EXECUTIVE SUMMARY

Background and motivation

A Water Research Commission sponsored workshop was held at Umgeni Water, Pietermaritzburg in February 1996 on "The occurrence and control of Cryptosporidium in the Umgeni catchment." The major stakeholders were present and discussed the present and future problems, research needs, collaborative partners and responsible researchers and laid out the aims of a research project. A collaborative effort was required to solve the problem of the source of the parasitic protozoa in the environment and assess the risks to health to both the rural communities who may use raw water as their only source and also the millions who are supplied with potable water directly, which could still be contaminated. The three organizations that were working on the parasitic protozoans in closely related areas, Umgeni Water, CSIR and University of Pretoria who had applied for funding were asked to combine projects and expertise.

A number of related WRC funded research projects have been undertaken at Umgeni Water. One is the investigation of the production of a novel Slide Immuno-Assay field test kit (report no. 825/1/03), for the protozoan parasites that could be tested in the high-risk areas pinpointed by this study. Another is Alternative Disinfection for the treatment of final wastewater effluents (report no. 1030/1/02); pilot studies have shown that large quantities of the parasites were present on occasions in some sewage effluents. Another project seeks to explore the relationship between the provision of rural water supply and community health, (Report no. 925/1/03).

Although there are many causes of diarrhoea, the enteric protozoons *Cryptosporidium parvum* and *Giardia lamblia* have been recognised as important causes of both outbreak-related and sporadic diarrhoea in humans (Casemore, 1990). Contamination of a water source may arise as a result of run-off, accidental spillage of farm slurry from agricultural land or from sewage effluent particularly from abattoirs or when infection exists in the community (Anon., 1990). In addition, the incidence of cryptosporidiosis and giardiasis is dependent on the lifestyle, socio-economic level and general health of the community (Fayer and Ungar, 1986; Feachem *et al.*, 1983). The largest waterborne outbreak in US history occurred in Milwaukee during spring 1993. Considerable epidemiological and environmental evidence indicates that the outbreak was caused by a large amount of *Cryptosporidium* passing through one of the drinking water treatment facilities of the Milwaukee Water Works. This waterborne disease outbreak caused illness in more than 400,000 people and resulted in several deaths (Solo-Gabriele and Neumeister, 1996).

From all assembled evidence it appears that occasional waterborne transmission of *Cryptosporidium* is likely. However, due to numbers likely to be present in raw waters and to probable immunity in the population, incidence is likely to be low. It is clear that there can never be absolute certainty that viable oocysts will not penetrate water treatment and so pass into supply. The problem therefore becomes one of risk assessment and minimisation by stricter regulations and guidelines to protect public health (Haas *et al.*, 1996). One of the requirements, therefore, is for good evidence on the occurrence of *Cryptosporidium* or *Giardia* in raw water sources. Using untreated surface water as a source of drinking water exposes a great number of the South African population to higher numbers of these pathogens and increases the risk of infection.

For the accurate enumeration of oocysts in samples, microscopy is still the method of choice. Under certain conditions the confirmation of microscopic results might be important, especially if oocysts are detected in treated drinking water. PCR (polymerase chain-reaction) is one of the techniques that could be used for this purpose.

Aims and objectives as specified in contract

The objectives of the project were:

(1) To establish a PCR method for confirming the presence of *Cryptosporidium* and *Giardia* in environmental water samples.

(2) To compare and contrast the PCR method of detection with immunofluorescence microscopy (CSIR; Umgeni Water) and flow cytometry (Rand Water) following concentration by cartridge filtration (CSIR), flocculation (Umgeni Water; Rand Water) or immuno-magnetic separation (Umgeni Water).

(3) To investigate the occurrence of parasitic protozoa in a catchment area.

(4) To establish to what extent diarrhoeal disease within a community is caused by *Cryptosporidium* and *Giardia*.

(5) To establish the presence of viable *Cryptosporidium* oocysts and *Giardia* cysts in the surface water of a high-risk community and any correlation with the occurrence of diarrhoea.

(6) To investigate the presence, viability and removal of parasitic protists during wastewater treatment processes and to evaluate the contribution of this source to their occurrence in the aquatic environment.

