EXECUTIVE SUMMARY OF RESEARCH ON

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

Report to the Water Research Commission

by

WOK Grabow, MB Taylor, JC Vivier, N Potgieter, and MG Gaobepe
Department of Medical Virology, University of Pretoria

November 2002

WRC report: 743/1/02
ISBN: 1 86845 859 8

Disclaimer

This report emanates from a project financed by the Water Research Commission (WRC) and is approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the WRC or the members of the project steering committee, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

EXECUTIVE SUMMARY

Contents

ACKNOWLEDGMENTS

- **Water Research Commission Project Steering Committee**

  Mrs APM Oelofse (Water Research Committion) Chairperson
  Dr N Mjoli (Water Research Commission)
  Prof CB Ijsselmuiden (University of Pretoria)
  Dr JH Olivier (City Council of Pretoria)
  Prof AD Steele (Medunsa)
  Ms B Genthe (Environmentek/CSIR)
  Dr PL Kempster (Department of Water Affairs and Forestry)
  Mr W van der Merwe (WaterWealth)
  Dr L Webber (University of Pretoria)
  Dr E Oosthuizen (City Council of Pretoria)
  Dr PGD Rautenbach (University of Pretoria)
  Mr DJ Marais (WRC Secretariat)

- **Research Team**

  Project Leaders:

  Prof W O K Grabow (University of Pretoria)
  Dr M B Taylor (University of Pretoria)

  Senior Partners:

  Ms J C Vivier (University of Pretoria)
  Dr C G Clay (University of Pretoria)
  Ms N Potgieter (University of Venda)
  Mr P Jagals (Technikon Free State)
  Ms M G Gaobepe (Technikon Soshanguwe)

  Research Staff and Students at the University of Pretoria:

  Ms K L Botma (MSc Student)
  Dr T Brisley (Medical Officer)
  Ms J C de Villiers (Research Assistant)
  Ms B Erasmus (Research Assistant)
  Mr F E Marx (PhD student)
  Ms L H Olivier (Secretariat)
  Me N Potgieter (MSc student)
  Dr P G D Rautenbach (Department of Community Health, University of Pretoria)
  Mr C Swanevelder (Control Technologist)
  Ms M A Vrey (Research assistant)

- **Collaboration**

The study has been carried out in collaboration with:

- Departments of Health Services and Sanitary Engineering, City Councils of Pretoria and Mamelodi, Gauteng.
- Town Council of Akasia, Gauteng.
- Department of Health, Northern Province.
- Department of Health, Botshabelo, Free State.

Support, information and guidance were rendered by:

- University of Chapel Hill, North Carolina, USA: Prof M D Sobsey, a world leader in the field concerned, visited the Department during the study to render assistance with establishment of new technology and the training of staff and students.
- University of Arizona, Tucson, USA: Prof C P Gerba and co-workers supplied valuable information on the typing of enteroviruses.
- Ruhr University, Bochum, Germany: Prof H Werchau and co-workers supplied valuable assistance with the typing of rotaviruses.
- National Institute for Virology, Johannesburg: Dr C Chezzi rendered valuable assistance with the typing of polioviruses.
- Medical University of Southern Africa, Medunsa: Prof A D Steele contributed to the planning of research and assisted with the training of Ms Potgieter in the typing of rotaviruses.
- Institute of Child Health, London: Drs W D Cubitt and S Parkers were actively participating partners in work on parts of the project.
- Water Research Commission, Steering Committee: Members of the Steering Committee made most valuable contributions in terms of advice and information.

1. INTRODUCTION

Infectious diseases are the most important concern about water quality (Craun et al, 1994). Authoritative estimates ascribe about 50 000 deaths per day in the world to waterborne and water-related infectious diseases. In addition to mortality, which affects mainly children in developing communities, waterborne diseases also have far-reaching socio-economic implications (Craun et al, 1994). Viruses feature prominently among the wide variety of pathogenic micro-organisms concerned.

The term "enteroviruses" refers to the following members of the family Picornaviridae: polio, coxsackie A and B, echo and entero viruses. Enteroviruses are common causes of localised and systemic infection in patients of all ages. Manifestations include paralytic poliomyelitis and other central nervous system diseases, myocarditis and other muscular diseases, etc. The group of enteroviruses belongs to the larger group of enteric viruses, all of which are typically transmitted by the faecal-oral route, ie, primarily faecally polluted water and food.

The importance of enteroviruses is highlighted by recent developments such as:

- The WHO puts every effort into a massive world-wide campaign for eradication of the poliovirus. No poliomyelitis cases have been reported in South Africa for some time now, but fears were raised by a recent outbreak in Namibia (Van Niekerk et al, 1994) and indications of the disease in Mozambique (Oostvogel et al, 1994).
- Concerns about the success of poliomyelitis control in South Africa are reflected by the recent declaration of "acute flaccid paralysis" (primarily caused by polio and other enteroviruses) a notifiable condition.
- Recent risk assessments based on the latest epidemiological data indicate that the health implications of waterborne coxsackieviruses are more serious than previously thought (Gerba et al, 1995).
- The latest data derived by new molecular techniques indicate that the role of enteroviruses in diseases of the heart is much larger than previously thought.
- Socio-economic developments in South Africa, such as informal settlements with inadequate sanitation, create conditions which are ideally suited for the spread of enteroviruses.

Many enteroviruses are cytopathogenic, ie, detectable by cell culture propagation. However, some enteroviruses, notably coxsackie A viruses, are not detectable by cell culture. Detection of these viruses requires inoculation of newborn mice, or recently developed molecular techniques.

Information on the incidence of enteroviruses in wastewater is of basic importance for controlling waterborne transmission of the viruses. Analysis of wastewater is being used as an indicator of enteroviruses circulating in communities, and is standard procedure for determining the presence of poliovirus or the success of poliomyelitis vaccination campaigns (Ivanova et al, 1995).

In a preceding project (WRC Project No 496/1/96) evidence has been presented that wastewater and diffuse effluents from informal settlements carry high loads of faecal pollution and viruses. However, the enteroviruses present in these effluents have not been typed to

meaningful extent, and the behaviour and survival of viruses in these effluents remain to be investigated. These details are essential for:

- Assessment of the risk of infection and waterborne transmission of enteroviruses constituted by effluents from informal settlements.
- Formulation of guidelines and policies for sanitation and wastewater disposal, as well as the protection of water resources.
- Investigation of enteroviruses circulating in the communities concerned. For instance, this gives an indication of the risk of poliomyelitis.

