Quantitative Investigation into the Link Between Irrigation Water Quality and Food Safety

VOLUME I: SYNTHESIS REPORT

Report to the
Water Research Commission

and

Department of Agriculture, Forestry and Fisheries

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EXECUTIVE SUMMARY

1. BACKGROUND AND MOTIVATION

In 2002-2005 there was a growing concern of the "safety status" of South African agricultural produce, especially those that are consumed raw. If these products are contaminated they will impact not only the health of the final consumer but also that of people living next to rivers and the producers. This will immediately impact both the national and international "trading status" and cause a suspension of exports. Furthermore, there were, and still are regular articles in the local press reporting on the shocking "environmental status" of local rivers. The Cape Town Regional Chamber of Commerce and Industry also had warnings from the European Union about fresh produce "watered" from polluted rivers.

Another important factor was the change in consumer nutritional trends that led to the belief that the consumption of raw, unpeeled, minimally and unprocessed products to preserve taste and heat labile nutrients, will enhance health. However, the consumption of these products has led to an increase in foodborne outbreaks internationally and these have been shown to be directly associated with the consumption of fresh produce.

The source of contamination of the agricultural products was identified as irrigation water that had been contaminated before irrigation took place. The health risks associated with the use of contaminated irrigation water on agricultural products thus became an increasing concern.

Little is known on a national level regarding the contribution of irrigation water and the associated, potentially contaminated raw produce and the burden of disease. Consequently little action is taken to remedy the situation. A clearer understanding of the problem is urgently required to make inputs for policy formulation and regulation to reduce contamination of irrigation water. Although the key is to stop pollution at the source, this is not realistically achievable over the short term. Thus it is important to evaluate the extent of the problem, regarding contamination of both irrigation water and raw food products, endeavour to establish links between the two and provide recommendations on the way forward in terms of treatment of irrigation water.

2. OBJECTIVES

The main objective of this Water Research Commission solicited research project was to do a quantitative investigation into the link between irrigation water quality and food safety. This was done by:

- performing a baseline study on the extent of contamination found in SA irrigation water that is used specifically for irrigation purposes at the selected sites in the different provinces;
- doing a baseline study on the extent (types and quantities) of contamination found on the irrigated raw produce (fruit and vegetables) at-harvest;
- performing an exploratory study on the extent (types and quantities) of contamination found on the fresh produce post-harvest and at-retail;
- investigating, on a field plot basis, the impact of environmental parameters on the growth kinetics of contaminants;
- establishing links between water quality and safety of fresh produce by means of standard microbial methods for the detection of indicator and potential pathogenic index organisms (bacteria, protozoa and viruses); and
- making recommendations for further research.

3. FINDINGS

3.1 Baseline study on contamination in SA river water that is used for irrigation purposes

Microbial results from the baseline study showed high concentrations of faecal indicators with concentrations at times reaching log 7 cell concentrations. The *Escherichia coli* concentrations in most cases exceeded the <1 000 cfu.100 mL^{-1} World Health Organisation (WHO) and Department of Water Affairs (DWA – formerly DWAF) guidelines. The presence of indicator organisms did not only indicate unsanitary conditions, but also the presence of other potential pathogens. These included aerobic and heterotrophic bacteria, aerobic and anaerobic spore formers, *Staphylococcus, Klebsiella*, *Listeria* and *Salmonella,* intestinal *Enterococcus*, different Gram negative coliforms, commensal and diarrhoeagenic *E. coli* and diarrhoea causing viruses (norovirus types GI and GII, and hepatitis A virus) and *Cryptosporidium* oocysts and *Giardia* cysts.

The presence of pathogenic *E. coli* strains including enteropathogenic *E. coli* (EPEC), enterohaemoragic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC) and enteroinvasive *E. coli* (EIEC), was also found which is a further indication that water from some of the rivers that were studied is unsafe for irrigation of fresh produce. Based on these results it was concluded that the rivers examined were of an unacceptable standard. It was also concluded that there is a high risk of exposure to human pathogens when water from the studied rivers are used to irrigate produce that is consumed raw or without any further processing steps.

The presence of pathogens in contaminated water sources is therefore a serious concern in view of irrigating fresh produce. Many of these organisms can cause gastro-enteric illnesses via the consumption of raw produce irrigated with contaminated water, route. According to the Department of Water Affairs (formerly DWAF) there is a correlation between the risk of being infected with the degree of produce contamination and the quantity of contaminated produce consumed. Therefore, higher counts of faecal coliforms in irrigation water can indicate an increased risk in contracting a waterborne disease, even if small quantities of this produce are consumed raw.

3.2 Baseline study on extent of contamination on irrigated fresh produce at-harvest and at-retail

During the baseline study on at-harvest irrigated produce, the total coliform (TC) and faecal counts (FC) were in most at-harvest cases much higher (>88%) than WHO standards and guidelines recommended by retailers. Typical growth of *E. coli* was also observed in most samples. The data obtained showed that in many cases (>60%) the aerobic colony counts (ACC) varied from zero to unacceptably high. Intestinal enterococci, *Salmonella, Listeria* and *Staphylococcus* were present in many produce samples indicating the presence of potential pathogens. The high counts clearly indicate that the produce studied may constitute a health risk. When comparing the data obtained with the "microbial guidelines" set by South African retailers, most of the fresh produce samples could be classed as microbiologically unsatisfactory.

In the exploratory study on produce available at-retail the microbial levels were in most cases of a more acceptable level.

Index organisms including human viruses, protozoa, intestinal enterococci, *Salmonella, Listeria monocytogenes* and *Staphylococcus aureus* were present on many produce samples indicating the presence of viruses, opportunistic and pathogenic bacteria. It is generally stipulated by the WHO that fresh produce likely to be eaten raw should not contain more than 200 total coliforms per gram and no *E. coli* present per gram to ensure safe consumption.

The presence of pathogenic *E. coli* (EC) strains was also found on some produce from subsistence farming areas. None were found on at-retail produce. Since the presence of EPEC, EHEC, ETEC, EAEC and EIEC strains have been shown, it is an indication that produce may be unsafe. Human pathogenic viruses were also detected in pre-harvest produce but at a lower frequency. The norovirus NoV GII.1 was detected on cabbage and hepatitis virus HAV IB on lettuce and tomato samples. From this study it was evident that the fresh produce samples collected at-retail were more likely to be contaminated with enteric viruses, with either NoV or HAV or both viruses being detected on 19% samples tested. It is important to note that the data showed that more viral contamination of the fresh produce occurred during the harvesting, processing and packaging steps.

As this study represented an exploratory study to ascertain the extent of contamination on fresh produce, further in-depth studies are required to determine what intervention strategies can be implemented to ensure the safety of the fresh produce.

3.3 Impact of environmental parameters on the growth kinetics of contaminants

Results obtained during the different studies on the impact of environmental parameters clearly showed that the time of sampling was of crucial importance to prevent gross underestimation or overestimation of the level of contamination in a river. It was additionally concluded that temperature and pH had no major impact on either the total coliform or *E. coli* counts. Based on the findings it is recommended that sampling always be done at the same daily time, but that a detailed preliminary study of a river is done before a decision is made on the sampling frequency.

The analysis of covariance showed no correlation between the physico-chemical (temperature and pH) and the microbial variables (total coliforms and *Escherichia coli*). However in a study under more controlled environmental conditions it was found that an increase in temperature leads to an increase in the growth of *E.coli* in non-sterile river water but this only takes place when sufficient nutrients, especially usable carbon, is available. Under ideal nutrient conditions and more optimal temperatures major growth increases of the faecal coliforms will occur. Overall it was clear that the carbon concentration of river water is the major impacter on microbial growth, survival and die-off. This is important in terms of pathogen carry-over and subsequent food safety. Currently there are no guidelines regarding carbon levels as a method to control microbial growth in river water, therefore it would be of value to implement such a pollution limiting guideline. Such a guideline would be of value in terms of carry-over loads and subsequent food safety.

The studies on attachment showed that pathogens like *Listeria monocytogenes* if present in irrigation water will rapidly attach to fresh produce and will remain viable for several days. However, the studies did show that attachment and survival varies from one type of vegetable to another.

3.4 Links between water quality and safety of fresh produce

The purpose of this research was to establish the link between water quality and safety of fresh produce by confirming the carry-over of specific indicator and index organisms (bacteria, protozoa and viruses) from the water to the produce.

In all the different selected sites in the provinces monitored during the study it was found that the indicator organisms' counts (TC and FC) were in most cases much higher than recommended WHO and

DWA guidelines. Faecal coliform counts of up to $1.6\times10^{6}.100\ mL^{-1}$ of irrigation water and $1.6\times10^{5}.g^{-1}$ of produce, were observed. In the general linking studies where strain identification, phenotypic and genotypic characterisations were undertaken, at least 30% direct linkages were made between isolates from the irrigation water and those from the fresh produce. It was thus concluded that specific species from the surface of produce were present as a result of transfer from the contaminated irrigation water. There can now be no doubt that specific carry-over does take place.

It is important to note that no carry-over to produce was observed when the counts of irrigation water were in the 10^{3} to 10^{4} $cfu.mL^{-1}$ range. This could be an indication that these are "safe" concentrations to irrigate fresh produce with and that zero or a minimum number of *E.coli* will be carried over.

4. GENERAL CONCLUSIONS

Based on the results from this research project, the microbial pollution levels of rivers and fresh produce monitored at selected sites in different provinces of South Africa over a period of 3-4 years were of an unacceptable microbiological standard and did not meet either the international or national faecal guidelines for safe irrigation or human consumption. Other potential waterborne bacterial, virus and protozoan pathogens were frequently recovered from both the water and the produce. It was concluded that there is a high risk of exposure to pathogens when water from these rivers is used to irrigate produce that is consumed raw or without any further processing steps.

In the research it was shown using phenotypic and genotypic identifications that direct water to produce linkages could be made. It was concluded that species from the surface of produce were present as a result of transfer from the contaminated irrigation water. There can now be no doubt that specific carry-over does take place. The potential of pathogenic organisms being transferred from irrigation water to the surface of fresh produce plus their ability to survive in these unfavourable conditions presents the scenario where consumers unknowingly face a high risk of being infected with harmful organisms when consuming fresh produce.

The potential risk of infection from contaminated fresh produce can be illustrated by the 2011 *E. coli* O104:H4 outbreak in Europe which resulted in 49 fatalities and left thousands seriously ill (>4 000). The outbreak led to fresh produce farmers in Spain loosing sales of more than 200 million Euros per week and put 70 000 jobs under threat. Based on the above situation and the levels of contamination shown in this WRC project (K5/1773/4) in/on selected irrigation waters and irrigated produce it is time to seriously pause to reflect on the situation. It has now become essential that systems be put in place to prevent a similar situation in South Africa.

RECOMMENDATIONS FOR FURTHER RESEARCH

1. Distribution profiles of pathogenic Enterobacteriaceae in SA irrigation water

To be able to define what constitutes risky and unacceptable irrigation water it is essential to know what the distribution of enterobacterial pathogens in SA irrigation waters are. Data from this solicited WRC project showed the presence of pathogenic *E. coli* including enteropathogenic *E. coli* (EPEC), enterohaemoragic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC) and enteroinvasive *E. coli* (EIEC) strains. This is an indication that water from rivers is unsafe for irrigation of fresh produce.

The 2011 *E. coli* O104:H4 outbreak in Europe which caused the highest frequency of haemolytic uremic syndrome (HUS) and deaths ever recorded should be seen as a warning to South Africa. The underlying mechanism behind the apparent increase in O104:H4 virulence is not known even though several virulence genes have been implicated. It is now known that these virulence genes play a role in colonisation and pathogenesis but how this happens is still unknown. But it is well known that these specific genes are key agents of change in microbial populations resulting in normal commensal *E.coli* acquiring genes to promote the dissemination of a variety of traits including virulence, drug resistance and the metabolism of rare substances. Thus it is essential to know which pathogenic strains are present in South African irrigation waters and which strain harbours multiple virulence genes.

2. *Cryptosporidium* and *Giardia* and other indicator organisms

No significant correlation was established between levels of faecal coliforms or *E. coli* in the water and presence of *Cryptosporidium* oocysts and *Giardia* cysts in this WRC study. It was thus concluded that faecal coliforms and *E. coli* are not good predictors of the presence of *Cryptosporidium* and *Giardia* in water. Therefore faecal coliforms or *E. coli* should not be used as the sole indicators of water quality. The link between the presence of *Cryptosporidium* and *Giardia* and other indicator organisms must be investigated to establish a suitable indicator for the presence of *Cryptosporidium* and *Giardia* in water.

