giardia* and *Cryptosporidium* (Personal communication). The results, however, can not be compared because the samples were not taken simultaneously. *Cryptosporidium* oocysts have been found in a greater number of samples than *Giardia* cysts and at higher concentrations in the USA (Rose *et al.*, 1991).

The average number of *Giardia* cysts detected in South African raw waters varied between 0 and 197 cysts/100ℓ and *Cryptosporidium* oocyst levels between 0 and 198/100ℓ. The highest maximum counts for *Giardia* cysts were observed at Potchefstroom (1000 cysts/100ℓ) and Wallmanstal (900 cysts/100ℓ). The highest maximum oocyst counts were recorded at Rietvlei (600 oocysts/100ℓ) and at Potchefstroom (900 oocysts/100ℓ). This is in contrast to 5300-5800 oocysts/10ℓ reported for polluted water in Arizona. However, streams and clean river waters in the USA were reported to have less than 8 oocysts/10ℓ (Rose *et al.*, 1991).

In this study, the percentage of samples positive for only *Giardia* cysts or only *Cryptosporidium* oocysts never exceeded 25%, except at Western Transvaal, Schoemansville and Wallmanstal (50%, 58% and 33% of the samples contained only cysts) and Rietvlei and Rand Water, where 33% and 40% respectively, of the samples tested positive for oocysts only. *Giardia* and *Cryptosporidium* occurred simultaneously in a high number of samples. Madore *et al.* (1987) also found that the two parasites often co-exist in polluted water.

All the final treated water samples evaluated in this study were free of *Cryptosporidium* oocysts. One sample from the Wallmannstal water works showed the presence of *Giardia* cysts (4 cysts/100ℓ). This represents 8% of the samples tested at Wallmannstal. In this study all water samples were collected at plants where treatment includes flocculation and sand filtration. In a previous study, a large number of samples were evaluated for the presence of protozoan parasites, including facilities not equipped with such purification treatment and it was found that 13.2% of drinking water samples tested positive for the presence of protozoan parasites (Kfir *et al.*, 1993).

Sixty three percent of the water purification works evaluated showed higher average counts for the presence of *Cryptosporidium* in raw source water during summer, while

75% of the waterworks showed higher average counts for *Giardia* during summer. Similar results were recorded by Rose *et al.* (1991), who found that average concentrations of cysts and oocysts were higher during summer and autumn. However, the percentage of samples positive for *Giardia* was higher during winter at 5 out of 8 water works evaluated, while the percentage of samples positive for *Cryptosporidium* oocysts were only higher during winter at two water works.

As a result of the high cyst and oocyst concentrations detected in South African source waters, care should be taken to ensure that treatment plants are functioning effectively. *Giardia* cysts and *Cryptosporidium* oocysts are known to be highly resistant to a range of environmental conditions and can survive for relatively long periods in water. These cysts and oocysts were also found to be more resistant to certain water purification processes than other bacterial indicators (Hibler and Hancock, 1990). Cysts can be effectively removed and inactivated in water supplies by a combination of filtration and chlorination. However, *Cryptosporidium* outbreaks have been reported where water has undergone treatment, including coagulation, sedimentation, sand filtration and chlorination, but due to poor operational practices oocysts were not inactivated or removed (Rose *et al.*, 1991).

The efficacy of water treatment works in removing protozoan parasites from source water was evaluated by studying water from Schoemansville and Temba at different stages of treatment, *i.e.* source water, after flocculation, after sand filtration and the final treated water. The treatment proved to be effective at both water works and only two samples, collected during May and July at Schoemansville, contained cysts and oocysts after flocculation. During January, *Cryptosporidium* oocysts, but no *Giardia* cysts, were detected after sand filtration in a sample from Temba and both *Giardia* cysts and *Cryptosporidium* oocysts were detected after flocculation during July. The treated water samples were free of protozoan parasite contamination. It is, however, important to test the final treated water from small plants and facilities where sand filtration is not used for the presence of protozoan parasites.

At present there is no simple test that can be used routinely to evaluate the occurrence of protozoan parasites in water. It was therefore decided to determine whether current microbial indicators of water quality and treatment processes, such as total and faecal coliforms can be used to give an indication of the presence of protozoan parasites in water.

The ideal indicator should be present when the pathogenic microorganisms of concern are present and absent in clean unpolluted water. The indicator should be present in numbers much greater than the pathogens it is intended to indicate and should respond to natural environmental conditions and water treatment processes in a manner similar to the pathogens of interest. The indicator should also be easy to isolate, identify and enumerate (Pipes, 1982).