(7) Once sources of contamination have been identified, the necessary health education and guidelines can be implemented according to the Risk Assessment to minimise the health risk to communities and to potable water supplies.

(8) To train technicians from previously disadvantaged backgrounds in parasitological techniques.

Study design

One main objective of this study was to investigate the use of PCR based methods for the confirmation of results and to evaluate it's compatibility with the commonly used concentration, purification and detection methods. It was decided that the research would focus on *Cryptosporidium parvum* in the light of the difficulties experienced during detection. The findings of this study could thereafter be applied to the detection of *Giardia* if shown to be a viable option.

The study consisted of two parts, first, PCR reactions detecting all species of *Cryptosporidium* or only *Cryptosporidium* parvum had to be established in the lab. It was decided to use the primers described by Rochelle *et al.* (1997) because these primer sets can not only be used for the standard detection of oocysts but also for RT-PRC to test for viability. They were also compatible and could be used as part of a multiplex PCR for the simultaneous detection of *Cryptosporidium* and *Giardia*. The second part of the project was to evaluate whether this method was compatible to be used in conjunction with other methods used for the concentration and separation of *Cryptosporidium* oocysts in source and treated water samples.

The Pietermaritzburg catchment in KwaZulu-Natal was chosen for investigation of protozoon incidence in its rivers as the area is impacted by rural, peri-urban and urban settlements. Water sampling sites were chosen along the uMsunduze River and its tributaries as they make their way through the rural and peri-urban areas of Vulindlela and through the city of Pietermaritzburg. In addition, influent, effluent and sludge samples were collected from Darvill wastewater works and the viability status of (oo)cysts determined. As sludge samples are disposed of onto land, the effect of desiccation on the viability of (oo)cysts present in these samples was evaluated. The Regional Veterinary laboratories and local veterinarians were contacted to obtain samples of dung and details of any *Cryptosporidium* detected.

This study tried to determine whether the cause of diarrhoea, suffered by some patients in the Pietermaritzburg catchment and the Vulindlela area in KwaZulu-Natal, was indeed caused by *Giardia* and *Cryptosporidium*. Stools were examined for cysts and oocysts and the extent of diarrhoeal diseases, in the Vulindlela area, was established using information obtained from five clinics. An attempt was made to correlate the water source types used for domestic purposes in the Vulindlela area with the occurrence of diarrhoea in the community. The risk of *Giardia* and *Cryptosporidium* infections was quantified based on results obtained for different sampling sites on the uMsunduze River and its tributaries and Darvill sewage treatment works' final effluent. The Risk Assessment aimed to provide a quantitative estimate of the probability of illness associated with environmental exposures and focused on human health risk assessment from *Giardia* cysts and *Cryptosporidium* oocysts present in river water.

Brief summary of results and conclusions

- PCR-based methods have the potential to be used to confirm the presence of protozoan cysts and oocysts in water samples but owing to sensitivity problems it would be difficult to implement this technology on a routine basis in the near future.
- Occurrence in rivers was sporadic with *Giardia* cysts detected in 8% of samples and in higher numbers than *Cryptosporidium* oocysts, detected in 2% of samples; mainly during the wet summer months (although this may have been due to sewer breaks).
- As *Cryptosporidium* and *Giardia* were detected widely in river and wastewater samples, they are probably ubiquitous in the Pietermaritzburg area community and possibly livestock.
- Darvill Wastewater Works was found to remove up to 99.9% of *Giardia* cysts, after the activated sludge process, chlorination and maturation, in the final effluent.