Additionally it is important to note, that although the WHO recommends that faecal coliforms be lower than 1 000 per 100 mL irrigation water for crops eaten raw, due to the greater risk posed by helminthic diseases, they recommend a limit of one or less helminthic eggs and one or less protozoan parasite cysts per litre. It is therefore strongly recommended that the quantification of these organisms and the extent of their occurrence be further investigated and regular monitoring facilities be established.

3. Seasonal variations in accurate monitoring of *E.coli* loads in irrigation water

From the data obtained for this WRC project it is clear that river water used for irrigation purposes carries unacceptably high microbial loads. This corresponds with data reported by researchers in the past who also claimed that the river water temperature was the main factor that impacted increases in *E.coli* loads. From the study data this has been shown not to be the case but then the question arises – are *E.coli* loads or just the loads of other thermotolerant coliforms being measuring. This is an important aspect that must be confirmed by future studies.

4. Bio-confirmation of links between pathogens in irrigation water and carry-over to produce

As part of this WRC study it has clearly been shown that if a microbial species is present in the irrigation water the chances are excellent that it will be carried-over to on irrigated produce. The fact that the data obtained clearly identifies potential pathogens of the same genus and species in both river water and on the irrigated produce and furthermore at times the loads are far higher than are acceptable in terms of food safety cannot be ignored. Given the margin of uncertainty, the only reliable resolution to this problem to establish whether the microbe in the water and the one on the produce are of the same genotype lies in the employment of more complex and expensive microbial source tracking (MST) methods.

In terms of MST there are two aspects that are of importance for future research in respect of further validation of results; identifying the source of the faecal contamination in the irrigation water, and tracking and identifying potential pathogens from the irrigation water to the final product.

5. Development of effective quality assurance measures for the detection of enteric viruses

The virological analysis of fresh produce and irrigation water is a complex multistep process, with each stage adding to the variability of the result. Furthermore, no standard procedure for the detection of enteric viruses in environmental samples currently exists. False negative results can occur due to low efficiency of recovery and concentration of the virus from the food matrix or water sample, low efficiency of nucleic acid extraction procedures and inhibition of the reverse transcription reaction, while false positive results usually result from cross-contamination. Further research should therefore focus on the development and validation of molecular assays for the routine detection of enteric viruses in food and water samples.

6. Bacterial adhesion to fresh produce

There is need for more research to study the aspect of bacterial adhesion to fruits and vegetables. This may lead to the development of more effective washing treatments to control microorganisms on produce after-harvest. Future research should also be done to improve the detection of foodborne pathogens and toxins in fresh produce. There should also be a study of intervention or hurdle strategies, such as the use of thermal treatment and irradiation, which could be applied to fresh produce to reduce the level of bacteria and viruses that are in or on the produce.

7. Treatment options

In terms of treatment options, the first question to be asked is “who is responsible for the treatment of polluted irrigation water?” Prevention of river and irrigation water pollution would be the ultimate solution, but in the interim cost-effective treatment techniques for irrigation water are needed. Conventional treatment methods (stabilisation ponds, storage reservoirs, slow-sand filtration, etc.) have been shown to be effective, but the inclusion/use of increasingly cost-effective technologies such as ozone and UV and the combination with filtration systems might exhibit potential. Investigating potential treatment options will have to take into consideration the volumes of water to be treated per unit time, the range of microbial loads, and the efficacy of the treatment technique on different microbes found in the irrigation water, the practicalities, maintenance, and operating costs and capital expenses.

Given the variable but high levels of contamination of surface water with micro-organisms and the related risk for food safety, different on-farm treatment options have to be investigated. This will give direction to further research on existing or new alternative treatment methods which are technically appropriate and financially feasible.

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LIST OF ABBREVIATIONS

AIDS	acquired immunodeficiency syndrome
BOD	biological oxygen demand
CDC	Centers for Disease Control and Prevention
COD	chemical oxygen demand
DAFF	Department of Agriculture, Forestry and Fisheries (previously known as DoA)
DAEC	diffusely adhering *E. coli*
DALY	disability-adjusted life year
DoA	Department of Agriculture (now known as DAFF)
DRA	dose response analysis
DWA	Department of Water Affairs (previously known as DWAF)
DWAF	Department of Water Affairs and Forestry
E. coli	*Escherichia coli*
EAEC	enteroaggregative *E. coli*
EC	*Escherichia coli*
EHEC	enterohaemoragic *E. coli*
EIEC	enteroinvasive *E. coli*
EPEC	enteropathogenic *E. coli*
ETEC	enterotoxigenic *E. coli*
EXPEC	extraintestinal *E. coli*
FC	faecal coliforms
GDP	gross domestic product
HACCP	hazard analysis and critical control point
HastV	human astroviruses
HAV	hepatitis A virus
HIV	human immunodeficiency virus
HRV	human rotavirus
HuCV	human caliciviruses
HUS	haemolytic uremic syndrome
MPF	minimally processed fruits and vegetables
MST	microbial source tracking
NMMP	National Microbial Monitoring Programme of DWAF
NoV	norovirus
PCR	polymerase chain reaction
QMRA	Quantitative Microbial Risk Assessment Modelling
RT-PCR	reverse transcriptase-polymerase chain reaction
TC	total coliforms
UK	United Kingdom
USA	United States of America
UV	ultraviolet irradiation
VTEC	vero cytotoxigenic *E. coli*
WHO	World Health Organisation
WRC	Water Research Commission

CHAPTER 1
LITERATURE REVIEW

by

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1. BACKGROUND

The South African local and export trading in fresh and processed fruit and vegetables are steadily growing. As healthy eating food trends among national and international consumers gain momentum, many other opportunities for increasing local trade and international exports will become evident.

Fresh and minimally processed fruits and vegetables (MPFs) provide most of our daily requirements for vitamins, minerals and fibre and their role in reducing the risk of lifestyle associated illnesses such as heart disease, diabetes and cancer has resulted in a further increase in their desirability and consumption. The WHO has issued reports claiming that correct fresh produce intake alone could save 2.7 million lives a year and that 31% of heart disease cases are due to an insufficient intake of such foods (Johnston *et al.*, 2006). As a result of such reports, fruit and vegetable consumption increased by 29% per capita in the United States between 1980 and 2000 (Matthews, 2006). A concern related to this increase in MPF consumption is the increased exposure to associated bacteria as well as an increase in bacterial numbers that are ingested, both of these increasing the chance of infection. An increase in salad bars and a trend towards healthier living has resulted in a much wider consumption of fresh salad products and healthier foods, and consumer demand is forcing shops to stock fresh produce that is prepared to a ready-to-eat level and also low in – or completely free of – preservatives (Johnston *et al.*, 2006).

There is a growing concern (Britz, 2005, Paper presented at the Cape Winelands District Municipality Imbizo, April 2005) over the safety status of agricultural products produced in South Africa especially those that are consumed raw or after minimal processing (Britz, 2005). Multiple surveys have shown increasing evidence that the consumption of irrigated fresh produce is a major factor contributing to gastrointestinal illness (Pachepsky *et al.*, 2011). The presence of foodborne pathogens on fruit and vegetables is known to vary from region to region and can be extremely high in some developing countries. If irrigated products are biologically contaminated they will affect not only the health of the final consumer but also that of people living next to rivers, producers, pickers, handlers, packers, and small, subsistence and commercial farmers. A decrease in food safety of the final agricultural product will immediately negatively impact both the national and international trading status and cause a suspension of exports and continued access to export markets. Subsequently, a devastating circle of economic downfalls, massive decrease in production of essential foods, loss of jobs, population movement to informal settlements, increased pollution of water sources, etc., will be started. The increasing evidence of the presence of potential pathogens in both irrigation water and on fresh produce as well as the growing scarcity of water resources leave little doubt about the need to pay more attention to the presence, fate and carry-over of pathogens from water to fresh produce.

Several South African rivers have been found to be unsuitable for irrigation of fresh produce (Barnes & Taylor 2004; Germs *et al.,* 2004; Olaniran *et al.,* 2009; Paulse *et al.,* 2009) mainly because of the high levels of faecal contamination. Indicator and index organisms exceeded the DWAF and WHO guidelines of 1 000 *E. coli* per 100 mL water for irrigation of fresh produce (WHO, 1989; DWAF, 1996b). The uMngeni River in KwaZulu-Natal was reported to have *E. coli* counts of 1 x 10^6 cfu per 100 mL water (Olaniran *et al.,* 2009). The Diep, Berg and Plankenburg Rivers in the Western Cape have also been reported to have heavy loads of faecal indicators sometimes with *E. coli* loads of >500 000 per 100 mL water (Paulse *et al.,* 2009). Such highly polluted river systems will certainly pose serious health risks (Zamxaka, 2004a) since in the above examples the rivers are used for drinking, irrigation and other recreational purposes.

For this reason, in 2007 the South African Water Research Commission initiated a research project to investigate the link between the quality of irrigation water and the safety of produce (Backeberg, 2006). The main objective of the research was to investigate which bacterial and viral contaminants are found in polluted irrigation water sources and highlight their potential risks and carryover potential to crops cultivated using such water sources. It was argued that this should give an indication whether faecally contaminated water used for irrigation of fresh produce can potentially lead to disease outbreaks.

2. WATER SITUATION IN SOUTH AFRICA

South Africa is located in a predominantly semi-arid part of the world, and as a result, the water resources are scarce and extremely limited (NWRS, 2004). The combined flow of all rivers in South Africa amounts about 49 000 million cubic metres per annum. Thus, as a result of the poor spatial rainfall distribution the natural availability of water across the country is very uneven. According to the documentation of the NWRS (2004), this situation is compounded by both the seasonality of rainfall and the high within-season rainfall variability over virtually the entire country.

The demands on South Africa’s already scarce water resources are increasing and projections are that, by 2025, there will be a national deficit in available water according to the recently published "South Africa Environment Outlook", a report on the state of the environment (DEAT, 2006). Already, in the year 2000, 10 out of 19 water management areas have been facing a water deficit (NWRS, 2004), with irrigation and water for basic needs in urban areas being the two largest consumers of water. Climate change is expected to increase the variability and intensity of rainfall, as well as increasing it along part of the eastern escarpment and decreasing it in the western parts of the country. Runoff in the western parts of the country may decline by 10% by 2015 (DEAT, 2006).

Water quality appears to be variable between catchments and over time (DEAT, 2006). Eight of the 19 catchments have quality restrictions that exceed target quality ranges. These and other factors have increased pressure on South Africa’s aquatic ecosystems, including wetlands. South Africa has severely degraded river ecosystems, and the discharge of untreated effluent into these rivers continues to grow

(DEAT, 2006). This water pollution is endangering human health and undermining economic development in South Africa.

Of the aquatic ecosystems, only 7% of total river length and 18% of wetlands are at present formally protected (DEAT, 2006). Aquatic ecosystems, including rivers and wetlands, are in the worst condition of all the ecosystems. Only 26% of South African rivers are intact, 54% are critically endangered, and more than 50% of wetlands have been destroyed.

The multitude of water demands (ecological, domestic, industrial, and agricultural) need to be balanced equitably, and the National Water Resource Strategy by the Department of Water Affairs and Forestry (DWAF) is seen as the main driver for ensuring the balance can be achieved. According to DWAF however, there should be sufficient water of suitable quality to meet South Africa's expectations with respect to maintaining a strong economy, improved social standards, and healthy aquatic ecosystems for the near future – provided that the resources are carefully managed and wisely allocated and utilized in line with the strategy. This pronouncement does, however, not take into account the expected effects of climate change on water resources or the degradation effects of non-compliance with legislation that can render large volumes of water unusable.

Urban areas and populations are increasing, which result in overwhelming development challenges (DEAT, 2006). Nearly 58% of South Africa's population now lives in urban areas. Settlements across the country vary in terms of quality of life and the social amenities that they offer. Depending on the type of settlement people live in, they enjoy greater or lesser measures of health, access to services, housing and safety. The successes in the delivery of electricity and water to many communities contrast with the inadequate access to sanitation, with 50% of the population still not receiving regular waste collection. This increased supply of basic household water without concomitant ability to treat the resultant wastewater, resulted in greatly increased pollution finding its way into the environment.

3. SOURCES OF WATER AVAILABLE

South Africa is particularly susceptible to the effects of climate change, and its effects on human and natural systems are becoming evident. Prognoses of the outcomes of climate change include a net drying of the western half of the country, a possible increase in rainfall along the eastern escarpment, with a shorter rainfall season possible in the Western Cape (DEAT, 2006). Some of the major impacts of the change and increasing variability of the climate include health issues (including the spread of malaria), changes in the distribution and availability of water resources, changes to biodiversity and ecosystems, and changes in patterns of agriculture.