In this study, the parasites were completely removed, while the coliforms were still present after treatment. In most cases, the bacterial indicators were present in high numbers, while no *Giardia* or *Cryptosporidium* could be detected. In some cases the presence of faecal coliforms at Temba gave an indication of the presence of *Giardia*

and *Cryptosporidium*, but in certain samples, faecal coliforms were present in the raw water, while the protozoan parasites were absent.

From the results obtained it can be seen that total and faecal coliforms are not good indicators of the presence of protozoan parasites in water. These findings are in agreement with results obtained by Rose *et al.* (1989) and Payment and Franco (1993) who also demonstrated that coliforms are inadequate indicators of the presence of pathogens, especially viruses and parasites.

Payment and Franko (1993) found *Clostridium* to be a good indicator of the presence of protozoan parasites. The suitability of *Candida albicans* and *Clostridium perfringens* as indicators for the presence of protozoan parasites was therefore investigated. It was found, however, that both *Candida* and *Clostridium* survive the treatment processes better than the parasites. *Candida, Clostridium* and the protozoan parasites did not always occur together in a sample e.g. during June high counts of both *Candida* and *Clostridium* were recorded in the raw water from Temba, but the water was free from protozoan parasites.

6. EVALUATION OF AVAILABLE SOUTH AFRICAN DRINKING WATER QUALITY GUIDELINES FOR THE PROVISION OF PARASITE-FREE DRINKING WATER

No clear standards or guidelines for levels of protozoan parasite cysts and oocysts are available worldwide. In many countries guidelines are still being developed. In the United States of America, the Surface Water Treatment Rule mandates that all surface water samples be treated to achieve at least a reduction of 10^{-3} (99.9 % removal) of *Giardia* cysts. The US Environmental Protection Agency has also recommended that a treatment be provided to ensure that populations are not subjected to risk of infection of greater than 1:10 000 (10^{-4}) for a yearly exposure and that this is an acceptable level of safety for potable waters (Rose and Gerba, 1991).

Protozoan parasites are not included in South African domestic water quality guidelines. The tentative guideline for protozoan parasites is based largely on South African expert opinion (Dept. Water Affairs and Forestry, 1993).

Draft guidelines for drinking water proposed by Rand Water suggest a recommended limit of no *Giardia* cysts/2ℓ, a negligible risk limit of 2 *Giardia* cysts/2ℓ and a low risk limit of 5 *Giardia* cysts/2ℓ. A recommended limit of no *Cryptosporidium* oocysts/2ℓ is proposed (Dept. Water Affairs and Forestry, 1993). However, although the recommended volume of 2ℓ facilitates analysis, it is likely to be too small to detect cysts or oocysts in domestic water, since they occur at extremely low levels. Risk assessment studies assessing fitness for use of drinking water indicated the need for increasing the volume of water samples tested for protozoan parasites (Rodda, 1993). At the time of publication of the guidelines, concentration and detection techniques for the examination of larger volumes of water were not available.

We recommend that drinking water should contain no *Giardia* cysts/100ℓ and no

Cryptosporidium oocysts/100ℓ. It is also recommended that water samples will be evaluated on a monthly basis.

7. CONCLUSIONS AND RECOMMENDATIONS

It is very important to concentrate large volume water samples, especially for drinking water, where low numbers of parasites are expected. Cuno wound filters proved to be effective, practical and affordable for the enumeration of *Giardia* cysts and *Cryptosporidium* oocysts. Results have indicated that where the presence of both protozoan parasites and enteric viruses have to be investigated, Filterite cartridge filters can be used, thereby shortening the time of sample preparation and reducing the cost per analysis.

South African source waters were found to be contaminated with both *Giardia* cysts and *Cryptosporidium* oocysts. The treatment at water purification works was found to be effective and the final treated waters were free from protozoan parasites. However, as a result of the high cyst and oocyst concentrations detected in South African source waters, care should be taken that the treatment plants are functioning effectively.

Current microbial indicators of water quality are inadequate indicators of the presence of protozoan parasites in water. Therefore, only direct monitoring can be relied on for determining the presence of *Giardia* and *Cryptosporidium* in water.

Protozoan parasites are not included in South African domestic water quality guidelines. As conventionally used microbial indicators can not be used to give an indication of the presence of protozoan parasites in water, water quality guidelines should be developed regarding the presence of these parasites in water.

8. FUTURE RESEARCH NEEDS

More data is required on the occurrence of protozoan parasites in rural areas where people use drinking water without any treatment or after limited treatment such as chlorination. We are presently busy with a study evaluating the occurrence of protozoan parasites in drinking water used by unserved rural communities. The data collected will be used to devise appropriate guidelines for the treatment of water by developing communities.