Details on enteroviruses have not been reported for a comparable situation anywhere in the world. The technology, expertise, manpower and infrastructure concerned is of fundamental importance for research on waterborne transmission of enteroviruses, the epidemiology of enteroviruses in general, and the safety of water supplies.

References

Craun GF et al (1994) Balancing chemical and microbial risks of drinking water disinfection, Part I. Benefits and potential risks. Aqua 43, 192-199.

Gerba CP et al (1995) Waterborne coxsackievirus: a quantitative risk assessment. International Congress on the Impact of Viral Diseases in Developing Countries, Johannesburg, 9-14 July.

Ivanova O et al (1995) Investigations on poliovirus circulation in environment in some regions of former USSR. International Congress on the Impact of Viral Diseases in Developing Countries, Johannesburg, 9-14 July.

Oostvogel PM et al (1994) Poliomyelitis outbreak in an unvaccinated community in the Nether-lands, 1992-93. Lancet 344, 665-670.

Van Niekerk ABW et al (1994) Outbreak of paralytic poliomyelitis in Namibia. The Lancet 344, 661-664.

2. OBJECTIVES

The following is a list of original objectives specified in the project agreement together with extensions recommended by the Steering Committee during the course of the study period. The aims were to:

- Optimise techniques for the recovery of viruses (cytopathogenic as well as non-cytopathogenic) from water
- Establish affinity chromatography techniques for the selective recovery of viruses
- Evaluate application of the L20B mouse cell line for the selective detection of polioviruses
- Evaluate plaque assays and most probable number procedures for the quantitative enumeration of cytopathogenic enteroviruses in water
- Optimise molecular techniques for the detection of viruses such as calici, astro, rota, hepatitis A and E, and certain enteroviruses including coxsackie A viruses, which are not detectable by conventional methods
- Assess the efficiency of a commercial point-of-use water treatment unit
- Investigate a typical outbreak of enteric viral disease

- Study the qualitative and quantitative incidence of viruses in waste water from selected informal settlements and polluted water sources
- Assess the correlation of viruses circulating in a community to viruses detectable in the waste water from the community
- Assess the extent to which waste water monitoring can serve as an indication of a risk of poliomyelitis in a community
- Assess the implications of viruses in waste water from informal settlements for sanitation and the protection of water sources
- Assess the risk of waterborne human viral infections constituted by animal wastes, notably rotaviruses
- Research on the role of water in enteric diseases in selected communities
- Evaluate the reliability of practical indicator organisms for assessment of the virological quality of water, notably faecal bacteria, bacteriophages and cytopathogenic viruses
- Formulate recommendations for the control of waterborne viral diseases
- Formulate recommendations for practical routine monitoring of the virological safety of water.

Research Products
Products:

- Techniques for detection of new viruses
- Data on risks of waterborne viruses
- Technology and expertise for safe water

Target Group:

- Water industry: safe water
- Health authorities
- Water industry: capacity building for safe water

Potential Application of Products

- Sanitation and wastewater disposal
- Control of waterborne diseases
- Assessment of the safety of water supplies
- Supply of safe water

Accomplishment of Objectives

All objectives have at least in principle been accomplished as defined above. Due to developments and progress during the study period, more than the original goals have eventually been achieved in many cases. However, it should be noted that much of the research did not have end-point goals. For instance, the development of new technology is an ongoing exercise. Although major progress has been made, the work revealed possibilities for further developments which need to be followed up. Likewise, valuable information has been gained on the quality of water supplies and possibilities for the control of water-borne diseases. However, no final solutions have been established, and new challenges emerge in the face of a variety of changing situations and conditions. Valuable

progress has also been made with capacity building and technology transfer, but this needs to be maintained and expanded. The facilities, expertise and infrastructure established by this project served as the foundations for a comprehensive and detailed study on the virological quality of drinking water supplies country-wide.

3. LITERATURE REVIEW

The latest literature on enteroviruses and other enteric viruses has been review in a number of publications (Grabow, 1996a,b, 1997; Grabow et al, 1996; Marx, 1997; Botma, 1999; Vivier, 2000). Additional details on specific viruses have been addressed in publications, dissertations and conference presentations listed in Chapter 11.

4. DEVELOPMENT OF NEW TECHNOLOGY AND EXPERTISE

Achievements on objectives which specifically address the development of new technology and expertise are summarised below with reference to further details published in peer review international scientific journals and reports available on request.

4.1 Optimise techniques for the recovery of enteroviruses (cytopathogenic as well as non-cytopathogenic) from water

A glass wool adsorption-elution technique for the recovery of viruses from water has been optimised and established in the laboratory (Grabow et al, 1999a). Ongoing literature surveys and consultations with overseas experts in the field failed to reveal indications of other procedures which may yield superior results. In an interlaboratory comparison study carried out in France efficiencies of recovery in excess of 80 % were recorded for raw and treated water supplies using basically the same method. This procedure is now being used in routine monitoring programmes, but results indicate that the method can be improved. This conclusion is based on results obtained from participation in an interlaboratory comparison study conducted by the Public Health Laboratory Service in the UK. Alternatives have been identified, and further research on techniques for the recovery of viruses from water is justified. In seeding experiments the efficiency of recovery was lower for rotaviruses than for poliovirus. Despite the relatively low efficiency of recovery, rotaviruses were recovered from a variety of waste waters and polluted rivers (Marx et al, 1997, 1998b; Taylor et al, 1997, 2000; Grabow et al, 2000a).

A procedure for the detection of viruses in water environments by the identification of their nucleic acid using molecular techniques based on the polymerase chain reaction (PCR) after amplification in cell cultures has been optimised for a spectrum of viruses. This procedure proved suitable and highly sensitive for both cytopathogenic and non-cytopathogenic viruses, and has been established successfully for routine detection of a spectrum of viruses (Grabow et al, 1999a,b; Vivier, 2000).

Progress with the development of advanced techniques for the typing of enteroviruses, as well as the application of this technology and expertise in practise, has been described (Vivier, 2000). Typing techniques are based predominantly on PCR using selected primers, hybridization using specific gene probes, nucleotide sequencing, and restriction enzyme analysis.

4.2 Establish affinity chromatography techniques for the selective recovery of viruses

An affinity chromatography (AC) procedure for the specific and selective recovery of viruses from water has been established (Potgieter et al, 1997). In experiments using coxsackie B virus as model, efficiencies of recovery exceeded 90 %. Unfortunately the AC procedure inactivated the viruses and they had to be detected by molecular techniques. The results suggest that the same principles may be applied for the selective recovery of other viruses. The principles involved offer opportunities for substantial further improvement, even with potential for the development of commercial products. AC has been identified as a priority for future research.