South Africa depends mainly on surface water resources (NWRS, 2004) for most of its urban, industrial and irrigation requirements. However, South Africa is a semi-arid country with only 8.6% of the rainfall available as surface water. This is one of the lowest conversion ratios in the world (Walmsley *et al.*, 1999). The run-off is furthermore not distributed evenly throughout the country, with the eastern seaboard receiving some 80% of the run-off. There is also great variability between years. In some areas over 50% of the wetlands have been converted for other land-use purposes, while both ground- and surface waters are being polluted at an alarming rate.

South Africa has a dam capacity of about 32 400 million cubic metres which is equivalent to 66% of the total mean annual runoff. Groundwater is also extensively utilised but this source is strongly limited by the geology of the country. According to the NWRS (2004), the water resources of the northern parts of the country are nearly fully developed and utilised while the reverse applies to the south-eastern regions where there are significant undeveloped resources.

The scarcity of freshwater resources has led to every major river in South Africa being exploited, with many catchments already experiencing a demand exceeding the supply. This situation is likely to worsen, especially in the Western Cape, as the effects of global climate change bite deeper. The future climate of the Western Cape is likely to be one that is warmer and drier than at present (Midgley *et al.*, 2005). This anticipated climate change is likely to put severe pressure on the rivers in the Western Cape.

4. DEFINITIONS OF VARIOUS FORMS OF RAW / UNTREATED WATER

Researchers studying the health aspects of irrigation water coming from less than pristine sources use various terms to describe the source or quality of the water used in their studies. Scientists, engineers and environmentalists use these terms in different ways, but a general consensus has emerged over time. The following brief descriptions will help to put the various studies quoted in the rest of this literature review into context (Smith & Scott, 2002; Murphy, 2006; Carden *et al.*, 2007).

Raw water usually refers to water obtained from natural sources (such as surface water from rivers or groundwater from wells) and the term simply indicates that such water has not been disinfected or otherwise improved. In this regard the reader should keep in mind the ecological slogan "We all live downstream", meaning that river water that constitutes a raw (untreated) source for one community may be the downstream (polluted) water from a community situated upstream along the same river. Since this pollution can reach all persons living in the environment via a multitude of pathways, there is no "clean upstream – we all suffer from the pollution in one way or another".

Wastewater is derived from various sources and is closely related to the quantity of water use. Put simply it represents return flows resulting from water use. In residential areas domestic wastewater is predominant, while industrial wastewater with a different chemical load originates from industrial areas.

Waste streams from residential areas can be divided into sullage (household greywater, excluding water from toilets), blackwater (septage = toilet waste), storm water and solid waste. Although the solid waste stream does not contain large quantities of water, in areas of poor solid waste management, this waste stream does, however, serve as a major source of contaminants of the other wastewater streams. In informal settlements and other areas with poor toilet provision, the solid waste contains large quantities of human excreta since underserved populations collect human excreta in containers and dispose of this into the few bulk solid waste containers available or on the ground. The leachate from solid waste delivers huge pathogen loads into the natural surface waters surrounding such settlements.

Household sullage (greywater) (used household water that is not toilet water) varies greatly in microbiological quality with faecal coliform levels varying from <10 to 2 000 000 cfu per 100 mL, depending on the sources in the home, possible treatment on-site and the storage system and storage time. Water from kitchen sinks are generally regarded as too contaminated for use (with faecal coliform levels varying from <10 to 4 000 000 cfu per 100 mL (Murphy, 2006), even on ornamental plants where humans can come into contact with the irrigated surfaces. Such water is usually excluded from greywater re-use systems, as is untreated sewage or partially treated sewage effluent. Thermotolerant *E. coli* levels of more than 84 000 000 cfu per 100 mL have been reported laundry water. Sullage also contains large amounts of solids (hair, lint, food particles, etc.), biological contaminants (blood, saliva, faecal matter), as well as chemical contaminants (products such as soaps and detergents, toothpaste, shampoo, bleach etc.). Such water clogs soil pores very quickly unless pre-filtered to some extent. Sullage flowing directly into rivers, especially in urban areas, creates particularly intractable problems for irrigation downstream.

Storm water in a built environment is produced from house roofs, paved areas and from roads during rainfall events. In addition, storm water is produced from the catchment of a stream or river upstream of the built-up area. The amount of storm water is therefore related to the amount of rainfall precipitation, and the nature of surfaces, with impervious surfaces producing more run-offs. During a storm event the peak flow is higher and duration shorter with an impervious surface, while the peak flow is lower and duration longer with a vegetated surface.

Open storm water drains are common in underdeveloped areas and in many cases act as open sewers, particularly for the conveyance of sullage (greywater). Often too, septic tanks act as vaults, with the overflow of sullage water being discharged direct to open storm water drains. In addition most industrial wastewater is discharged into these same drains. This practice tends to change the characteristics of wastewater drastically, and invariably compromises the function of such drains. Storm water run-off may contain as much solids as household sullage, depending on the debris and pollutants in the path of the storm water run-off.

Effluent from municipal wastewater treatment works (often referred to as sewage works or even sewage farms) is returned to the nearest natural surface water course in order to make a contribution to the river ecology. Whether this contribution is beneficial or detrimental depends on the quality and efficiency of the treatment processes. Unfortunately the municipal wastewater treatment works in South Africa is generally in a very poor state and this effluent often do not meet the discharge requirements set by the Department of Water Affairs and Forestry. As an example the Stellenbosch Wastewater treatment works has been discharging substandard effluent into the Eerste River for a number of years, although plans are in place to remedy this situation (Barnes & Taylor, 2004). This effluent discharge comprises 80% of the summer flow in the ecologically sensitive Eerste River and has a serious effect on the degradation of the river and the adjacent agricultural and tourist activities along the river.

5. QUALITY OF SOUTH AFRICAN RIVERS

Water is an indispensable natural resource and fundamental to life. It is a prerequisite for maintaining the integrity of the environment, for food production, hygiene and sanitation and for economic development. It is also essential for water to be of an acceptable quality for human and other uses. According to the NWRS (2004), the deterioration of the quality of the South African surface water resources is one of the major threats the country's capability to provide sufficient water of appropriate quality to meet its needs and to ensure environmental sustainability. The Minister of Water Affairs and Forestry furthermore stated (NWRS, 2004) that bacteriological contamination of the water sources, arising from the absence or poorly maintained sanitation facilities, is widespread in our country and that this is one of the main sources of disease in South Africa. This unhealthy state of the rivers in South Africa regarding microbiological pollution is therefore a source of great concern.

Escalating rates of urbanization, industrialization and population growth have aggravated water pollution as a threat to the economy and well-being of South Africa. One of the major sources of faecal pollution of natural water courses are the many un-serviced informal settlements that have been established near rivers in the last two decades as the process of urbanization of poverty stricken rural people gather momentum. The other major contributor to the dangerously high levels of pollution in many of the rivers in South Africa is the failing sewage disposal systems of a large number of villages, towns and cities. These systems in total leak huge amounts of raw sewage into the rivers, either from inadequate sanitation in low-income housing areas or from poor maintenance of sewage reticulation systems and inadequate wastewater treatment works.

There is a crucial need for scientifically sound answers to the problems of contamination of water sources with potentially hazardous microbial organisms of human origin and the assessment of the risks posed by such pollution. Research on the microbiological contamination of water sources and measures to alleviate the situation has not received sustained attention. The burden of disease caused by waterborne infections is of particular importance since many of these cases could theoretically be prevented by provision of sanitation, adequate waste disposal and proper water treatment.

Testing for every potential pathogenic organism in a river in order to monitor water quality is expensive and technically problematic. Thus, the concept of an 'indicator organism' is used to indicate the possible presence of other disease-causing constituents. Gut bacteria of warm-blooded animals are used as indicators of sewage pollution in natural waters. The coliform group of bacteria and specifically *E. coli* are used as such indicators of the levels of faecal pollution of river water. Once substantial numbers of *E. coli* are detected, it is prudent to assume that such water has been contaminated with faecal waste and that other harmful or disease-causing organisms (pathogens) are also present.

The microbiological health status of river water is gauged by determining the levels of certain organisms that occur naturally in the digestive tract of mammals, namely the coliform group of bacteria and specifically *E. coli*. High concentrations of *E. coli* are seen internationally as an indication that such water is contaminated by untreated sewage. Drinking water should contain no *E. coli* organisms, while the safe limit for swimming and other recreational use of rivers is taken to be no more than 400 *E. coli* organisms per 100 mL water. The limit above which the risk of transmission of disease starts rising for irrigation water is taken internationally to be 1 000 *E. coli* organisms per 100 mL water, while the DWAF (1996b) considers a count of 1 000 *E. coli* organisms per 100 mL water to pose a low risk. Measurements between 1 000 and 4 000 are considered to indicate a ‘moderate’ risk, and above 4 000 ‘high risk’. The National Microbial Monitoring Programme of DWAF (NMMP, 2002) has set a guideline of >4 000 *E. coli* organisms per 100 mL water before they classify water as a high risk when irrigating crops that are eaten raw.

The rivers in our urban areas unfortunately regularly measure hundreds of thousands or even millions of *E. coli* organisms per 100 mL water (Barnes & Taylor,2004). The Jukskei River in Gauteng has been reported in 2003 to measure 13 million *E. coli* organisms per 100 mL water, while the uMngeni River in Natal measured up to 1 080 000 *E. coli* organisms per 100 mL water. The Plankenburg River running past Stellenbosch peaked at 560 000 000 *E. coli* organisms per 100 mL water in 2004 and even in January 2006 the Plankenburg still measured 9 200 000 *E. coli* organisms per 100 mL water (Barnes, 2003; Barnes & Taylor, 20043). The Berg River below the confluence with the Stiebeuel in Franschhoek measured 92 080 *E. coli* organisms per 100 mL water while the storm water ditches joining the Berg River from the informal settlement of Mbekweni at Paarl measured 2 440 000 000 *E. coli* organisms per 100 mL water in 2004. Data on microbiological pollution of South African rivers have been extremely difficult to obtain in the public arena over the past three years, but these figures are enough to show that several South African rivers and streams are unacceptably polluted.

A note on quality groundwater (mostly by means of boreholes or wells) is needed here. Quality of groundwater is heavily dependent on the sources of contamination that exist in the immediate vicinity of the borehole or well. This contamination can arise from the nature of the soil around the borehole or well (high salt content, brack conditions, disturbed chemical water profile, etc. or it can be microbiological due to animal and human contamination of the water). Contamination can seep along cracks in the soil for some distance, but contaminated groundwater is almost always a highly variable but local problem. It is thus not so easy to make general statements about groundwater quality as about river quality.

Water quality problems in rivers arise from a few key sources: natural climatic conditions (e.g. low rainfall and high evaporation rates) over which there is no control, domestic and sewage effluents, industrial effluents, surface (road) runoff and in rural areas, seepage and runoff from animal husbandry. Each of these aforementioned effluents includes different characteristic types of pollutants. Domestic and sewage effluents primarily contribute to the nutrient enrichment of the river system through inputs of nitrogenous and phosphate compounds as well as huge loads of disease-causing organisms. The exact nature of industrial effluents largely depends upon the industrial processes being used. Heavy industrial processes such as metal working for instance, may create an increase in metal concentrations within the river (copper, lead, aluminium, iron, cadmium etc.). Surface runoff from roads increases levels of soot, grease, fuels and heavy metals such as lead.

Besides storm water runoff, water quality is greatly reduced in urban catchments by the deliberate discharge or leakage of sewage effluent into the river system from poorly maintained sewerage pipelines or poorly operated wastewater treatment works.

The other major contributor of untreated sewage to the river courses in urban areas is the discharges of untreated sewage and run-off from informal settlements around the cities. While some of these settlements have sprung up over recent years, some of them have been in place for many years while still lacking adequate toilets. The lack of sanitation provision for these inhabitants, coupled with increased water use in the form of free basic water allocations, led to large amounts of highly contaminated wastewater and raw sewage making its way to the streams and rivers nearby. In some settlements in the Western Cape, for instance, there are from 60 to 100 persons per toilet (Barnes, 2003). There are areas surrounding South African cities where there are no toilet facilities available and the inhabitants must make use of nearby open ground. The run-off from such areas is highly contaminated as well.

The provision of free basic water for most of South Africa's inhabitants did not go hand in hand with a concomitant effort to provide basic sanitation or waste water treatment. The driving forces behind the provision of free basic water, namely increased hygiene and improved living conditions are thus negated by the lack of provision for adequate disposal once the water had been used.