- Some 70% of Darvill effluent samples were positive for the protozoa, however, containing up to 520 *Giardia* cysts 10 ℓ^{-1} and 110 10 ℓ^{-1} *Cryptosporidium* oocysts with up to 200 cysts 10 ℓ^{-1} in the river downstream.
- The potential Darvill effluent loading could be some 2.5 Billion cysts and 0.5 Billion oocysts per day into the uMsunduze River (at a dry weather flow of some 50 Mℓ /day), which constitues half the dry weather (winter) flow of the river.
- Fresh sludge disposed onto land was found to contain up to 3000 oocysts ℓ^{-1} and 700 000 cysts ℓ^{-1} and needs to be monitored if used in sensitive locations; desiccation experiments proved that viability could be reduced significantly.
- Neither the activated sludge process, nor the anaerobic digestion of sludge, appeared to significantly affect the viability status of the *Cryptosporidium* or *Giardia*.
- Treated effluents need to be monitored prior to discharge into rivers, which could be a source of water for both humans and animals and possibly treated further to remove them.
- The potential risk of infection for *Giardia and Cryptosporidium* posed by the river water, downstream of sewage effluents would appear to be high, if used for drinking or recreation.
- The occurrence of Giardia cysts throughout the year in the effluent samples indicates that giardiasis is probably endemic in the Pietermaritzburg population, while cryptosporidiosis infections may be sporadic and quite possibly asymtomatic.
- *Giardia* cysts were present in (black) schoolchildren's stool samples analysed, with 9.5% prevalence from the Kranskop area and 5.5% from semi-rural Vulindlela; *Cryptosporidium* oocysts were not detected, however.
- Reported incidence of diarrhoea was high in the Vulindlela area, with 39% overall and 49% in children under 5.
- A total of 20 household and source water samples were analysed from families who reported diarrhoea, but all were negative for *Giardia and Cryptosporidium*
- The actual cause of diarrhoea could generally not be established from local clinic, hospital or laboratory records; very few stools tested were positive for *Giardia* but approximately 3% were *Cryptosporidium* (mostly AIDS related therefore probably not of significance for water use).
- It would appear that diseases caused by these pathogens are not prevalent in livestock in this area or in South Africa in general, despite international reports they are the most common cause of diarrhoea in calves, foals and lambs worldwide.

Extent to which objectives were reached and actions to be taken as a result of the findings

The above objectives were generally achieved as described above, except for a part of number 7. The sources of contamination were identified and some preliminary guidelines drawn up for the community, veterinarians and for safe-guarding potable water supplies, but the implementation of these is the responsibly of the relevant authorities.

Health education and guidelines (for Schools, Depts. Educations and Health, Environmental Health Officers, Veterinarians, DWAF, farmers etc)

- The potential risk of infection downstream of wastewater effluents, for human consumption and recreation (swimming), would appear to be high. Therefore the community, especially children must be made aware of this and steps taken to restrict access.
- The effluents themselves and maturation ponds etc are often used, as they appear clean, for portable water, swimming and fishing. Access control for these areas should be considered, along with warning signs and pictures in various languages.
- Livestock is also at risk from the above
- Health education at schools should include the above and that even chlorination will not remove these protozoa.
- Direct faecal-oral transmission amongst children especially at crèches would seem to be important, therefore personal hygiene should also be emphasised at school.
- Screening of high-risk school children would enable carriers of intestinal parasites to be identified, as these infections can seriously affect cognitive ability.
- Access by livestock to areas of fresh sewage sludge should also be restricted and the sludge should not be used to fertilise crops that are eaten raw.
- If used as soil conditioner etc the sludge should be dried first and monitored for the protists before being transported off-site.
- Veterinarians should be encouraged to send dung samples to Veterinary laboratories for identification purposes.

General guidelines (for Municipal Managers, Water Boards, DWAF, farmers etc)

Tertiary treatment should be considered at wastewater works or alternative disinfectants eg UV, which would inactivate (oo)cysts.

- Computerisation of hospital, clinic and laboratory records to aid prevalence studies.
- Catchment management to ensure recreational and abstraction points are not close downstream to wastewater works
- Catchment surveys to identify water treatment plant abstraction points at risk from broken sewers or septic tank effluents; these should be monitored regularly.

Recommendations for further research and technology transfer

1. Intensive monitoring of specific areas is the only way to get an accurate picture of the presence of parasites, as these are likely to be sporadic, reflecting infection in the community and rainfall events.
2. The data acquired from such monitoring is invaluable to indicate which sources of contamination need to be addressed as a priority as well as providing data about which types of future development are likely to be potentially the most dangerous.
3. Risk assessment of each Water Treatment Plant is necessary to prioritise resources for monitoring and should comprise both process and catchment assessments.
4. The role of livestock (and possibly wild animals), as the source of the protozoan parasites in rivers has not been properly investigated, although indications are that this is relatively unimportant and surveys of feedlots and farms needs to be undertaken in this regard.