4.3 Evaluate application of the L20B mouse cell line for the selective detection of polioviruses

The L20B mouse cell line has been studied comprehensively in laboratory tests as well as field investigations. The cells proved superior to a number of cell lines and primary vervet kidney cells for the detection of polioviruses. However, the cells were not absolutely selective for polioviruses. A small number of other viruses, apparently of animal origin, were also isolated on these cells. Best results for the isolation of polioviruses were obtained by using the L20B mouse cell line in combination with the PLC/PRF/5 human liver cell line and the BGM monkey kidney cell line. L20B cells are now part of the battery of cell culture systems routinely used for the detection of polioviruses in patient specimens as well as environmental samples (Grabow et al, 1999a).

4.4 Evaluate plaque assays and most probable number procedures for the quantitative enumeration of cytopathogenic enteroviruses in wastewater

A number of cell cultures has been compared for detecting viruses using plaque assays (PA) and most probable number assays (MPN). PA proved well suited for the accurate enumeration of rapidly growing highly cytopathogenic viruses, such as poliovirus, when these viruses are present in relative high numbers in test samples. PA have shortcomings for slow growing viruses such as reovirus and injured enteroviruses because it is not possible to keep the cell cultures viable for long enough in these assays. MPN proved more sensitive because cells can be kept viable for longer periods. However, since counts of viruses in test samples are statistically calculated from numbers of positive and negative wells, MPN is less accurate than PA where viruses are physically counted as plaques on a lawn of host cells. The most sensitive method for the detection of small numbers of viruses was by inoculating relatively large volumes of test samples into flasks with cell cultures. This basically qualitative detection method has the benefit that cells can be kept viable for prolonged periods of time and it is easy to passage viruses to fresh cells in new flasks for repeated cultivation. This allows ample time for recovery or adaptation to produce a visible cytopathogenic effect (CPE). The inoculation of relatively large volumes of test samples into flasks with cell cultures proved the method of choice for the qualitative detection of small numbers of viruses. The ability of a number of compounds to enhance viral CPE has been investigated as part of this study. 5-Iodo-2-deoxy-uridine (IDU) has been found to enhance CPE of a number of laboratory strains of viruses in both PA and MPN assays. IDU also increased PA and MPN counts of cytopathogenic viruses in waste water and raw water sources to meaningful extent. Other chemicals associated with enhancement of CPE, failed to yield

meaningful results. These included polyethylene-imine, cholesterol and indomethacin. Mixed cell cultures likewise failed to stand up to expectations created by some reports. Findings and recommendations have been recorded by Botma (1999).

4.5 Optimise molecular techniques for the detection of viruses such as calici, astro, rota, hepatitis A and E, and certain enteroviruses including coxsackie A viruses, which are not detectable by conventional methods

This was a major activity of the project. New techniques have been developed for the detection of a wide spectrum of viruses. The new technology is now being used routinely in research on viruses in water, the efficiency of water treatment processes and the safety of water supplies. The new technology has established this laboratory as a centre of excellence on viruses in water, thus enabling the laboratory to offer services and the training of manpower which compare favourably with the best in the world. However, this is a rapidly developing field of expertise with many attractive opportunities for further development. An important goal is to reduce the cost of virological analysis and to develop techniques which are within reach of a wider spectrum of laboratories. Further work on viral detection techniques has therefore been identified as a high future priority.

Details on this work have been published (Marx et al, 1997, 1998a,b; Taylor et al, 1997; Webber et al, 1998; Grabow et al, 1999a,b; Vivier, 2000) and presented at a number of conferences (Chapter 11).

5. INVESTIGATION OF A TYPICAL OUTBREAK OF ENTERIC VIRAL DISEASE

An outbreak of gastroenteritis in the infant-toddler unit of a child care centre in Pretoria was investigated for possible viral enteropathogens. Three different enteric viruses were detected in patient stool specimens from 10 patients. Two patients had co-infection with rota- and astroviruses, and one had co-infection with rota-, astro- and enteric adenovirus. The results highlight the diversity of viral enteropathogens that may be associated with a diarrhoeal outbreak and emphasise the need to investigate the possibility that multiple enteropathogens may simultaneously cause a single outbreak of diarrhoea. It was not possible to determine the origin of the infection. However, all three the viruses concerned are typically transmitted by the faecal-oral route and waterborne transmission of these viruses is well documented.

Details of the study have been published by Taylor et al (1997). Further epidemiological data on the incidence in South Africa of viral infections typically associated with water-borne transmission have been published by Grabow et al (1996), Grabow (1997), Taylor et al (1996), Wolfaardt et al (1997) and Marx et al (1998a).

6. ASSESSMENT OF THE EFFICIENCY OF A POINT-OF-USE WATER TREATMENT UNIT

The efficiency of the *Aquaguard* point-of-use domestic water treatment unit has been evaluated in collaboration with the manufacturers of the unit, Eureka Forbes Ltd, Bangalore, India. The removal and inactivation of a spectrum of pathogens and indicators have been investigated. The study offered an ideal opportunity to assess and apply in practice new techniques developed in this project. The unit proved highly efficient, and could certainly be used to alleviate the quality problems with domestic drinking water supplies in developing

communities. However, the unit also had shortcomings for this particular purpose. The most important of these shortcomings were high cost, dependence on electricity and a need for advanced operation and maintenance. Details of the study have been published by Grabow et al (1999b).

7. RESEARCH ON THE ROLE OF WATER IN ENTERIC DISEASES IN SELECTED COMMUNITIES

The following objectives of the project were addressed in work covered by this Chapter:

- Study the qualitative and quantitative incidence of enteroviruses in wastewater effluents from selected informal settlements and polluted water sources
- Assess the correlation of enteroviruses circulating in a community to enteroviruses detectable in the wastewater from the community
- Assess the extent to which wastewater monitoring can serve as an indication of a risk of poliomyelitis in a community
- Assess the implications of enteroviruses in wastewater from informal settlements for sanitation and the protection of water sources

Waste water from a number of informal settlements and polluted water sources such as selected rivers, have been found to contain high numbers of a wide spectrum of viruses. At least some of the rivers serve as the only source of domestic water supply to certain rural communities. Data on the incidence and types of viruses in the waste waters and polluted sources confirm a potential health risk, and emphasise the need for improvement of sanitation and water supply in many communities. In economic considerations regarding improvement of these services, the cost of improving sanitation and water supply should be weighed against the cost of the impact of the water-borne and -related diseases concerned. This is illustrated by the health impact and related costs of the cholera epidemic which currently afflicts parts of the country. Studies focussed on the following communities:

7.1 Molopo, North West

Substantial inputs had been made into endeavours to carry out a study on the incidence of enteric viral infections in developing communities in the Molopo region near Mmabatho. The intention was to correlate the incidence of infections with the presence of viruses in water supplies used by these communities. The study was due to be carried out in collaboration with the University of the North West in Mmabatho. Preliminary door-to-door surveys had been completed, and the quality of a number of water sources had been analysed. The key role player at the University had intentions of using the study for a PhD, but resigned. Attempts to recruit a suitable candidate at the University of the North West to carry on with the project were not successful and the study could not proceed.