The population of urban poor is increasing faster than service delivery due to urban migration and population increases. There is a huge backlog in services such as housing and provision of sanitation and clean drinking water in all cities in South Africa. The lack of adequate and timely removal of solid waste causes further seepage from bins and bulk rubbish containers, joining the toxic flow reaching the rivers.

Poor people and environmental damage are often caught in a downward spiral. Past resource degradation deepens today’s poverty, while the poverty of today makes it very hard to care for or restore the environmental resource base. Poor people are forced to deplete resources to survive and this degradation further impoverishes them. When this downward spiral becomes extreme, poor people are forced onto marginal land and fragile ecosystems in ever increasing numbers. It is this spiral that has seriously damaged many river systems in South Africa and consequently gave rise to use of polluted irrigation water in food production.

6. WATER FOR AGRICULTURAL USES

The agro-climatologic zone of a country determines the agricultural and cultivation practices (Schultz, 2001). Generally, South Africa has mainly a dual agricultural economy, comprising a well-developed commercial sector and a predominantly subsistence-oriented sector in the rural areas. Only about 13% of South Africa's surface area can be used for crop production, of which just 22% can be seen as high-potential land. The most important factor limiting agricultural production is the availability of water. Rainfall is distributed unevenly across the country, with almost 50% of water being used for agricultural purposes. Primary agriculture contributes about 2.6% to the gross domestic product (GDP) of South Africa and almost 9% of formal employment (WESGRO, 2006). According to Nieuwoudt and Groenewald (2003), the contribution that agriculture makes to the GDP is low but the real contribution is heavily underestimated and obscured when only direct measurements are applied. The true value lies in the contribution it makes in its backward and forward linkages (Backeberg, 1996; Backeberg *et al.*, 1996; Nieuwoudt & Groenewald, 2003). The more accurate estimates of the sectors contribution is considered to be in the region of 20-30% with the more important contributions being food supply, earning foreign exchange, economic inter-regional linkages and direct and indirect employment. For example, the Western Cape Province is the largest producer of fruit and vegetables and includes the production and distribution of dried, canned and juice products. Exports from this province alone have increased from R 6 billion in value in 2001 to R 8 billion in 2003 (WESGRO, 2006). Presently, this industry is considered to be a primary employer in the Western Cape representing commercial, new development farmers and more than 220 000 farm workers who all in turn support over more than 1.5 million dependents (WESGRO, 2006).

In South Africa there is a high demand for fresh water in agriculture and between 60% and 67% of the nation's fresh water is used by farmers with about 33% being utilised for the irrigation of crops with some 35% of all domestic foodstuffs and 85% of all agricultural exports being derived from irrigated lands with more than 1.3 million hectares (ha) under irrigation (Backeberg, 1996). The importance of irrigation water to any type of farming, whether it is commercial or subsistence cannot be overemphasized since South Africa is a country that is in the arid and semi- arid agro-climatologic zone (FAO, 2005). A major constraint to South African agriculture (Zimmerman, 2000) is its climate and agro-ecological potential that, throughout most of the country, is more suited for livestock-grazing than crop production.

Over a 30 year period (1956-1986) as much as 27% of the country was drought-stricken for more than 50% of the time (Cowling, 1991). According to the Thompson (1999) and the FAO (2005), the area equipped for irrigation in SA at that time was 1 498 000 ha and the distribution of land suitable for irrigation differs in the nine SA provinces (Table 1). The main irrigated crops are fodder crops, wheat, sugar cane, vegetable and pulses. However (Backeberg, 2006) of the >1.3 million ha being irrigated, only 100 000 ha are food plots and smallholder irrigation schemes. Irrigation contributes 25-30% of commercial agricultural production for local and export and specifically ranges from maize (10%), 30% for wheat and up to 90% of vegetable, grape, citrus and deciduous fruit. According to Backeberg (2006), no information is currently available on the production of staple foods under irrigation for household consumption.

One very important contribution of irrigation farming not always appreciated is the economic linkages between irrigation farming, agriculture, and directly and indirectly to the rest of the South African economy. As shown by Backeberg *et al.* already in 1996, the importance of any economic activity goes much further than its direct contributions to revenue generation. Such activities often cause ‘ripple’ effects that beside negative or positive changes in revenue generation may heavily impact employment, production, trading status, manufacturing and up-field processing capabilities, packaging, storage, transport, distribution and consumer preference and health. A regional study by Kirsten and Van Zyl (1990) showed that irrigated agriculture has similar forward and backward economic linkages as main stream agriculture. However, for irrigation farming backward and forward linkages, especially employment (Backeberg *et al.*, 1996), will depend on the degree to which inputs or products are locally provided and processed but also on the availability and quality of usable water. The use of irrigation for food production is a vital sector in the economy of many regions and provinces of South Africa and any negative impact will lead to negative linkages which could, unless managed very carefully, lead to a devastating cycle of economic downfalls.

Table 1. Distribution of irrigated areas in South Africa per Province in 1999 (Thompson, 1999; FAO, 2005).

Province	**Commercial irrigation, permanent (ha)**	**Commercial, temporary (ha)**	**Total area equipped for irrigation (ha)**
Eastern Cape	11 070	179 995	191 065
Free State	46	68 764	68 810
Gauteng	18	16 330	16 348
KwaZulu-Natal	2 747	131 974	134 722
Mpumalanga	18 498	116 977	135 475
North West	706	114 094	114 801
Northern Cape	34 759	130 181	164 940
Limpopo	58 704	160 617	219 321
Western Cape	290 204	162 325	452 529
Total	416 753	1 081 257	1 498 010

In South Africa the high usage of fresh water could be problematic if this water was to become microbially contaminated to high levels since the water use is so widespread that no alternative resource would be possible. It is therefore of the utmost importance that the quality and safety of South Africa’s fresh water resources be improved and maintained (Backeberg *et al.*, 1996; DWAF, 1996a, b; Reinders, 2000; NWRS, 2004).

The common sources of irrigation water used in SA are large reservoirs, farm dams, rivers, ground water, municipal supplies and industrial effluent (DWAF, 1996a,b). In 1996, it was estimated (Backeberg *et al.*, 1996) that irrigation farming in SA was made up of approximately 40 000 small-scale farmers, 15 000 medium-to-large-scale farmers, 120 000 permanent workers and an unknown number of seasonal workers. For that year it was found that about 51% of South Africa’s water was utilised in irrigation farming which gave an agricultural output of between 25 and 30%. In contrast in the USA, among different of sources of irrigation water, the most common source is deep ground wells, with 51% of the vegetable and 39% of the fruit growers reporting this source of water. Flowing surface water was the next most common source of irrigation water, with 38% of fruit growers and 19% of the vegetable growers drawing water from this source. About 5% of produce growers reported using municipal water (FDA, 2001). Other sources of irrigation water used are run-off water and reclaimed water. In the USA there are standard conventions in irrigation management and local or regional incentive programs for collection and recycling run-off water for on-farm or downstream irrigation. A long-standing solution to both wastewater management and water availability needs has been the use of reclaimed water in agriculture, including irrigation of fruits and vegetables. Reclaimed water has been increasingly used for irrigation and to recharge ground water since the 1980s (FDA/CFSAN, 2001).

Many factors, such as water availability and cost, soil type, slope, depth of water table, economics, and cropping rotations, determine the choice of irrigation mode (FDA/CFSAN, 2001). World-wide the three main irrigation application designs are 55-65% for surface irrigation; 75-85% for mechanized and non-mechanized sprinkler systems and 85-95% for localized irrigation (FAO, 2005). In the USA (USDA, 1998), four main methods of irrigation are common, gravity flow irrigation (flood or furrow); sprinkler irrigation; drip/trickle irrigation and sub-irrigation. In Germany, three main types of irrigation methods are used: flush irrigation technologies; sprinkler irrigation; and drip irrigation. The EWTSIM (2005) reported that flush irrigation technologies were used from about 1890-1970 for production of crops like vegetables, potatoes and grain. Starting from the early 20th century, irrigation development moved towards sprinkler irrigation, 1950 hand moved and from 1960 portable sprinklers with quick-coupling pipes. Sprinkler irrigation was only used for vegetables crops. The development continued to the production of hose reel irrigation machines. Drip irrigation is now mainly used for vineyard and orchard irrigation. In South Africa, permanent irrigation is considered to be the most important type of irrigation practised in all rainfall regions (Backeberg & Odendaal, 1998). Other irrigation methods used are flood irrigation (32.8%), sprinkler (54.4%) and micro-irrigation (11.8%). Several variations of these methods are also used by small-scale and subsistence farmers. These farmers are also known to apply innovative adaptions such as short-furrow irrigation.

7. ECONOMIC SITUATION OF THE SOUTH AFRICAN FRUIT AND VEGETABLE INDUSTRY

South Africa has a market economy that is largely based on services, manufacturing and mining. In 2010, the primary agricultural sector sectors contributed 2.5% of the GDP, down from the 7.1% in 1970 (DAFF, 2011a). There are, however strong linkages (backward and forward) into the economy, so that the agro-industrial sectors contribution is estimated at 12% of the GDP (GCIS, 2011).

South Africa is the major and leading exporter of fresh fruits and vegetables in Africa. Ndiame and Jaffee (2005) reported that 73% of fruits and vegetables exported to the USA under the AGOA preference from Africa (African Growth and Opportunity Act) were from SA. SA also recently unseated Turkey in the EU market and is now the largest third world supplier of fruits and vegetables to the European Union (EU). South Africa has 31% of total EU imported fruit market share (Ndiame & Jaffee, 2005). Several countries in Sub-Saharan Africa export vegetables but three, Côte d'Ivoire, Kenya and the SA, account for nearly 90% of the region’s trade to international market with South Africa the leading exporter (Ndiame & Jaffee, 2005). For some produce, especially fruits, SA ranks between number 1 and number 20 among the world fresh produce exporting countries in terms of monetary value (Table 2) (FAO, 2004).

According to a WESGRO (2006) agriculture sector brief report on fruit processing, the fruit industry is very important to the South African economy contributing 20%, or four million tons, to total agricultural production. Sixty per cent of fresh fruits produced in SA are exported. After Chile, SA is the 2nd largest southern hemisphere exporter of deciduous fruit, apples and pears, and stone fruit, nectarines, peaches and plums. For citrus fruit, SA is ranked 3rd in the world after Spain and the USA. Apart from the exported fresh fruit, 20% is consumed locally, while the remaining 20% is processed into juices, which are sold at retail outlets like Woolworths, Pick and Pay, Spar, Shoprite and Checkers (WESGRO, 2006).

Table 2. Produce exported from South Africa and food pathogen outbreaks associated with these produce internationally (Harris *et al*., 2003; FAO, 2004).

Produce	Quantity (Mt)	Value (000, USA $)	Unit value ($)	Position in 1st 20 leading exporting countries in the world	Pathogens implicated in outbreaks associated with irrigation water	Country where outbreak occurred
Apple	305 190	181 020	593	9		
Avocados	28 585	21 153	740	7		
Carrot	1 832	1 944	1 061	19	*E. coli*	USA
Grapes	237 110	282 786	1 193	4		
Mangoes	9 919	8 236	830	13		
Orange	736 592	270 667	367	3	*Salmonella*	USA
Pears	138 836	79 626	574	8		
Pineapples	3 774	3 325	881	20		
Potatoes	30 319	9 733	321	20		
Sweet Potatoes	470	267	568	20		

Of the nine provinces, the Western Cape has the highest rate of growth and development in agriculture, especially in fruits and vegetables. About 25% of the South African agricultural sector's total gross income was generated by the Western Cape Province and it also accounts for more than 50% of exported produce (WESGRO, 2006). This is made possible because of the suitable climatic and physical geographic conditions in the Western Cape. Seventy per cent of fruit produced in SA is from various areas in the Western Cape. Apples and pears are mostly produced in Ceres, Elgin is known for apple production and the Small Karoo is renowned for apricots, plumbs, peaches and nectarines, while the Hex River Valley for grapes. The Western Cape produces 15-20% of the total citrus fruit produced in SA, which constitutes 8.5% of total world export (WESGRO, 2006).