A disadvantage of the methods currently being used is that one can not distinguish between viable and non-viable cysts and oocysts. Assays are needed to determine the viability of protozoan parasites in water supplies.

Methods for the detection of *Giardia* and *Cryptosporidium* can be improved. Several reports indicate that alternate enumeration and detection techniques yield higher recoveries. These methods should be evaluated and incorporated in current methods if found to be applicable.

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APPENDIX A

LITERATURE STUDY ON ENUMERATION TECHNIQUES FOR THE DETECTION OF PROTOZOAN PARASITES IN DRINKING WATER

1. INTRODUCTION

The protozoan parasites, *Giardia* and *Cryptosporidium*, are environmentally resistant intestinal parasites that can cause gastroenteritis in humans when ingested. *Cryptosporidium* has been the responsible agent of 23% of diarrhoeal cases worldwide (Fayer and Ungar, 1986), while the prevalence of *Giardia* in the population has been indicated to be as high as 24%, depending on the life style, socio-economic class and the general condition of the community (Feachem *et al.*, 1983). In recent years the incidence of Giardiasis and Cryptosporidiosis has reached endemic proportions throughout the world and at present *Giardia* and *Cryptosporidium* are implicated as the main parasitic causes of waterborne epidemic disease.

These parasitic diseases cause acute, sporadic gastroenteritis in otherwise healthy subjects, particularly children, in both developed and under-developed countries and in travellers, most of whom are adults. They are potentially fatal infections in the immunocompromised (Casemore, 1990).

Giardia cysts and *Cryptosporidium* oocycts are excreted in the faeces of infected individuals and are transmitted by the faecal-oral route. Both these parasites share a common mode of transmission *via* water or food polluted by sewage thus, many of the studies on detection methods used for the enumeration of these parasites address the two simultaneously. Their existence in water is often a result of the contamination of watersheds, from which large segments of the population derive their drinking water, by faecal waste from human and animal origin. Both cysts and oocysts are known to be highly resistant to environmental stress and can withstand extreme environmental conditions. Cysts and oocysts have been shown to remain viable for months at 4 - 10°C in source water. These cysts and oocysts were also found to be more resistant to certain water purification processes than other bacterial indicators (Hibler and Hancock, 1990; Rose, 1990). Generally, conventional treatment processes within a treatment plant should effectively remove or inactivate cysts and oocysts from source water (99.9% removal) (LeChevallier *et al.*, 1991a) and if protozoan cysts and oocysts are detected in samples from purification plants, it indicates some sort of breakdown in the treatment processes at the plant (Regli *et al.*, 1988; Logsdon, 1988). However, in a number of parasite-related diarrhoeal outbreaks reported in literature, the water quality was within the drinking water bacteriological standards or guidelines.

No clear standards for permitted levels of the protozoan parasites cysts or oocysts in water are available worldwide. The US Environmental Protection Agency has established treatment guidelines of 3 logs reduction for *Giardia* cysts, to achieve an annual risk of not more than 1 infection in 10 000 (Rose and Gerba, 1991).

2. DETECTION AND RECOVERY

The techniques used to isolate *Giardia* cysts and *Cryptosporidium* oocysts are similar and their efficacy does not differ significantly. The basic techniques to be described for the enumeration of protozoan parasites from water address sample collection and concentration, elution and recovery of the parasites and their identification.

2.1. Sampling

Ideally sampling should be carried out directly from either a tap or surface water source (such as streams), where the rate of flow can be monitored. The volumes of water should be controlled.

Sample transport and holding time should be minimized as much as possible. Samples should be protected from heat and sunlight during collection and transport. After collecting samples, the filters should be removed and placed in a large plastic bag and shipped on ice to the laboratories for processing (Rose *et al.*, 1991).

2.2. Concentration of samples

2.2.1. Filtration

Laboratories performing parasitic analyses on environmental samples prefer different filtering techniques. Filters used include either surface-type (flat-bed) filters or cartridge filters made of various materials such as yarn wound (orlon), polypropylene, cotton or acetate. No rigorous or controlled comparative evaluations of filter types have been done for both protozoan parasites and the selection is based mostly on laboratory preference, but filters should have a porosity of not greater than 1μm.

The surface-type filters such as epoxy fibreglass tube or the membrane filters may have a distinct disadvantage because they become clogged with material suspended in the water (Jakubowski, 1984). However, the physical retention of parasites larger than 1μm is successfully achieved by these types of filters and up to 71% of the parasites from seeded experiments could be eluted. Although elution is successfully achieved the reconcentration of the sample is more difficult, as found by other researchers attempting to detect parasites in water samples. Up to 52% of the added cysts in seeding experiments could be recovered by using density-gradient flotation (Jakubowski, 1984).