7.2 Atteridgeville, Gauteng

A study on the role of water in the transmission of viruses has been completed. Vaccine poliovirus was used as model at times when vaccination campaigns were carried out in the community. The results revealed that the virus was detectable in water environments within a day or two after the onset of vaccination of children. These findings revealed that enteric

viruses from infected individuals in communities are released rapidly into the environment and water resources. The results also illustrate the value of monitoring waste water as a tool to screen viruses circulating in communities. The findings confirm the value of water quality monitoring to obtain important information on the epidemiology of waterborne viruses, and the role of water in their transmission. Among other things, the results imply that wild type polioviruses would be detectable in waste waters almost immediately after the onset of an outbreak in a community. The evidence that such viruses would be detectable in environmental waters even in communities without conventional sewerage systems is of particular importance. In this case study the analysis of waste waters yielded valuable information on the efficiency of the poliomyelitis vaccination campaign carried out in the community. For further details see Grabow et al (1999a) and Rautenbach (1996).

7.3 Soshanguwe, Gauteng

The study at Soshanguwe was carried out in collaboration with the Town Council of Akasia and community authorities concerned. The investigation formed part of an environmental health and hygiene awareness campaign launched by the Council. The key role player in the study had intentions of using the study for a PhD degree. A research protocol was formulated and a questionnaire for door-to-door surveys has been designed taking into consideration recommendations of the Water Research Commission Steering Committee. The study has been launched successfully. Students at the Soshanguwe Technikon were employed in door-to-door surveys and the collection of specimens. The study yielded valuable information. For instance, the results indicated that households with in-house piped water supplies had lower incidences of gastroenteritis than households where drinking water supplies had to be collected in containers some distance away and stored in the home. A report on preliminary results is available (Gaobepe, 1998). Details on rotaviruses isolated from patients in the communities concerned have also been included in another report (Grabow et al, 2000a). Unfortunately the researcher resigned from the Soshanguwe Technikon and took up new employment where it was not possible to carry on with the study. This is most unfortunate because an infrastructure and opportunities were in place to do most valuable research.

7.4 Botshabelo, Free State

Studies at Botshabelo were predominantly carried out at the Technikon Free State in Bloemfontein. In a comprehensive survey meaningful evidence has been presented that elevated incidences of water-related infections were associated with the collection of drinking water supplies in containers from communal sources for storage in homes. This was confirmed by an intervention study in which meaningful evidence was presented that the in-house supply of piped water may reduce the incidence of water-related infections. Details have been published (Jagals et al, 1997, 1999) and presented at conferences (Chapter 11).

7.5 Venda, Northern Province

The work at Venda was predominantly carried out with staff and students at the Department of Biochemistry and Microbiology, University of Venda. The Head of the Department, and colleagues from other Departments at the University, made most important contributions to the planning and execution of the research.

Work in Venda focussed on rotavirus as a model for enteroviruses and other enteric viruses.

Reasons included the higher incidence of clinical symptoms caused by rotavirus infections compared to enteroviruses. This made it easier to trace infected patients in the community than individuals infected by enteroviruses. Also, the information on rotaviruses is of particular interest because these viruses remain the single most important cause of infantile death in the world.

The homes of patients who presented with rotavirus-diarrhoea at hospitals and clinics were visited and their drinking water supplies were analysed. Detailed questionnaires on the incidence of enteric infections in these and control homes were conducted by undergraduate students. Raw water sources were also analysed. These included the Levuvhu River, boreholes and springs, as well as treated supplies delivered by pipeline or tanker trucks. The survival of indicators and pathogens in water supplies stored in containers for daily use in homes has also been investigated. The results illustrate the potential for waterborne transmission of viruses and other pathogens in these communities.

Special attention was given to the possible water-borne transmission of rotaviruses from animals to humans. This was done because it is generally believed that rotaviruses are highly host specific and that animal strains of rotavirus will not readily infect humans. However, recent reports from some parts of the world suggest that under circumstances rotaviruses may indeed cross species barriers. Venda offers an ideal opportunity for research along these lines because many people there live in close contact with animals and exposure to animal wastes. Partners in this part of the study included international experts on rotaviruses, notably Prof H Werchau, Department of Virology, Faculty of Medicine, Ruhr University, Bochum, Germany, and Prof AD Steele, MRC Research Unit for Enteric Viruses, Medunsa.

Stool specimens from patients with rotavirus gastroenteritis were collected in the Pretoria area and rural areas of Venda. These study areas were selected to represent communities with limited exposure to animals (Pretoria) and communities with substantial exposure to animals (Venda).

Specimens from the Pretoria area were predominantly obtained from the Department's diagnostic laboratory where these stool specimens were received from various hospitals, clinics and medical practitioners for diagnosis of the infections. Specimens from the Venda area were predominantly collected from clinics and hospitals. A total of 87 Pretoria rotavirus isolates and 108 Venda isolates were typed in detail. The results show that all infections in both areas were caused by strains of rotaviruses typically associated with infections in humans. There were no meaningful indications of infections in humans which may be of animal origin.

Rotaviruses were also detected in 2 samples of waste water from an informal settlement, 2 samples of water from a polluted river, 3 samples of dam water, and 2 samples of waste water from one abattoir and 13 samples of waste water from another abattoir. However, these viruses were detected by molecular techniques and typing of the viruses concerned was not possible. The number of rotaviruses isolated from environmental waters was smaller that would be expected in view of the incidence of rotavirus infections in study communities. Although most valuable new techniques for the detection and typing of rotaviruses have been established as part of this project, it was evident that technology for the recovery and detection of rotaviruses in water environments was in need of improvement. This has been identified as a major priority for future research.