Apart from cultivation of fruit, the Western Cape is also the leading province in the production of vegetables, representing 12% of the total vegetable production in SA. Examples of vegetables produced by commercial farmers in the region are onions, potatoes, carrots, cabbages and brassica (WESGRO, 2003). It is not the international market alone that is in high demand of fruit and vegetables from SA commercial farmers. Fruit and vegetables sales from the major fresh produce markets and in local supermarket chains in SA have increased due to high preference by SA consumers for the fruit and vegetables produced in SA (Table 3) (WESGRO, 2003; NDA, 2007)

According to the National Department of Agriculture and Forestry (DAFF, 2011b) the major fresh produce markets selling vegetables increased from 14 in 1989 to 19 in 2010. The total quantity of the most important vegetables increased from 1 885 500 tons in 2000 to 2 094 000 tons in 2010. The most important vegetables sold on the 19 fresh produce markets are potatoes, 935 800 t; tomatoes, 258 900 t; cabbage, 113 300 t; onions, 311 100 t, pumpkins, 50 400 t, carrots, 85 000 t and butternut squashes, 91 200 t, the major contributors. If one looks at the production of vegetables during the 90s until 2010, there has been a steady increase in the production of most of the major vegetables sold at the fresh produce markets, with the exception of green peas, cauliflower, cabbage and green beans (DAFF, 2011b).

Table 3. Gross value of major vegetables produced in South Africa and food pathogen outbreaks associated with those vegetables internationally (Harris *et al*., 2003; Buchanan, 2006; NDA, 2007; DAFF, 2011a, b, c, d; Taban & Halkman, 2011).

Vegetable	**2009/10 R 1000**	**Pathogens implicated in outbreaks associated with irrigation water**	**Country where outbreak occurred**
Potatoes	5 155 176		
Cauliflower	45 300		
Pumpkins	277 911		
Beetroot	106 120		
Lettuce	125 860	*E. coli, Shigella, L. monocytogenes*	USA, Norway
Carrots	340 719	*E. coli*	USA
Green beans	100 788		
Green peas	58 942		
Cabbage	161 768	*C. botulinum*	USA
Gem squashes	52 029		
Sweet potatoes	120 396		
Tomatoes	1 555 089	*E. coli, Salmonella*	USA
Onions	1 210 635	*E. coli, Salmonella*	USA
Strawberries	80 874	*Salmonella*	
Apples	2 869 740		
Avocados	365 082		
Grapes	7 026 321		
Oranges	4 036 371		
Mangoes	186 308		
Naartjies	93 020		
Pears	1 418 911		
Peaches	550 216		
Bananas	1 217 775		
Grapefruit	860 902		
Pineapples	174 448		
Lemons	367 178		
Watermelons and melons	195 810		

All fruit production values given in the subsequent sections have been calculated from data given by the DAFF (2011a). The total production of apples increased from 394 164 t in 1980/81 to 780 687 t during 2009/10, with 20% sold on the national fresh produce markets, 43% exported, 30% processed and <1% dried during 2009/10. The total value of production during this period was R 2 869 740 (DAFF, 2011a, b).

The production of apricots varied substantially from 1980/81 to 2009/10, with a total production of 58 942 t during 2009/10, and a total value of production of R 129 854 000 (DAFF, 2011a, b). Two-and-a-half per cent of apricots produced are sold on the national fresh produce markets, 8% exported, 71% processed and 18% dried during 2009/10.

Similar to apples, the total production of pears increased from 136 208 t in 1980/81 to 366 216 t during 2009/10 (BFAP, 2011), with the bulk being exported, 50% and processed, 34% during 2009/10. The total value of production during this year was R 1 418 911 000 (DAFF, 2011a).

The total production of peaches decreased from 165 871 t in 1980/81 to 158 123 t during 2009/10, with 62% of the production purchased for processing (2009/10), total value of production during this year was R 550 216 000. Seventy-for per cent of all plums, production 55 769 t (2009/10) increasing from a low production base of 9 539 to (1980/81), are exported. The total value of production was R 388 027 000 (DAFF, 2011a, b, c, d).

Although strawberries are possibly a high risk product regarding food safety, the production in comparison to other fruit and vegetables in SA is low, 5 487 t in 2009/10, with 35% sold on fresh markets and the others processed, gross value R 61 671 000 (DAFF, 2011a, b).

In 2009/10, 23 907 tons of guavas were produced with a gross value of R 44 391 000 and 88% of the production was processed. Economic data is also available for mangoes, paw-paws, bananas, pineapples, citrus fruit, granadillas, litchis, avocados and watermelons. However, since most of these fruits are peeled before consumption, food safety risk associated with these fruits should be low (DAFF, 2011a, b, c, d).

It is clear from the previous sections that fruit and vegetables are important to the SA economy. A foodborne outbreak can have a severe impact on the economics of the fresh produce market (Calvin, 2006). A typical example of how a foodborne outbreak can impact an industry is the following: in 2005, leafy greens, head lettuce, leaf lettuce, romaine and spinach, were the most important of the top five fresh-market vegetables in the USA with a farm production value of 2 140 million USA$. After an FDA announcement regarding an *E. coli* O157:H7 – spinach outbreak during September 2006, the price, sales and shipments of spinach, were affected severely (Calvin, 2006; Calvin *et al*., 2006).

It is therefore important to evaluate the microbiological safety of vegetables and fruits emanating from SA (Tables 2 and 3), because an outbreak of foodborne illnesses as a result of consumption of fresh vegetables and fruits originating from South Africa to Europe or other countries will lead to banning of such produce into overseas countries.

8. COMMUNITY IMPORTANCE OF IRRIGATED CROPS

Agriculture is the basis of food security which together with several other factors (Wenhold & Faber, 2006) influence a community's food nutritional intake. Based on the fact that South Africa is a net exporter of agricultural products, the country has at the national level South Africa been classified as "food secure", but it is well known that approximately 35% of the population are vulnerable to food insecurity (Wenhold & Faber, 2006). The National Food Consumption Survey (NFCS, 2000) reported that in SA only one out of four households appears to be food secure and the diet of the great majority of children in SA is deficient in energy and of poor nutrient density. Children from rural areas are the most affected.

Vegetables are generally considered to be the most affordable and sustainable dietary source of vitamins, trace elements and other bioactive compounds. They are the major source of micronutrients (NFCS, 2000; Wenhold & Faber, 2006) and offer the most practical and sustainable way to ensure micronutrients is supplied through the diet (Chada & Oluoch, 2003). However, fruits and vegetables are seldom consumed by economically and socially deprived communities in developing countries and dietary intakes usually consist of plant-based staple foods (Faber & Benadé, 2003). Nutritionists have indicated that the local production of vegetables and fruits could alleviate vitamin, iron and micronutrient deficiencies in rural SA. Nutritionists promote home-based gardens as a strategy to alleviate the mentioned deficiencies (Chada & Oluoch, 2003; Faber & Benadé, 2003). They have found that these home-garden interventions are effective and should be encouraged. However, sadly these communities are also usually most susceptible to food-borne infections and intoxications. Therefore, if any intervention in rural areas to increase the production and consumption of vegetables is associated with contaminated irrigation water, these communities will be seriously affected.

In South Africa there is a typical example: Within the Limpopo Province, the Vhembe region is considered a rural area with inadequate water and sanitation infrastructures (DWAF, 1996a). Almost all farmers in this region have no alternative but to use wastewater or faecally contaminated surface water sources to irrigate their crops. The dominant crops grown by farmers in this region include tomatoes, onions, cabbages, sugarcane, bananas, carrots, beetroots and spinach, which grow in different types of soil. Waste water also carries potential health risks for farmers, crop-handlers and consumers who eat the raw produced crops due to the prevalence of opportunistic and pathogenic microorganisms (Havelaar & Melse, 2001). These farmers practice unrestricted irrigation with both treated and untreated wastewater. Unrestricted irrigation is the irrigation of all crops such as tomatoes, cabbage and carrots that can be eaten uncooked. It is well known that the use of untreated wastewater for unrestricted irrigation tends to increase the outbreak of diseases such as diarrhoea, skin irritation, typhoid and cholera (WHO, 1989). In addition, inadequate sanitation has also been shown to be a sources of pathogens on contaminated fruits and vegetables that have subsequently been associated with the cause of human disease outbreaks (Mederios *et al.*, 2001). It is also well known that irrigation water polluted with raw sewage or improperly treated effluents released from sewage treatment plants may contain hepatitis A virus, noroviruses and enteroviruses (WHO, 2000).

If people eat fruits and vegetables which were irrigated with contaminated irrigation water without washing or cooking, they are at great risk of being infected with pathogenic and opportunistic organisms. Inadequate sanitation and persistent faecal contamination of water sources is responsible for a large percentage of people in both developed and developing countries not having access to microbiologically safe drinking water and suffering from diarrhoeal diseases (WHO, 2000; WHO, 2001a, b).

The use of untreated or inadequately treated wastewater in agriculture is occurring more frequently because of water scarcity and population growth (Beuchat, 1986). It is often the poorest households in a country that rely on wastewater resources to secure their livelihood (Faruqui, 2001; Faruqui *et al.*, 2004) and holds negative health implications to these households. Already in 1993, Smith *et al.* estimated that one tenth or more of the world's population consumes food produced on land irrigated with wastewater. In 2000, Gilbert and co-workers reported that treated wastewater produces more than 40% of all food produced in the world. The WHO reported that at least 20 million hectors (ha) of the farms/schemes in 50 countries are irrigated with raw or partially treated wastewater (WHO, 1989).

Outbreaks of food infections associated with consumption of raw fruits, vegetables and unpasteurised fruits juices have been increasing (Beans *et al.*, 1997; Parish, 1997; De Roever, 1998; Beuchat, 2002) and this constitutes a worldwide public health concern (FAO/WHO, 2006). Recently, in September 2006, pre-packaged fresh spinach was recalled by the FDA in the USA as a result of an *Escherichia coli* outbreak. In the same month, fresh tomatoes consumed at restaurants in the USA were responsible for an outbreak of typhoid. There was also an *E. coli* O157:H7 outbreak linked to lettuce from Taco Bell restaurants in the northern USA (FAO, 2007). During 1998-2006 five commodity groups made up 75% of fresh produce related to foodborne outbreaks in the USA (Buchanan, 2006). These were lettuce/leafy greens 30%; tomatoes 17%; cantaloupe 13%; herbs (basil, parsley) 11% and green onions 5%.

The increase in foodborne outbreaks due to fresh produce is as a result of changes in dietary habits, including a higher per capita consumption of fresh or minimally processed fruits and vegetables, and the increased use of salad bars and meals eaten outside the home (Altekruse & Swerdlow, 1996; Alzamora *et al.*, 2000). According to Alzamora *et al.* (2000), yearly consumption of fresh fruits and vegetables in the USA has increased by 20 pounds per person from 1988 to 1996 mostly because of the belief that fruits and vegetables are healthier. Changes in production and processing methods; agronomic, harvesting; distribution and consumption patterns and practices are other factors (Calvin, 2006) that have also contributed to the increase (Beuchat & Ryu, 1997; Hedberg *et al.*, 1999). Other reasons given by the FAO and WHO (2006) are: microbial adaptation; increase in international trade; increase in susceptible population and increase in travel; change to a lifestyle of convenience and consumer demands regarding healthy food with no chemical preservatives with an extended shelf life; changes in human demographics and behaviour. Even though there is no data available on foodborne outbreaks as a result of the consumption of fresh produce in South Africa, the reasons given for the increase of outbreaks in USA are also applicable to SA.

As can be seen from the above data, the horticultural industry is extensive and represents a large financial investment. From these data the extent of the risk that polluted irrigation water poses to informal, small and commercial farming can be deducted. The increasing demand for fresh products especially presents a challenge for government, researchers and processors to ensure microbiological stability and safety.

9. EPIDEMIOLOGICAL HEALTH ASSESSMENT OF SA ENVIRONMENT

[Note: the following assessment is based on data in the Health Systems Trust Health Statistics database (Health Systems Trust, 2007) and the review of general population health in a South African Health Review of the Health Systems Trust (Bradshaw & Nannan, 2004), unless otherwise referenced.]

9.1 Background

The South African population increased exponentially from 5.17 million people in 1904 to 46.9 million in 2004, with a high average annual growth rate of 3.34% since 1975 (DEAT, 2006). This means that there are eight times as many people as there were a century ago trying to survive on the same amount (and in some cases less) of resources such as food, water, shelter, sanitation, clothing, energy, transport, education, and employment. Along with the growth rate that has decreased since 1995, life expectancy has declined dramatically since 1998, to below 50 years, which is largely attributable to the effects of HIV and AIDS. South Africa has experienced a widening of the wealth gap, with more poor people being vulnerable to droughts and floods and hazardous environments.

There is a clear link between access to water and socio-economic well-being (Dlamini & Schultze, 2005). Water can be viewed as a commodity, of which availability is a very real limit-to-growth upon economies. Water is an essential input or infrastructure resource to much of agriculture, energy production, industrial manufacture, mining, water transport and water-related recreation industries. The availability of water has an important role in poverty alleviation, especially in developing countries where water is still needed primarily for food production and rural development (Chartuvedi, 2000). Seven of the eight Millennium Development Goals of the United Nations Development Programme (2003) relate directly or indirectly to water (United Nations, 2000). The key effects of the health linkages between water and poverty are summarised by Dlamini and Schultze (2005) as: water and sanitation related diseases; stunting from diarrhoea-caused malnutrition; and reduced life expectancy.