2.2.2. Flocculation

Vesey *et al.* (1994) proposed a new method using calcium carbonate flocculation for concentrating water samples. The method is apparently less time-consuming and labour-intensive than the cartridge filter method. They claim that by using flocculation, smaller more managable samples may be used (10ℓ-20ℓ), than with cartridge filters whilst maintaining the same degree of sensitivity due to improved recovery rates.

3. Filter elution techniques

Filters are usually processed within 2 to 4 days after sample collection, however in some cases filters can be kept longer at 4° C prior to processing.

Extraction of suspended material from filter cartridges is carried out by hand, using

distilled water (Hibler, 1988) or with water containing 0.1% Tween 80 (Rose *et al.*, 1991). Extraction from membranes or the epoxy fibreglass tube is performed by a backwash procedure.

Some laboratories using the yarn wound (orlon) filter cartridge, unwind the yarn, separate the yarn into sections, and hand wash it (Jakubowski *et al.*, 1978). This is a laborious time-consuming procedure and does not necessarily mean that the recovery of cysts will be any better.

A fast and efficient procedure is to slice the cartridge to the core with a razor knife. This produces fibres that are teased apart and placed in a stomacher bag (3.5ℓ-capacity) in 1.75 litres of phosphate-buffered saline (PBS pH 7.4) containing 0.1% sodium dodecyl sulfate (SDS) and 0.1% Tween 80. The filter material is homogenized repeatedly for 3 minutes over a 15 minute period. Between each homogenization period, the filter material is hand kneaded to redistribute the fibres in the bag (LeChevallier *et al.*, 1991a).

Studies indicated that irrespective of the filter cartridge used, or the specific elution protocol followed by different laboratories, the first and potentially the greatest loss of cysts occurs during this washing procedure (Jakubowski *et al.*, 1978; Madore *et al.*, 1987). The washed filter material is discarded, the eluate is combined and centrifuged at 1 200g for 10 minutes.

The pellets are then washed and resuspended in 10mℓ of Tween 80/SDS solution and homogenized, and one drop of antifoam agent is added. The mixture is sonicated for 4 minutes in a waterbath and layered onto sucrose (1.24 specific gravity; g/mℓ) for *Cryptosporidium* oocysts identification and onto potassium citrate (1.24g/mℓ ±56%) for the identification of *Giardia* cysts. After the final concentration, the concentrate is filtered through a 13mm-diameter cellulose nitrate membrane of 1.2µm porosity for *Cryptosporidium* oocysts and 5.0µm porosity for *Giardia* cysts (Musial *et al.*, 1987; Rose *et al.*, 1991).

4. Identification of protozoan parasite cysts and oocysts

Methods used for the detection of cysts and oocysts include direct microscopy, using stains such as Lugol's iodine and trichome as well as immunofluorescence techniques (Mahbubani *et al.*, 1991).

The principle method for cyst and oocyst identification is an immunofluorescence technique. The stain is added directly to the filtered samples while in the housing, using either direct or indirect immunofluorescent procedures, depending on the antibodies in use. The development and evaluation of this technique and methodology is described by Ongerth and Stibbs (1987); Rose *et al.* (1991) and Sterling *et al.* (1987).

There are a few kits available for the detection of cysts and oocysts. A Hydrofluor Combo kit obtainable from Meridian Diagnostics (Cincinnati OH, USA), providing for the simultaneous detection of *Giardia* cysts and *Cryptosporidium* oocysts from

environmental sources is used worldwide. The kit contains monoclonal antibodies targeting the cysts and the oocysts of the two protozoan parasites and the reaction is made visible by the addition of a fluorescent isothiocyanate (FITC)-conjugated anti-immunoglobulin. This kit utilizes the principle of indirect immunofluorescence, whereby the prepared pellet is incubated with murine monoclonal antibodies directed against surface antigen components of *Giardia* cysts and *Cryptosporidium* oocysts. Thereafter the sample is rinsed and incubated with a FITC-conjugated anti-murine immunoglobulin. Unbound conjugate is rinsed away and the preparation is counterstained with Evans Blue. The filters are mounted with glycerol phosphate buffered saline or commercially available glycerol and the entire filter is examined using an epifluorescent microscope (Rose *et al.*, 1991).

Cryptosporidium oocysts and *Giardia* cysts are identified by the following criteria:

a) Bright apple-green fluorescence on the outside wall of the cyst or oocyst like objects.

b) Appropriate size and shape, as well as characteristic folding of oocyst wall.