Details on the work have been recorded in a report (Grabow et al, 2000a) and have been presented at conferences (Chapter 11).

The project at Venda made a major contribution to the establishment of an infrastructure for most important further research on the control of waterborne diseases in rural and developing communities. This includes the training of researchers at the University of Venda where research on viruses has not previously been carried out. The involvement of undergraduate students in the project has stimulated interest which is due to have major benefits for their education and future careers. A number of postgraduate students are now engaged in studies on the microbiological quality of water. Involvement of communities by door-to-door surveys created opportunities for education on rotaviruses and basic methods for control of the infections. This certainly had benefits for at least those households that were visited.

7.6 Mamelodi, Gauteng

Detailed studies carried out on waste waters at Mamelodi showed the presence of exceptionally high numbers of a spectrum of enteric viruses in diffuse effluents from an informal settlement (Grabow et al, 1996a). These waste waters heavily polluted a stream running through the area. Although direct survival studies were not carried out to meaningful extent in this study, it is known that viruses may remain viable for extended periods of time in water environments of this kind. This pollution does, therefore, constitute significant health risks for the utilisation of the water downstream. The findings underline the need for sanitation and the protection of water resources. Results for similar studies carried out in other areas are in agreement with the findings for Mamelodi (Grabow et al, 1996a).

8. CONCLUSIONS AND RECOMMENDATIONS

8.1 Reliability of conventional indicator organisms

In extensive analysis of raw water sources (river and dam water) viruses were rarely detected in the absence of indicators such as coliform bacteria, enterococci and somatic or F-RNA coliphages. These results suggest that indicators of faecal pollution rarely fail to indicate the potential presence of viruses in waste water and polluted water environments (Grabow et al, 1996a).

However, viruses were detected in treated drinking water supplies which met generally accepted quality limits for heterotrophic plate counts, total coliform bacteria, enterococci, and somatic and F-RNA coliphages. These results show that conventional indicator tests have shortcoming for assessing the presence of viruses in treated drinking water supplies. The findings have substantial implications for international quality guidelines which recommend the absence of viruses from drinking water supplies (Grabow, 1996a,b; Grabow et al, 1999a, 2000b).

8.2 Recommendations for the control of water-borne viral diseases

In the high risk communities addressed in this project domestic drinking water supplies were largely based on the collection of water from various sources using a variety of containers for indoor storage and daily use. At Botshabelo and Soshanguwe these drinking water supplies were predominantly collected from good quality water at communal standpipes and tanker trucks. In Venda some communities collected domestic water supplies from polluted

sources. Evidence has been presented that the quality of the drinking water supplies tends to deteriorate during storage. This deterioration was due to growth of heterotrophic bacteria, or to contamination during storage. Although the health risk constituted by these water supplies has not been clearly defined, there is little doubt that they constitute a potential risk of infection. Intervention studies were carried out by using clean and decontaminated vessels with a screw-cap at the top and tap at the bottom (Gaobepe, 1998). These findings confirmed that the risk of infection constituted by indoor stored drinking water supplies can be reduced extensively by education, training and minor modifications to water storage practices.

The extent to which practices of indoor water storage may affect viral infections in consumers was not an objective of this project and is not yet altogether clear. However, results obtained indicate that a meaningful reduction in viral infections may require a more holistic approach including sanitation, food hygiene and personal hygiene. Evidence has also been presented that the quality of indoor stored drinking water supplies can be improved extensively by bringing the source of safe water closer to residences. At Botshabelo it was shown that this strategy resulted in a meaningful reduction in the periods of storage and the volumes of water that were stored, and more frequent collection of water supplies in smaller containers. This reduced opportunities for quality deterioration by growth of heterotrophic bacteria, as well as contamination during storage by handling, dust, flies, etc (Jagals et al, 1997, 1999).

Recommendations for the control of waterborne viral diseases in high risk communities are, therefore, largely based on education and training regarding the collection and storage of indoor drinking water supplies. This addresses short-term immediate interventions at minimum cost. The need for supplying safe water closer to homes is also emphasised. This option is more expensive and will take longer to implement. The ultimate goal is to supply running tap water inside households, but this is the most expensive and time consuming option.

8.3 Recommendations for the routine monitoring of water quality

Evidence has been presented that conventional indicators such as heterotrophic plate counts, coliform bacteria, enterococci and coliphages serve a valuable purpose for indicating faecal pollution in waste water and polluted raw water sources. This faecal pollution indicates the potential presence of pathogens such as viruses. However, there is no direct correlation between any indicators and the incidence of viruses. Indicators of faecal pollution, notably coliphages and clostridia, may also give a valuable indication of the survival of viruses in the environment, raw water sources, and water treatment and disinfection processes. The absence of a direct correlation between any indicators and viruses in treated drinking water supplies was clearly illustrated by the detection of viruses in drinking water supplies which met quality limits for all conventional indicators (Grabow, 1996a,b, Grabow et al, 2000b) A particularly interesting case of evidence in this regard was the drinking water supplies stored indoors in informal residences. These storage vessels tended to have high counts of heterotrophic and coliform bacteria, and the counts of these bacteria tended to increase during storage, while no viruses were detected. Even if viruses were present initially, they could not multiply like heterotrophic and coliform bacteria.

These findings show that conventional indicators have shortcomings for assessment of the virological safety of drinking water supplies (Grabow et al, 2000b). Although conventional indicators certainly offer valuable indications of the potential presence of pathogens, ultimate

confirmation of virological safety requires additional strategies. These strategies may include analyses for viruses themselves. Progress in technology and expertise increasingly renders this option more practical and feasible. Where the inclusion of virological analysis in routine monitoring programmes is not possible, as in the case of the high risk communities addressed in this project, alternative strategies are required. These would include appropriate sanitary surveys of water sources as well as the handling and storage of drinking water. This would have to be supplemented by using containers which prevent contamination during storage, and by cleaning and decontaminating storage vessels by procedures known to inactivate or remove pathogens like viruses (Grabow et al, 2000a).

9. RESEARCH PRODUCTS

9.1 Research products

9.1.1 Techniques for detection of new viruses

Substantial progress has been made in the development of techniques for the detection of viruses in water which were not previously detectable. New techniques are predominantly based on amplification of viral nucleic acid in selected cell cultures followed by molecular detection of the nucleic acid using molecular techniques.