The health of the South African population has declined rapidly in the last decade, as evidenced by a decreasing life expectancy. This has been the result of the rapid spread of an HIV epidemic of profound proportions. The HIV/AIDS epidemic has exacerbated an already serious TB epidemic, while also increasing mortality due to pneumonia, diarrhoea and other infections. Apart from this huge disease burden, large numbers of South Africans also live in poverty and suffer from chronic malnutrition. This means that a considerable proportion of the population can already be classified as "vulnerable". The serious state of pollution of many major river systems in South Africa and the all too frequent outbreaks of waterborne diseases such as cholera, typhoid and gastroenteritis, cause many poverty-stricken communities in South Africa to exist on the edge of disaster already. The following few indicators of health status are discussed briefly:

9.2 HIV and AIDS

According to the HST database, based on actuarial projections by the AIDS Committee of the Actuarial Society of South Africa, there are at present 5.51 million South Africans living with HIV. This translates to 11.4% of the total population, which masks much higher incidence in vulnerable subgroups. For instance, in KwaZulu-Natal, 26.1% of the population aged 15 to 49 years is estimated to be HIV positive, while Free State is estimated to have 22.3% of its population aged 15 to 49 years HIV positive. The corresponding rate for Gauteng is 22.5%.

9.3 Child health

Child mortality refers to the number of children aged 12 months to 5 years who die in a year, per 1 000 live births. The overall child mortality for 2003 was 15.8 per 1000 live births for the whole country, with the highest figure (21.1 per 1 000 live births) recorded for the Free State and the lowest (9.4 deaths per 1 000 live births) recorded for Gauteng.

The annualised rate of severe malnutrition is the number of children who weigh below 60% of expected weight for age per 1 000 children in the target population. The countrywide rate is 9.8 per 1 000 children for 2004 (down from 25 per 1 000 in 2001). KwaZulu-Natal again fares the worst at 20.5 per 1 000 children.

The situation regarding childhood diarrhoea is of especial concern. Diarrhoea incidence in children under 5 years of age per 1 000 of the target population was reported to be 268.7 per 1 000 in 2005. KwaZulu-Natal is the province with the highest reported incidence of childhood diarrhoea at 487 children per 1 000 of the target population – this translates to almost half of the target population in a recording year suffering from diarrhoea.

9.4 Lifestyle-induced risk factors for ill health

The percentage of the population who at the time were current smokers was recorded in 2003 as 31.1% for males and 8.4% for females. The Northern Cape had the highest number of male smokers, namely 48.8% of the population. In 2002 during a survey of learners in school grades 8 to 10, the number of male smokers recorded was 34.3% and the female smokers were 21.6% of the population surveyed. The Western Cape had the highest number of male smokers amongst learners at 46.9%. In the same survey it was recorded that 16.2% of male smokers and 15.3% of female smokers amongst the school learners started smoking before the age of 10 years. This implies that, even at a relatively young age, these learners would have been smoking for a long time.

The only data for alcohol dependence were reported for the 1998 Demographic and Health Survey. In that year, 27.8% of males were identified by the CAGE alcohol dependence questionnaire as alcohol dependent while 9.9% of females were so identified. During the same survey a further 32.3% of males and 32% of females were classified as "risky" drinkers – i.e. 'binge' drinkers over weekends.

9.5 Indicators of infectious disease

The cholera outbreak that started in KwaZulu-Natal in 2000 (a total of 106 389 cases were reported in 2000/2001 season) dwindled to 2 780 cases in 2004. The last year for which statistics are available in the database was 2005 with no cholera cases. Similarly, the last cases of typhoid reported in the database are from 1998. There were 0.7 cases of viral hepatitis per 100 000 of the population reported for 2004 in the database (70 per 1 000). The provincial breakdown figures are only available for 1998, where the Western Cape had the highest number of reported cases, namely 7.8 per 100 000 and the country as a whole only 2.6 cases per 100 000.

9.6 Environmental and sanitation aspects

In 2005, 84.9% of households had access to piped water, albeit not all inside their dwellings. The Western Cape had the highest percentage namely 98.3% and the Eastern Cape the lowest namely 65.4%. The proportion of the population with sustainable access to an improved water source was 87%. In contrast to this, in 2005 the percentage of households that had no toilet of any kind was 10.9% for the whole country, while 27.3% of households in the Eastern Cape had no toilet. In 2002, 67% of persons in the country had access to improved sanitation of some kind. In 2001, only 55.4% of households in South Africa had refuse removal with only 14.2% of Limpopo Province enjoying such a facility.

9.7 Conclusion

From the above few statistical indicators, it can clearly be seen that the pre-existing health status of a large proportion of the South African population is already cause for serious concern. A significant segment of the population is living with serious health conditions, risky lifestyles and/or food insecurity.

9.8 Vulnerability of people

In South Africa, the interaction between socio-political circumstances and environmental conditions determines the vulnerability of people. The major causes include deepening poverty, unemployment and HIV and AIDS, poor levels of disaster readiness, susceptibility to climate change and variability, and people's inability to cope with extreme weather events including droughts and floods. Household food security is a major concern in the face of climate variability. In addition, a deteriorating state of the environment, poor past land-use planning, and patchy success in the delivery of services such as sanitation and clean water, are increasing the exposure of people to environmental disasters. These include dangers arising from contaminated water sources, and high levels of environmental degradation and pollution. The most vulnerable people include those who are marginalized, those who lack access to land, capital, literacy, and other assets, and those who are often female, young, sick, or disabled. These groups lack the capacity to cope with environmental stresses. This means that this segment of the population is especially vulnerable to infections from water- and food-related pathogens. This makes the study of the links between polluted water and food-related illness of strategic concern.

10. RISK ASSESSMENT OF PATHOGENS ASSOCIATED WITH VEGETABLES AND FRUIT

10.1 Aspects of risk assessment methodology

Awareness is growing that fresh and minimally processed fruit and vegetables can be sources of disease-causing bacteria, viruses, protozoa and helminths. Irrigation with poor quality water is one way that fruit and vegetables can become contaminated with foodborne pathogens. Groundwater, surface water and wastewater are commonly used for irrigation and these water sources carrying varying degrees of contamination risk.

Risk is a combination of hazard, vulnerability and the capacity to cope with a hazard. Quantifiable risk assessment was initially developed to assess human health risks associated with exposure to chemicals and, in its simplest form, consists of four steps, namely: hazard assessment; exposure assessment; dose-response analysis; and risk characterization.

The output from these steps feeds into a risk management process. This basic model (often referred to as the chemical risk paradigm) has been extended to account for the dynamic and epidemiological characteristics of infectious disease processes (Haas & Eisenberg, 2001).

10.2 Hazard assessment

For micro-organisms involved in pollution of irrigation water, hazard assessment (i.e. the identification of a pathogen as an agent of potential significance) can be a straightforward task in well-resourced and properly managed environments. Such identification however, depends on availability of technological resources and skills which may be in short supply in resource-poor environments or in developing countries. On the other hand, not all of the microbial hazards (pathogens) are easily recognized (especially by less sophisticated means) and many cannot be readily enumerated or studied (Bartram *et al.*, 2001).

One outcome of a hazard analysis is a decision about the principal consequence(s) to be quantified in the formal risk assessment. With microorganisms, consequences may include infection (without apparent illness), morbidity or mortality. Moreover, the causal links between identified pathogens in irrigation water, those pathogens transferred from such water to food and the eventual rates of disease in the general population are complex and often obscured. Adverse health effects may arise after a single exposure, yet water quality varies continuously, widely and rapidly.

10.3 Exposure assessment

Selection of indicators – The purpose of an exposure assessment is to determine the microbial doses typically consumed by the direct user of water or food. This is complicated when the water used for irrigation originates from the same (contaminated) source as the drinking water available to a community. This may necessitate the estimation of raw water micro-organism levels followed by estimation of the likely changes in microbial concentrations with treatment, storage and distribution to the end-user. A second issue arising in exposure assessment is the amount of ingested material per ‘exposure’.

The use of indicators of contamination are preferred to measurements of pathogenic organisms in the water due to the low numbers of some kinds of pathogenic organisms present, the difficulties in detecting them and the expense involved. Indicators should be selected that are appropriate to the water being studied e.g. thermotolerant coliforms or *E. coli* are used in assessing the quality of drinking water whereas these are less suitable for assessing the quality of coastal recreational waters where enterococci and faecal streptococci are generally preferred. Where the density of an indicator does not accurately reflect the relative density of the underlying pathogenic organism, then it is not a valid indicator organism. This is a particular concern when bacterial indicators are used to indicate the presence of both bacterial and viral pathogens, as treatment methods are often less effective against viruses.

Measurements of exposure and disease status – Measurements of exposure and disease status need to be made in the study population while minimizing the various types of error that can occur. Where errors occur, this results in misclassification of the risks faced by individuals or the population as a whole.

For *exposure* to occur, an individual must have contact with water of a given quality or contaminated food produced with the aid of such water. It is preferable to measure exposure at an individual level, but in many studies exposure status is measured at a group level, which can give rise to misclassification of exposure for the individual. Not all individuals are exposed to the same food produced by means of contaminated water. Differential misclassification can either overestimate or underestimate the effect of exposure on disease. One source of misclassification of exposure results from the limited precision of current techniques for the enumeration of indicator organisms. This has not been taken into account in many experimental studies of the health impact of contaminated water.

Analysis of the relationship between exposure and disease – The basic measures of disease frequency in each population are described by using the prevalence rate (which is the proportion of the population that has the disease at a specific point in time) or the incidence rate (the number of *new* cases of disease per unit of person-time): when water is unsafe, conventional testing almost always indicates this only after exposure has occurred, i.e. too late to contribute to disease prevention; important health effects (both acute and delayed) may occur as a result of short-term exposure.

Hamilton *et al.* (2006b) constructed QMRA models for estimating the annual risk of enteric virus infection associated with consuming raw vegetables that have been overhead irrigated with non-disinfected secondary treated reclaimed water. They ran models for several different scenarios of crop type, viral concentration in effluent, and time since last irrigation event. The mean annual risk of infection was always less for cucumber than for broccoli, cabbage, or lettuce. Across the various crops, effluent qualities, and viral decay rates considered, the annual risk of infection ranged from 10^{-3} to 10^{-1} when reclaimed-water irrigation ceased 1 day before harvest and from 10^{-9} to 10^{-3} when it ceased 2 weeks before harvest. Two previously published decay coefficients were used to describe the die-off of viruses in the environment. For all combinations of crop type and effluent quality, application of the more aggressive decay coefficient led to annual risks of infection that satisfied the commonly propounded benchmark of $<10^{-4}$, i.e., one infection or less per 10 000 people per year, providing that 14 days had elapsed since irrigation with reclaimed water. Conversely, this benchmark was not attained for any combination of crop and water quality when this withholding period was 1 day. The lower decay rate conferred markedly less protection, with broccoli and cucumber being the only crops satisfying the 10^{-4} standard for all water qualities after a 14-day withholding period. Sensitivity analyses on the models revealed that in nearly all cases, variation in the amount of produce consumed had the most significant effect on the total uncertainty surrounding the estimate of annual infection risk. The models presented cover what would generally be considered to be worst-case scenarios: overhead irrigation and consumption of vegetables raw. Practices such as subsurface, furrow, or drip irrigation and post-harvest washing/disinfection and food preparation could substantially lower risks and need to be considered in future models, particularly for developed nations where these extra risk reduction measures are more common.

The United States Protection Agency recommends that any water treatment (or any other human health risks for that matter) should not subject a person to a risk of infection of more than 1 per 10 000 per year (Pepper *et al.*, 1996). In a residential greywater re-use study where contaminated household water was used for irrigation, the risk of infection surpassed the risk of 1 in 10 000 in almost all households (Water Conservation Alliance of Southern Arizona, 2004)

Mukerjee *et al.* (2006) carried out a longitudinal microbiological survey of fresh produce from 14 farms using organic production methods, 30 semi-organic farms Using organic methods but not yet certified) and 19 farms using conventional farming methods in the upper Midwest of the USA. They analysed 473 organic, 911 organic and 645 conventionally produced food samples of lettuces, leafy greens, cabbages, broccoli, peppers, tomatoes, zucchini, summer squash, cucumbers and berries. Conventional produce either had significantly lower or similar coliform populations compared with the semi-organic or organic produce. *E. coli* was detected in 8% of the samples and leafy greens, lettuces and cabbages had significantly higher *E. coli* counts than all the other product types.