These criteria would render the sample presumptive positive. Confirmed positive test results must meet the above criteria, as well as, the following:

a) *Giardia* - two to three of the following internal structures: nuclei, median bodies and fibrils.

b) *Cryptosporidium* - sporozoites visible as four crescent or sausage shaped structures within the oocysts.

A microscope equipped with phase contrast is necessary for the confirmation of protozoan parasites in these environmental samples.

One of the difficulties encountered using monoclonal antibodies for the identification of *Giardia* and *Cryptosporidium* is the ability of the antibodies to bind to other microorganisms, i.e. to stain non-specifically. Algae, other diatoms and invertebrate eggs have the capacity to bind with these antibodies and appear fluorescent. For this reason the immunoflourescence assay is used only to screen organisms as presumptive cysts or oocysts. Confirmation can only be made using phase contrast or differential interference contrast (Clancy *et al.*, 1994).

The immunofluorescence assay for cysts and oocysts is time-consuming, labour-intensive and requires a large degree of analytical expertise. Moreover the recovery efficiency of the procedure, especially for *Cryptosporidium* oocysts is relatively low (LeChevallier *et al.*, 1994). Therefore, research is being done to develop new more sensitive and specific methods for the detection of cysts and oocysts.

Enzyme-linked immunosorbent assays (ELISA) of faecal specimens for *Giardia* and *Cryptosporidium* antigens have emerged as a sensitive and objective method for diagnosing giardiasis and cryptosporidiosis. Several ELISA kits for detecting *Giardia*

and *Cryptosporidium* are now commercially available. These include kits from LMD Laboratories Inc, Cambridge Biotech Corporation and Alexon Inc., but have been developed for analysing stool samples. The kits will have to be evaluated for use in the water field.

Gene probe hybridization and PCR techniques are exciting possibilities, offering means of species-specific identification and good sensitivity detection. Because of the low concentration of *Giardia* and *Cryptosporidium* in natural waters, large volumes of water must be filtered to obtain samples to analyze. Through filtering not only the cysts and oocysts present are concentrated, but also organic material from the water, which is thought to interfere with both hybridization and PCR (Rodgers *et al.*, 1993).

Abbaszadegan *et al.* (1991) developed a ribosomal cDNA probe that could detect *Giardia* cysts. The disadvantage of probes is that the sensitivity is very low and this level of sensitivity is inadequate for environmental monitoring (Mahbubani *et al.*, 1991).

Pilot studies suggest that polymerase chain reaction (PCR) amplification of *Giardia* DNA is a sensitive method for detecting cysts in water samples. Mahbubani *et al.* (1991) developed a method for the detection of *Giardia* cysts by using PCR and the giardin gene as target.

Vesey *et al.* (1993) developed a flow cytometric method for the routine analysis of environmental samples for the presence of *Cryptosporidium* oocysts. Vesey *et al.* (1994) combined the method with the flocculation method for the concentration and detection of cysts and oocysts. The concentrated sample is stained in suspension with FITC-labeled monoclonal antibodies specific to *Giardia* cyst walls and *Cryptosporidium* oocyst walls before being analysed using a Coulter Elite flow cytometer. Particles with the fluorescence and light scatter characteristics of cysts and oocysts are sorted onto a microscope slide. Once the sample has been analysed, the microscope slide is dried, examined using epifluorescence microscopy and differential interference contrast (DIC) and cysts and oocysts are counted (Vesey *et al.*, 1994).

They claim that the flocculation method and the flow cytometry technique combined are a considerable improvement over the USA and UK accredited methods for concentrating and detecting *Giardia* and *Cryptosporidium* in water samples. However, the technique is not ideal due to the high cost of a flow cytometer incorporating cell sorting and the requirement for a highly qualified operator.

5. SUMMARY

Currently the proposed American Society for Testing and Materials (ASTM) analytical procedure is considered to be the method of choice for detecting *Giardia* and *Cryptosporidium* (LeChevallier *et al.*, 1991b; Clancy *et al.*, 1994) and is the accredited method for the detection of *Giardia* and *Cryptosporidium* in the US and UK. The method employs wound cartridge filters to collect particulate matter from large volumes of water. Captured particles are recovered by dissecting the filter and washing with detergent. The wash waters are collected before further concentration

by centrifugation. The sample is stained with FITC-labelled monoclonal antibodies specific to cyst and oocyst surface antigens. Detection of cysts and oocysts relies on examination of the sample by epifluorescence microscopy for fluorescing particles that have the morphological characteristics of cysts and oocysts.

More sensitive and rapid methods are being developed for the detection of cysts and oocysts, but most of these methods are still in experimental phase and will have to be optimized and evaluated for use in the water field.

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