9.1.2 Data on risks of waterborne viruses

Data have been recorded on the incidence of potentially water-borne viruses in water resources and treated drinking water supplies, as well as infections in patients caused by viruses which are potentially water-borne. This information underlines the potential role of water in the spread of enteric viral diseases.

9.1.3 Technology and expertise for safe water

Evidence has been presented that in high risk communities such as those which practice the collection of drinking water supplies in a variety of containers for indoor storage and daily use, the risk of infection may be reduced substantially by a variety of intervention strategies. These may include the use of vessels which appropriately protect the water from contamination, and the proper cleaning and decontamination of containers prior to filling them with safe water. The success of these and related intervention strategies heavily depend upon education and training of user communities.

9.2 Target Group

9.2.1 Water industry: safe water

Techniques and standard operating procedures for routine monitoring of drinking water supplies have been established and are ready for direct application in practice.

Information of fundamental importance to the supply of safe drinking water to high risk communities, notably those in informal settlements and rural areas, has been

recorded.

The results on the incidence of viruses in waste waters and water resources underline the importance of sanitation aimed at protecting water sources from pollution with waste waters which contain viruses and other pathogens.

9.2.2 Health authorities

The results of the project offer valuable guidelines to health authorities on strategies that may be followed to reduce the risk of water-borne diseases. Various options for addressing risks associated with indoor storage of drinking water supplies have been recorded. The importance of education and training as a strategy for controlling intestinal infections has been emphasised. Risks of viral infections have been identified as a point in case illustrating the importance of a holistic approach to the control of enteric diseases involving sanitation, food and personal hygiene, and appropriate sanitary surveillance in addition to safe water supplies.

9.2.3 Water industry: capacity building for safe water

A number of postgraduate students and research assistants have been trained in advanced technology and expertise of fundamental importance to the water industry. This includes the training of post- and undergraduate students at the Bloemfontein Technikon and the University of Venda. Undergraduate students were involved in the collection of samples, door-to-door surveys to collect information and samples, and basic laboratory tests. This involvement of young students contributed to the creation of awareness and interest in the water industry. Visits to households in informal settlements and rural communities, and the involvement of residents in experiments on different types of water containers, contributed to education and training.

9.3 Potential application of Products

9.3.1 Sanitation and waste water disposal

Technology and expertise have been developed for assessment of the virological quality of waste water, and the efficiency of waste water treatment and disposal practices.

9.3.2 Control of water-borne diseases

Strategies for the control of water-borne viral diseases in high risk communities have been defined. A number of options for reducing the risk of viral infections have been proposed.

9.3.3 Assessment of the safety of water supplies

An advanced standard operating procedure for monitoring the virological safety of water supplies has been established.

9.3.4 Supply of safe water

Strategies for improving the safety of drinking water supplies in high risk communities such as informal settlements and rural communities where drinking water supplies are stored indoors in various types of containers, have been formulated.

10. RECOMMENDATIONS FOR FURTHER RESEARCH

Progress in technology development and findings on water-borne transmission of viral diseases in high risk communities recorded in this project reveal a number of areas for essential further research. The most important of these include:

10.1 Technology development

Potential has been identified for the further improvement of techniques for the recovery of viruses from large volumes of water, and the subsequent detection and characterisation of these viruses. This includes application of the principles of affinity chromatography and new approaches to the recovery of viruses by filtration procedures followed by the direct cultivation of viruses on membranes. A highly successful infrastructure for progress in the development of new techniques to detect and characterise viruses in water has been established. This should be duly utilised not only for the development of new technology and expertise, but also for capacity building and the training of manpower in a field of fundamental importance to the water industry and related disciplines. There is great potential for increasing the sensitivity and reliability of detection methods, as well as the reduction of costs. In addition, viral detection techniques may be simplified to bring virological analyses within reach of the technological and financial capabilities of many more laboratories.

10.2 Water-borne transmission of viruses from animals to humans

Observations that enteric viruses seem to be highly host specific and that animal viruses are rarely if ever transmitted by water to humans, have important implications for the epidemiology of water-borne diseases. Indications are that the hepatitis E virus may be the only exception to the rule. This needs to be confirmed because evidence has been presented that hepatitis E is endemic in South Africa and that the virus does infect at least pigs in many parts of the country.

10.3 Indicators

Evidence on the host specificity of viruses places new emphasis on the importance of distinguishing between faecal pollution of human and animal origin for assessment of the safety of water supplies and risks of infection. This underlines the need for further research and optimisation of techniques to use phages for this purpose, notably serogroups of F-RNA coliphages and *Bacteroides fragilis* HSP40 phages.

10.4 Control of water-borne diseases in high risk communities

This project has made fundamental contributions to the establishment of an infrastructure and opportunities for most valuable research on the control of water-borne diseases in high risk communities. It is now possible to conduct qualitative and quantitative assessments of

the risks of infection constituted by viruses and other pathogens in drinking water supplies. This information is essential to calculate the feasibility and cost effectiveness of intervention strategies. The project has also disclosed valuable opportunities for intervention in the transmission of these diseases. An attractive variety of intervention strategies are available, and their application should be investigated. This will inevitably lead to major progress in the control of water-borne diseases in high risk communities.

10.5 Technology transfer and capacity building:

New technology has been transferred to establishments such as the Universities of Venda and Fort Hare, and the Technikon Free State. Other historically disadvantaged tertiary education facilities and laboratories have been targeted for future activities. A number of students have been earmarked for postgraduate studies on topics initiated by this project.

11. RESEARCH OUTPUTS

The following outputs generated by the project are available in the literature or on request. The peer review publications in international scientific journals and reports contain further details on work summarised in this report.

11.1 Publications

Gaobepe M (1998) A preliminary epidemiological study of the association of the quality of water and diarrhoeal diseases in a developing community in South Africa. Internal Progress Report, Department of Medical Virology, University of Pretoria.

Grabow W O K (1996a) Water-borne diseases: Update on water quality assessment and control. Water SA 22, 193-202.

Grabow W O K (1996b) New challenges in monitoring water for pathogens. Water, Sewage & Effluent 16, 51-54.

Grabow W O K (1997) Hepatitis viruses in water: update on risk and control. Water SA 23, 379-386.

Grabow W O K , Botma K L, de Villiers J C, Clay C G, Erasmus B (1999a) Assessment of cell culture and polymerase chain reaction procedures for the detection of polioviruses in wastewater. Bulletin of the World Health Organization 77, 973-980.