In general, bacteria and protozoa tend to show the poorest survival outside the human host, whereas viruses and helminths can remain infective for months to years (Steele and Odumeru, 2004).

10.4 Dose-response analysis

There are difficulties associated with dose-response assessments for microbiological contaminants of water. Such assessments are faced with the large variation that can be encountered both in human responses and in the microbiology of the pathogens themselves. The likelihood of contracting an infection is influenced by factors such as immune status, immunity imparted by previous exposure and the virulence of the particular type or strain of micro-organism.

Evaluating causal linkages between estimated doses of pathogens ingested and consequent disease, generally involves a considerable amount of uncertainty that necessitates the employment of assumptions in the statistical models used to predict risk. Predictions are often based on extrapolation of reaction following high doses to chronic low-level exposures in humans.

The risk of disease transmission from pathogenic microorganisms present in irrigation water is influenced by the level of contamination; the persistence of the pathogens in water; in soil, and on crops; and the route of exposure (Steele & Odumeru, 2004). Hamilton *et al.* (2006a) considered various factors when assessing whether the risk of illness from microbial pathogens are different for different population groups. They used Quantitative Microbial Risk Assessment Modelling (QMRA) and considered the concentration of pathogens in the source water; water treatment efficiency; the volume of water coming into contact with the crop; the die-off rate of the pathogens in the environment; as well as the amount of food consumed. They found that despite the disparities in consumption rates by different ethnics groups, they all faced comparable levels of risk. This is important for the present study to know, since amounts of potentially contaminated foods consumed by various income groups and ethnically diverse communities in South Africa vary widely.

It is generally necessary to fit a parametric dose-response relationship to experimental data since the desired risk (and dose) which will serve to protect public health is often far lower than can be directly measured in experimental subjects (at practical numbers of subjects). Hence it is necessary to extrapolate a fitted dose-response curve into the low-dose region. For some micro-organisms, human dose-response studies are available in QMRA which can be used to estimate the effects of low level exposure to micro-organisms. It has to be noted however, that generally, several models may fit available data in a statistically acceptable sense, and yet provide very different estimates for the risk at an extrapolated low dose.

In an exponential model, which may be derived from the assumption of random occurrence of micro-organisms along with a constant probability of initiation of infection by a single organism (r), the probability of infection (P_I) is given as a function of the ingested dose (d) by: $P_I = 1- \exp(-rd)$.

For many micro-organisms, the dose-response relationship is shallower than reflected by curve produced by this equation, suggesting some degree of heterogeneity in the micro-organism-host interaction. (Haas & Eisenberg, 2001)

10.5 Risk characterization

The process of risk characterization combines the information on exposure and dose-response into an overall estimation of likelihood of an adverse consequence (Haas & Eisenberg, 2001). This may be done in two basic ways. First, a single point estimate of exposure (i.e. number of organisms ingested) can be combined with a single point estimate of the dose-response parameters to compute a point estimate of risk. This may be done using a 'best' estimate, designed to obtain a measure of central tendency, or using an extreme estimate (worst-case scenario), designed to obtain a measure of consequence in some more adversely affected circumstance. An alternative approach, which is currently receiving increasing favour, is to characterize the full distribution of exposure and dose-response relationships, and to combine these using various tools (for example, Monte Carlo analysis) into a distribution of risk. This approach conveys important information on the relative imprecision of the risk estimate, as well as measures of central tendency (e.g. means, medians) and extreme values (Burmaster & Anderson, 1994).

One important outcome of the risk characterization process using a Monte Carlo approach is the assessment of the relative contribution of uncertainty and variability to a risk estimate (Haas & Eisenberg, 2001). Variability may be defined as the intrinsic heterogeneity that leads to differential risk among sectors of the exposed group, perhaps resulting from differential sensitivities or differential exposures. Uncertainty may be defined as the factors of imprecision and inaccuracy that limit the ability to exactly quantify risk. Uncertainty may be reduced by additional resources, for example devoted to characterization of the dose-response relationship.

Some aspects of risk characterization deserve further comment. In general, all available dose-response information for micro-organisms (human or animal) pertains to response to *single* (bolus) doses. In actual environmental (or food) exposures, doses may occur over time (or may even be relatively continuous). In the absence of specific data on the impact of prior exposure on risk, the assumption used in projecting risk to a series of doses has been that the risks are independent (Haas, 1996).

It is important to keep in mind in all judgments on risks carried by polluted irrigation water that, because the pathogens of concern are widespread and because their occurrence varies widely and rapidly in time and space, the absence of safeguards in itself constitutes a hazard. This approach is derived from traditional ‘hygiene’ but is reflected in modern risk management such as the hazard analysis and critical control point (HACCP) principles that are used in the food industry. A further concern to keep in mind is that management actions are rarely of consistent effectiveness, and their outcome may be difficult to predict. In this regard, the willingness of regulatory and oversight bodies to act timeously when pollution incidents are suspected or uncovered is of crucial importance to the successful protection of the population.

11. PATHOGENS ASSOCIATED WITH VEGETABLES AND FRUIT

11.1 Background

Despite major advances in preventative health care and food technology water and foodborne diseases remain a widespread and growing public health problem in the industrialized and developing world (Leggitt & Jaykus, 2000; Satcher, 2000; Parashar & Monroe, 2001; Egli *et al.*, 2002), and the burden of foodborne disease is grossly underestimated (Marx, 1997; Egli *et al.*, 2002; Koopmans *et al.*, 2002; Carter, 2005; Newell *et al.*, 2010). In industrialized countries foodborne diseases have generated much media attention and governments across the globe are increasingly finding themselves urgently in need of upgrading their domestic food safety systems. However, in many developing countries, such as South Africa, there is no comprehensive food safety system in place to upgrade. Factors such as changing lifestyles and demographics, changing farming practices and intrusion of man into former undeveloped areas, increased numbers of immunocompromised individuals, faster and more frequent travel, decreasing water supplies in certain countries and enhanced importation of foods have contributed to the increase in food and waterborne infections (Cuthbert, 2001; Koopmans *et al.*, 2002; Newell *et al.*, 2010). The greatest demand on fresh water sources worldwide is for irrigation and the scarcity of water has resulted in the use of wastewater for irrigation (Moe & Rheingans, 2006). The use of wastewater for irrigation purposes has been responsible for many disease outbreaks caused by bacteria, protozoa, parasitic helminths and viruses (Bitton, 1980; Parashar & Monroe, 2001) and the health risks associated with using wastewater in irrigation have been quantified (Mara *et al.*, 2007).

All produce has the potential to harbour pathogens (Brackett, 1999), and *Shigella*, *Salmonella, enterotoxigenic* and *enterohemorrhagic E. coli, Campylobacter, Listeria monocytogenes, Yersinia enterocolitica, Bacillus cereus, Clostridium botulinum,* viruses and parasites such as *Giardia lamblia, Cyclospora cayetanensis,* and *Cryptosporidium parvum* are of public health concern (Beuchat, 1996; Ortega *et al.*, 1997; De Roever, 1998; Beuchat, 2002). Most of these have been associated with bacterial infections associated with fruit, unpasteurised fruit juice and vegetables (Tables 4 and 5) and the acidic property of some of these produce do not always prevent the survival and growth of these pathogens (ECSCF, 2002).

According to Beuchat (1998), the frequencies of pathogens on vegetables vary. The prevalence of *Campylobacter* is mostly at levels of <3%, whereas the prevalence of *Salmonella* is higher, between 4 and 8%. *Escherichia coli* O157:H7 and *L. monocytogenes* were found more frequently compared to *Salmonella* (ECSCF, 2002). In some studies pathogens were not detected at all on raw vegetables. In a survey done by McMahon and Wilson (2001) on 86 organic vegetable samples in Northern Ireland, *Salmonella*, *Campylobacter, E. coli, E. coli* O157:H7 or *Listeria* spp. were not found on the organic vegetables examined.

According to Matthews (2006), the FDA found that 1% of high-volume vegetables including cantaloupe, celery and loose-leaf lettuce were positive for either *Salmonella* or *Shigella* spp. during a survey of domestic fresh produce. No samples were positive for *E coli* O157:H7. Similar results were obtained for imported produce, where 4.4% were positive for either *Salmonella* or *Shigella* spp. and no samples were positive for *E coli* O157:H7.

11.2 Indicator organisms

In the analysis of products for pathogenic loads, it is impractical as well as uneconomical to always perform a whole range of tests for the presence of different organisms. Therefore, the practice of monitoring a certain organism, whose presence and number will give a fairly accurate indication of the type of pathogens present and in what quantity they are, has become popular and widespread. The organisms that are monitored in these tests are referred to as 'indicator' organisms. An indicator organism has been defined by the FDA as 'a microorganism or group of them that indicate that food has been exposed to conditions that pose to increase the risk of food becoming contaminated with a pathogen or held under conditions conducive for pathogenic growth' (James, 2006).

Table 4. Members of bacterial genera that have been reported to be associated with raw and MPF products (Matthews, 2006).

Acinetobacter *Aeromonas* *Bacillus* *Bacteriodes* *Bifidobacterium* *Brucella* *Campylobacter* *Citrobacter* *Clostridium* *Edwardsiella* *Escherichia*	*Enterobacter* *Enterococcus* *Franciella* *Hafnia* *Klebsiella* *Listeria* *Leptospira* *Mycobacterium* *Pasteurella* *Plesiomonas* *Proteus*	*Providencia* *Pseudomonas* *Salmonella* *Serratia* *Shigella* *Staphylococcus* *Streptococci* *Vibrio* *Yersinia*

The requirements of an indicator is that they should have very broad survival criteria with regards to aspects such as type of water, they should be more numerous than the pathogens themselves, unable to multiply in an aquatic environment, have a moderate to long survival time, correlate in number to the degree of pollution of the water and be absent in the absence of pollution, not multiply in the water and relatively safe to work with in the laboratory. The environmental survival rate of an indicator should be as similar to other pathogens also found in wastewater as possible.

Escherichia coli is probably the most common indicator organism and is used particularly for the detection of faecal matter as well as for the prediction of faecal coliform counts, especially in drinking water. The presence of *E. coli* is never beneficial to a consumer and always points to faecal contamination. Its presence, therefore, should not be ignored if it is detected in a sample. Confirmation of faecal contamination can be found with a positive indole test with *E. coli*. Faecal streptococci occur in much lower numbers than *E. coli* and are not as easy to detect. This group is, however, more resistant that *E. coli* and therefore has a higher survival rate over time. While the low numbers do not make it a suitable substitute test, it is a good test to be performed in addition to the indicator test for the confirmation of faecal contamination and as an indication of the levels present. Another indicator for faecal contamination is somatic coliphages which attack and infect the *E. coli* host. The test for somatic coliphages is a simple one but their survival curves follow more closely those of the human viruses than of E. coli and other bacteria of faecal origin. It has been found that the most reliable and representative of all indicator tests are microbiological tests. However, a major drawback of microbiological tests is that they are slow which results in a time delay – and thus a greater influence of variables – before the results can be obtained. This problem can only be dealt with through strict standardisation of methods in order to minimise the influence of external variables.

Table 5. Outbreaks of bacterial infections associated with fruits, unpasteurised fruit juice and vegetables (Beuchat, 2002).

Bacteria	Year	Country	Source
Bacillus cereus	1973	USA	Seed sprouts
Clostridium botulinum	1987	USA	Cabbage
E. coli O157:H7	1991	USA	Apple cider
	1995	USA	Lettuce
	1996	USA	Apple juice
	1997	Japan	Radish sprouts
	1997	USA	Alfalfa sprouts
E. coli (enterotoxigenic)	1993	USA	Carrots
Listeria monocytogenes	1979	USA	Celery, lettuce, tomato, cabbage
Listeria monocytogenes	1979	Canada	Watermelon
Salmonella			
Miami	1954	USA	Apple cider
Typhimurium	1974	USA	Watermelon
Oranienburg	1979	USA	Mungbean sprouts
Saintpaul	1988	UK	Cantaloupes
Chester	1989- 90	USA	Tomatoes
Javiana	1990	USA	Cantaloupes
Poona	1991	USA/ Canada	Tomatoes
Montevideo	1993	USA	Alfalfa sprouts
Bovismorbificans	1994	Sweden/Finland	Orange juice
Hartford	1995	USA	Alfalfa sprouts
Stanley	1995	USA	Alfalfa sprouts
Montevideo	1996	USA	Mamey
Typhi	1998- 1999	USA	Alfalfa sprouts
Mbandaka	1999	USA	Fruit salad
Shigella flexneri	1998	UK	Lettuce
S. sonnei	1986	USA	Lettuce
	1994	Norway	Parsley
	1998	USA	Scallions
Vibrio cholerae	1995	USA	Vegetables
	1970	Israel	Coconut
	1991	USA	Vegetables

Results of a survey of *Salmonella, Shigella*, and enteropathogenic *E. coli* on fruits and vegetables suggested that the frequency with which target pathogens could be isolated from irrigation water was inversely correlated with crop height (Velaudapillai *et al.*, 1969; FDA/CFSAN, 2001). Plants, such as spinach and cabbage, had a higher frequency of confirmed positive isolation of pathogens than taller chilli peppers or tomatoes, while contamination of tree fruit was negligible. Other factors according to (FDA/ CFSAN, 2001) that may cause the persistence of pathogens are plant surface hydrophobicity and contours.