5. Typing of the different strains is now possible and early data suggests that there are different types for animals and human sources.
6. Investigation into resistance patterns in humans, to the parasitic protozoa could establish how widespread these infections are.

Technology transfer

The following workshops were attended/given to publicise the research work:

- A KZN Parasite Control Programme workshop, hosted by the Provincial Environmental Health department, was attended by Mr Bailey and Ms Jarmey-Swan, who presented an overview of the occurrence of *Cryptosporidium* and *Giardia* in faecal and water samples with particular reference to the Pietermaritzburg area.
- A KZN Regional Communicable Disease Control meeting was also attended at which a similar presentation was made to highlight the occurrence of these protists in KwaZulu-Natal.
- The Allerton Regional Veterinary laboratories and local veterinarians were visited and the project described to them and the significance of the protozoan parasites was explained. Training was given on laboratory procedures especially the immuno-fluorescence method for their detection.

Publications emanating from this project

C Jarmey-Swan, IW Bailey and AR Howgrave-Graham. 1997. Detection, occurrence and epidemiology of *Cryptosporidium* in KwaZulu-Natal, South Africa. In *1997 International Symposium on Waterborne Cryptosporidium Proc.*, Editors: CR Fricker, JL Clancy and PA Rochelle, 159-170.

C Jarmey-Swan, RA Gibbs, GE Ho, IW Bailey, and AR Howgrave-Graham, 2000. A novel method for detection of viable *Giardia* cysts in water samples. *Wat. Res.* **34** (6), 1948-1951.

C Jarmey-Swan, IW Bailey and AR Howgrave-Graham. 2000. Ubiquity of the waterborne pathogens *Cryptosporidium* and *Giardia* in KwaZulu-Natal populations. *Water SA* January (1) 2000.

C Jarmey-Swan, IW Bailey and C Johnson 2000. Occurrence and source of *Cryptosporidium* and *Giardia* in catchment areas and wastewater works in KwaZulu-Natal. In *The Water Institute of Southern Africa (WISA) biennial conference and exhibition: Health Impacts Proc.*

Archiving

The raw data for the relevant sections is archived as follows:

Umgeni Water:	Occurrence of parasitic protozoa in a catchment area.
CSIR:	Occurrence of *Cryptosporidium and Giardia* in a community
University of Pretoria:	Development of a PCR technique for *Cryptosporidium* and *Giardia*

Capacity building

Six previously disadvantaged individuals were employed during the duration of this project namely Ms Lungile Mthembu, Ms Zola Msiska, Ms Vashnee Chinnah, Ms Nosipho Gulwa, Ms Shantel Pecku Ms Caron Johnson and Ms Tracy Schmidt They were trained in concentration, detection and viability staining of protists in water, wastewater and sludge samples. Ms Johnson was trained in techniques for identifying *Cryptosporidium* and *Giardia* in stool samples under the auspices of the Medical Research Council, Durban. Ms Tracy Schmidt was trained in *Cryptosporidium* and *Giardia* concentration and detection techniques

Ms Mthembu has subsequently been employed as a permanent technician with the Microbiology and Public Health section of Analytical Services, Umgeni Water. Ms Msiska is employed as a Plant Pathologist with the Agricultural Research Council while Ms Chinnah declined a position as Senior Technologist with the Medical Research Council. Ms Gulwa is now employed as a microbiologist with Amatola Water in the Eastern Cape. Ms Johnson obtained a post with the Dept Health in Malaria Research, while Ms Schmidt was subsequently employed by the Allerton State Veterinary Laboratories.

Ms Zola Msiska obtained a BSc Hons
Ms Shantel Pecku obtained a BSc Hons
Ms Claire Jarmey-Swan obtained an MSc

User groups

Water Boards, local and national Departments of Health, Environmental Health Officers and other medical personnel interested in epidemiology within communities, Department of Water Affairs and Forestry, Municipal Managers, farmers and veterinarians.