Grabow W O K, Clay C G, Dhaliwal W, Vrey M A and Müller E E (1999b) Elimination of viruses, phages, bacteria and *Cryptosporidium* by a new generation *Aquaguard* point-of-use water treatment unit. Zentralblatt für Hygiene und Umweltmedizin 202, 399-410.

Grabow W O K (1999). Enteric hepatitis viruses. In : WHO Guidelines for Drinking Water. World Health Organization, Geneva (in press).

Grabow W O K, De Villiers J C, Erasmus B, Erasmus D, Engelbrecht L (1996a) Viruses in waste water from an informal settlement. Proceedings of the Biennial Conference of the Water Institute of Southern Africa, Port Elizabeth, 20-23 May, Vol 2, Paper 73, 1-8. Water Institute of Southern Africa, Halfway House.

Grabow W O K, Potgieter N, Werchau H and Steele A D (2000a) Research on waterborne viruses in urban and rural communities using rotavirus as model. Internal Report, Department of Medical Virology, University of Pretoria.

Grabow W O K, Taylor M B, Clay C G, de Villiers J C (2000b) Viruses in drinking water. Proceedings of the Biennial Conference of the Water Institute of Southern Africa, Sun City, 28 May to 1 June. Water Institute of Southern Africa, Midrand. In preparation. See also: Editorial (2000) Viruses found in SA drinking water. Water Sewage & Effluent June/July, 17.

Grabow W O K, Taylor M B and Webber L M (1996b) Hepatitis E virus in South Africa. South African Journal of Science 92, 178-180.

Jagals P, Bokako T C, Grabow W O K (1999) Changing consumer water-use patterns and their effect on microbiological water quality as a result of an engineering intervention. Water SA 25, 297-300.

Jagals P, Grabow W O K, Williams E (1997) The effects of supplied water quality on human health in an urban development with limited basic subsistence facilities. Water SA 23, 373-378.

Marx F E, Taylor M B and Grabow W O K (1997) A comparison of two sets of primers for the RT-PCR detection of astroviruses in environmental samples. Water SA 23, 257-262.

Marx F E, Taylor M B, Grabow W O K (1998a) The prevalence of human astrovirus and enteric adenovirus infection in South African patients with gastroenteritis. Southern African Journal of Epidemiology and Infection 13, 5-9.

Marx F E, Taylor M B, Grabow W O K (1998b) The application of a reverse transcriptase-polymerase chain reaction-oligonucleotide probe assay for the detection of human astroviruses in environmental water. Water Research 32, 2147-2153.

Potgieter N, Van Wyngaardt S, Grabow W O K, Verschoor J A (1997) An anti-coxsackie B1 monoclonal antibody suitable for affinity chromatography. South African Journal of Science 93, 75-80.

Taylor M B, Cox N, Vrey M A, Grabow W O K (2000) The occurrence of hepatitis A and astroviruses in selected river and dam waters in South Africa. Water Research (in press).

Taylor M B, Grabow W O K, Cubitt W D (1997) Propagation of human astrovirus in the PLC/PRF/5 hepatoma cell line. Journal of Virological Methods 67, 13-18.

Taylor M B, Marx F E, Grabow W O K (1997) Rotavirus, astrovirus and adenovirus associated with an outbreak of gastroenteritis in a South African child care centre. Epidemiology and Infection 119, 227-230.

Taylor M B, Parker S, Grabow W O K and Cubitt W D (1996) An epidemiological investigation of Norwalk virus infection in South Africa. Epidemiology and Infection 116, 203-206.

Webber L M, Grabow W O K, Favorov M O, Fields H A (1998) Comparison of two enzyme immunoassays for the detection of hepatitis E virus antibodies. Southern African Journal of Epidemiology and Infection 13,16-19.

Wolfaardt M, Taylor M B, Booysen H F, Engelbrecht L, Grabow W O K and Jiang X (1997) Incidence of human calicivirus and rotavirus infection in patients with gastroenteritis in South Africa. Journal of Medical Virology 51, 290-296.

11.2 Conference Presentations

Botma K L, Grabow W O K (1998) Modification of cell culture sensitivity for the detection of enteric viruses. Oral paper: Virology Congress of Southern Africa, Breakwater Lodge, Cape Town, 27-30 October. Book of Abstracts.

Clay C G, Vivier C, Grabow W O K (1998) The value of enteroviral RT-PCR in the diagnosis of acute viral central nervous system disease. Oral paper: Virology Congress of Southern Africa, Breakwater Lodge, Cape Town, 27-30 October. Book of Abstracts.

Erasmus B, Botma K, de Villiers J C, Vrey A, Vivier C, Uys M, van der Watt E, Cox N, Clay C G, Grabow WOK (1999) Detection of enteric viruses in recreational waters by the polymerase chain reaction. Faculty Day, Faculty of Medicine, University of Pretoria, 25 August.

Grabow W O K (1996) Why monitor viruses in water and food. Invited paper: Symposium on Ecotoxicology, City University, Hong Kong, 4 July.

Grabow W O K (1996) Control of waterborne viral diseases. Invited oral paper: International Congress on Waterborne Pathogens, jointly organised by the German Association for Hygiene and Microbiology, and the World Health Organization, Bonn, Germany, 22-24 May. Proceedings in press.

Grabow W O K (1997) Environmental surveillance for polioviruses in South Africa. Invited oral presentation: Meeting of a Working Group on Environmental Surveillance for Wild Poliovirus, World Health Organization, Geneva,Switzerland, 17-18 February.

Grabow W O K (1997) Overcoming new challenges in controlling waterborne diseases. Invited oral paper: Water Management, Ownership and Distribution Summit, Institute for International Research, BIFSA Conference Centre, Midrand, 16-18 July.

Grabow W O K (1997) Pathogens and indicators of faecal pollution. Invited oral paper: International Seminar on Water Supply, Fundacao Universidade do Rio Grande (FURG) and Associacao Brasileira de Engenharia Sanitaria e Ambiental (ABES), Rio Grande, Brazil, 24-28 November.

Grabow W O K, Botma K L, de Villiers J C, Clay C G, Rautenbach P G D (1998) An assessment of cell culture and PCR procedures for the detection of polio viruses in waste water. Poster: Virology Congress of Southern Africa, Breakwater Lodge, Cape Town, 27-30 October. Book of Abstracts.

Grabow W O K, de Villiers J C, Clay C G, Erasmus B, Botma K L (1998) An evaluation of cell culture and PCR procedures for the detection of polio viruses in waste water. International Symposium on Health-Related Water Microbiology, IAWQ Biennial Conference, Trade and Convention Centre, Vancouver, Canada, 21-26 June.

Grabow W O K, Vivier J C, Clay C G, Webber L M (1998) Detection of the hepatitis E virus in serum and stool specimens of South African patients. Oral paper: Virology Congress of Southern Africa, Breakwater Lodge, Cape Town, 27-30 October. Book of Abstracts.

Grabow W O K, (1998). We and viruses on the road ahead : Opportunities and challenges for both. Invited H W Snyman Memorial Lecture, Faculty Day, Faculty of Medicine, University of Pretoria, 25 - 26 August.

Grabow, W O K, Taylor M B, Clay C G, De Villiers J C (1999). Molecular detection of viruses in drinking water : implications for safety and disinfection. Poster : Second Conference of the International Life Sciences Institute : The Safety of Water Disinfection : Balancing Chemical and Microbial Risks. Radisson Deauville Resort, Miami Beach, Florida, USA, 15-17 November

Griesel, M; Jagals, P and Grabow, W O K (2000). Infection risk for riparian users of water from a catchment drain receiving treated wastewater and polluted urban discharges. Oral paper: Biennial Conference of the Water Institute of Southern Africa, Sun City, 28 May to 1 June.

Griesel M, Jagals P, Grabow W O K (2000) Infection risk for riparian users of waters receiving treated wastewater and other urban discharges. Poster: Symposium on Health-Related Water Microbiology, World Water Congress of the International Water Association, Paris, France, 4-7 July 2000.

Marx F E, Taylor M B, Grabow W O K (1996) The detection of human astroviruses in stools of patients with diarrhoea using molecular techniques. Poster: Congress of the Federation of South African Societies of Pathology, Karos Kruger Lodge, 29 June - 3 July.

Marx F E, Taylor M B, Grabow W O K (1996) PCR detection of human astroviruses in environmental water samples. Oral paper: IAWQ International Symposium on Health-Related Water Microbiology, Mallorca, Spain, 6-10 October.

Müller E E, Clay C G, Grabow W O K (1999). Detection and isolation of *Escherichia coli* O157:H7 from sewage and environmental waters using immunomagnetic separation. Faculty Day, Faculty of Medicine, University of Pretoria, 25 August. Faculty Day, Faculty of Medicine, University of Pretoria, 25 August 1999.

Potgieter N, Vrey A, Mavhungu N J, Mushau F M G, Musie E, du Toit P, Grabow W O K (2000) The quality of water supply, handling and usage in Venda, South Africa. Oral paper: Biennial Conference of the Water Institute of Southern Africa, Sun City, 28 May to 1 June. Proceedings in press.

Potgieter N, Vrey M A, Steele A D, Mashau F M G, Musie E, Dagada T, Mavhungu J, du Toit P J, Grabow W O K (2000) Incidence of rota viral infections in relation to the quality of drinking water in a rural area of South Africa. Poster: Symposium on Health-Related Water Microbiology, World Water Congress of the International Water Association, Paris, France, 4-7 July 2000.

Retief J, Webber L M, Vivier C, Davel G, Grabow W O K (1998). Acute hepatitis E : A case study. Oral paper. Annual Congress of the Federation of South African Societies of Pathology, University of Pretoria, 28 June - 1 July. Book of Abstracts, V4, p289.

Taylor M B, Cox N, Vrey M A, Grabow W O K (2000) The detection of hepatitis A and astroviruses in river and dam water. Poster: Biennial Conference of the Water Institute of Southern Africa, Sun City, 28 May to 1 June. Proceedings in press.

Taylor M B, Wolfaardt M, Grabow W O K (1996) Molecular epidemiology of South African strains of hepatitis A virus: 1984-1995. Poster: International Congress of Virology, Jerusalem, Israel, 11-16 August.

Taylor M, (1998). Viruses in Aquacise : In there cause for concern? S A Water Fitness Association, National Convention, Boksburg North Indoor Pool, Boksburg, 24 - 27 September Convention Proceedings.

Uys M, Clay C G, Grabow W O K (1998). Molecular characterisation of F-RNA coliphages in South Africa. Oral paper : Faculty Day, Faculty of Medicine, University of Pretoria, 25 - 26 August. Book of Abstracts IIB : PV11.

Van Wyk, J E; Steynberg, M C; van Zyl, G and Grabow, W O K (2000). An epidemiological study of water-borne pathogens amongst the participants in the Iron Man Competition, March 1999. Poster: Biennial Conference of the Water Institute of Southern Africa, Sun City, 28 May to 1 June. Proceedings in press

Vivier J C, Clay C G, Botha M E, Grabow W O K (1999) Molecular typing of enteroviruses. Poster IDP 37, Programme and Abstract Book: Lesedi Africa '99 Congress, 21-24 November.

Vivier C, Clay C, Grabow W O K (1998) The value of enteroviral RT-PCR in the diagnosis of acute viral central nervous system disease. Oral paper: Faculty Day, Faculty of Medicine, University of Pretoria, 25-26 August. Book of Abstracts IIA:PV7.

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

Vivier J C, Clay C G, Grabow W O K (2000) Detection and rapid differentiation of human enteroviruses in water sources by restriction enzyme analysis. Poster: Symposium on Health-Related Water Microbiology, World Water Congress of the International Water Association, Paris, France, 4-7 July 2000. Submitted for publication.

Vrey M A, Grabow W O K, Taylor M B (1998) Detection of human astroviruses in river and dam water. Oral paper: Faculty Day, Faculty of Medicine, University of Pretoria, 25-26 August. Book of Abstracts IIA:PV13.

11.3 Dissertations

Botma K L (1999) Studies on methods for increasing the susceptibility of cell cultures to enteric viruses. MSc Thesis, Department of Medical Virology, University of Pretoria.

Marx F E (1997) Detection of human astroviruses in South Africa. PhD Thesis, Department of Medical Virology, University of Pretoria.

Müller E E (Mr), BscHons (Medical Virology), Dissertation : "An evaluation of methods for the detection of F-RNA coliphages and *Bacteroides fragilis* HSP40 phages in large volumes of water."

Rautenbach, P G D (1996) An overview of environmental monitoring for polio virus with an application in an informal settlement area. MMed Dissertation, Department of Community Health, University of Pretoria.

Uys, M (1999). Molecular Characterisation of F-Specific RNA Phages in South Africa. MSc (Med Virol), Department of Medical Virology, University of Pretoria.

Vivier, J C (2000) Molecular typing of enteroviruses. MSc Thesis, Department of Medical Virology, University of Pretoria.