In another study, during a seven-month microbiological survey of vegetables, higher total coliform counts were recorded when the sprinkler irrigation water source was of poor microbiological quality than when water of acceptable microbial quality was used (FDA/CFSAN, 2001; Armon *et al.*, 2002). In addition, the coliform levels depended on the product, possibly its structural features. *Salmonella* spp. were detected only on vegetables that had been irrigated with poor quality water.

Another type of indicator apart from that discussed above is a surrogate. This is an organism that mimics the growth of pathogens as well as the effects that different conditions will have on them. They can be injected into products in known concentrations and then be tested for at the end of a certain process in order to monitor the effect of that process on a pathogen. This result can be used to predict the risks that different processes pose to the safety of the end product. This vital information can, therefore, all be obtained without releasing a pathogen into a production plant. A surrogate can either be a non-pathogenic strain of the same genus i.e. *Listeria innocua* is used as a surrogate for *Listeria monocytogenes* or it can simply be a different organism responds and behaves in a similar way to the pathogen i.e. *Bacillus stearothermophilus* is used as a surrogate for *Clostridium botulinum* (James, 2006).

Cryptosporidium oocysts and *Giardia* cysts are spread through contaminated faeces. Run-off water from informal settlements, farms and effluent from faulty water treatment plants can thus contaminate rivers and dams with *Cryptosporidium* oocysts and *Giardia* cysts. According to Beuchat (1996), human specific enteric pathogens such as S*almonella* spp., S*higella* spp., Hepatitis A virus and Noroviruses are more likely to be found in water contaminated with human faeces. Animal faeces can also be source of various serotypes of *Salmonella*, *E. coli*, *Giardia* and *Cryptosporidium* (Beuchat, 1996). Also, most pathogens associated with fresh produce originate from enteric environment. That means that they are found in the intestinal tracts and faecal material of humans and animals (Harris *et al*., 2003). This is why indicator organism are used to indicate faecal pollution and the possible presence of pathogens.

Faecal coliforms are commonly used as indicator organism to indicate faecal contamination and to predict the presence of enteric pathogens (Rhodes & Kator, 1988; Tallon *et al*., 2005) since the presence of thermo tolerant coliforms and *E. coli* has been found to have significant predictive value for the presence of enteropathogens (Horman *et al.,* 2004). However, it has now been observed that the ecology, prevalence and resistance to stress of faecal coliforms differ from those of many pathogens (Scott *et al.,* 2002). The use of other indicator organisms such as *E. coli*, *Enterococcus* spp., *Clostridium perfringens* and bacteriophage has thus been suggested (Scott *et al.,* 2002; Harwood *et al*., 2005). *E. coli* has long been used as an indicator of faecal pollution. It meets many of the criteria of a good indicator organism as it is not normally pathogenic to human and is present in higher number than the pathogens it predicts. In warmer climate, *E. coli* is however able to replicate in contaminated soil which may decrease its reliability as an indicator under such condition (Desmarais *et al*., 2002). The enterococcus group is a subgroup of faecal streptococci and has been found to be good indicators of faecal pollution especially in marine environment and recreational water (Griffin *et al*., 2001). *Clostridium perfringens* is an enteric, gram-positive, anaerobic, spore-forming, pathogenic bacterium and is found in human and animal faeces (Scott *et al.,* 2002). It has the ability to survive for longer period of time in the environment and has thus been used to predict the presence of viruses or remote faecal pollution (Payment & Franco, 1993). Bacteriophages have been suggested as indicator of enteric viruses as their morphology and survival characteristic are similar to those of enteric viruses (Havelaar *et al*., 1993). Enteric viruses have however been detected in drinking-water supplies that met accepted specifications for treatment, disinfection and conventional indicator organisms, including somatic bacteriophages (Grabow *et al.,* 2001; van Heerden *et al.,* 2005).

Certain protozoa are more resistant to environmental stresses than bacteria. *Cryptosporidium* and *Giardia* have a distinct resting stage, the oocyst or cyst respectively, which is able to survive for a long time in the environment. So once water has been contaminated with either *Cryptosporidium* and or *Giardia*, these parasites will remain viable in the water for longer than bacteria. According to Rose *et al*. (1996), *Cryptosporidium* oocysts are able to survive in river water for 176 days at 5-10˚C. *Giardia* cysts were shown to remain viable for 56 days in river water (deRegnier *et al*., 1989), while *E.coli* has been shown to die off in rivers after only 4 to 11 days at 4˚C (Flint, 1986). So water contaminated with *Cryptosporidium* or *Giardia* might not necessarily test positive for *E.coli* due to the longer survival of those protozoa in water. In previous studies, no correlation was found between the concentration of indicator organisms and presence or absence of *Cryptosporidium* and *Giardia* in surface water and water effluent from water treatment plant (Harwood *et al.*, 2005; Horman *et al*., 2004). It has also been suggested that monitoring of faecal coliforms levels on fresh produce might not adequately reflect the occurrence *Cryptosporidium* and *Giardia* due to coliforms' high susceptibility to chemical disinfection compared to the two protozoa (Harwood *et al.*, 2005). The monitoring of several indicators as well as some key pathogens was suggested as a more accurate alternative to the one-indicator system to predict the presence of human pathogen in water (Harwood *et al.,* 2005). This group is classified as protozoa and is considered a serious human pathogen. This organism is often isolated from surface waters (Fayer *et al*., 1992). They occur as oocysts, and Fayer *et al.* (1992) reported that 72% of surface water samples taken in the USA tested positive for these oocysts.

11.3 Bacterial pathogens

11.3.1 Escherichia coli

Escherichia coli is a member of the genus Enterobacteriaceae and most strains are normal inhabitants of the intestinal tract and are practically always present in faeces and thus also in faecally contaminated water. This has resulted in the almost universal use of *E. coli* as the standard indicator for faecal contamination (Francis *et al.*, 1999). However, not all strains are harmless and the major pathogenic strain, *E. coli* O157:H7, has been identified in several MPF-related food outbreaks. If ingested, this strain commonly results in haemorrhagic colitis, gastroenteritis and kidney failure (Francis *et al.*, 1999) while it less commonly results in thrombocytopenic purpura and haemolytic uremic syndrome (Gil & Selma, 2006). Serious cases can even result in death.

The monitoring of faecal matter in rivers and on the MPFs is therefore of great importance since there is very little control possible over animals faeces entering the river (Francis *et al.*, 1999). Over the last 25 years, *E. coli* O157:H7 has turned from being basically unheard of as a fresh produce pathogen to being responsible for 34% out of all the *E. coli* outbreaks, of which none were a result of contamination during preparation. This correlates directly to the increasing levels being found in river systems (Matthews, 2006). Another finding is that the general survival ability of *E. coli* O157:H7 increases upon exposure to one environmental stress which indicates that it is able to activate survival mechanisms when it is threatened (Maciorowski *et al*., 2007). *E. coli* is known to be able to withstand very highly acidic environments and can survive at pH ranges as low as 3.3-4.2 (Johnston *et al.*, 2006). The number of *E. coli* present in an environment was found to increase logarithmically with an increase in oxygen, indicating that *E. coli* has a high chemical oxygen demand and therefore grows better under aerobic conditions. While a relationship exists between the chemical oxygen demand and the *E. coli* load, there is no direct correlation between the two values and thus an accurate estimation of the microbiological status of the water cannot be made (Johnston *et al.*, 2006).

Outbreaks of enterohemorrhagic *E. coli* O157:H7 infections associated with lettuce and other leaf crops have occurred frequently in recent years (Mahbub *et al.*, 2004). Burnett and Beuchat (2001), indicated that *Escherichia coli* O157:H7 has been associated with lettuce, alfalfa sprouts and apple juice, and enterotoxigenic *E. coli* has been linked to carrots and spinach. Produce on which it has been more regularly detected are celery, coriander, cilantro mixed raw vegetable products (Johnston *et al.*, 2006), lettuce (Gil & Selma, 2006; Mena, 2006), radishes and peas (Matthews, 2006), parsley (Matthews, 2006) and alfalfa sprouts (Gil & Selma, 2006; Mena, 2006). Gil & Selma, (2006) also reported no effect of modified air packaging (MAP) on the growth of *E. coli*.

In 2011 in Germany, 32 HUS- associated deaths were recorded and 852 patients were confirmed as having HUS. In the USA there were 5 confirmed cases of people that had travelled to Germany with STEC O104:H4 infections. A single lot of fenugreek seeds from an exporter in Egypt was identified as the most likely source of sprouts linked to these two outbreaks.

11.3.2 Listeria

Listeria monocytogenes is a very invasive, non-spore-forming pathogen (Maciorowski *et al*., 2007) and is widely distributed in the environment, where it is associated with decaying vegetation, soil, sewage and faeces of animals, and has been isolated from celery, lettuce, tomato and cabbage in USA and Canada (Beuchat, 1996, 2002). It has the ability to survive in a wide range of environmental conditions including high moisture concentrations, low oxygen concentrations, become facultatively anaerobic and grow at refrigeration temperatures as low as 0.5°C (Francis *et al.*, 1999; Johnston *et al.*, 2006), making it an ideal waterborne pathogen (Maciorowski *et al*., 2007). The only condition under which it cannot cope is osmotic stress, which is experienced during desiccation. An unusually long incubation time makes an infection caused by *L. monocytogenes* very difficult to trace back to a specific product (Johnston *et al.*, 2006). Soil containing *L. monocytogenes* can contaminate plants during fertilisation as well as by water droplets splashing from the soil onto the plants (Johnston *et al.*, 2006). *L. monocytogenes* can survive for extremely long periods of time and has been reported to remain viable for 10-12 years (Maciorowski *et al*., 2007).

Infection caused by *L. monocytogenes* is extremely serious and contact with the eye can cause eye-infections while infections in humans and ruminants can result in meningitis, septicaemia, still-births and abortions (Francis *et al.*, 1999; Maciorowski *et al*., 2007). Should *L. monocytogenes* manage to penetrate the central nervous system once inside the body; the infection can be fatal (Gil & Selma, 2006). The seriousness of the infections caused by exposure to *L. monocytogenes* has resulted in strict legislation regarding its presence on produce. France and Germany permit levels of up to 102 cfu.g^{-1} while in America and England complete of the organism is required in a 25 g sample and any sample found to contain colonies are rejected (Francis *et al.*, 1999; Gil & Selma, 2006).

According to Beuchat (1996) cases of human listeriosis that have been associated with the consumption of raw vegetables are likely, in part, due to contamination by manure from ruminants. Prazak *et al.* (2002) looked at the prevalence of *L. monocytogenes* during the production and post-harvest processing of cabbage and they found that out of 855 samples, 425 cabbage, 205 water and 225 environment sponge samples, examined *L. monocytogenes* was isolated from 3% of samples, 26 of 855. Twenty of these isolates were obtained from cabbage; three from water samples and another three were environmental sponge samples of packing shed surfaces.

Listeria monocytogenes often causes pathogenic problem in silage but it has also been found in wheat as well as in other vegetables such as cabbage (Francis *et al.*, 1999; Matthews, 2006), bean sprouts, lettuce, potatoes, radish, broccoli, cucumber, leafy vegetables, green pepper, mushrooms, field cress and tomatoes. It has also been found in ready-to-use products such as salad packs, pre-mixed vegetable packs and coleslaw (Francis *et al.*, 1999; Johnston *et al.*, 2006; Matthews, 2006). This wide selection of fresh produce on which *L. monocytogenes* is found makes it a very real pathogen threat in river-irrigated MPFs (Maciorowski *et al*., 2007).

11.3.3 Campylobacter

Campylobacter jejuni is a pathogen of animal origin and it is transmitted to the soil by these animals. Contact of a meat product or animal faeces with raw or uncooked foods is its primary vehicle for infection. It has been found in lettuce, parsley, spring onions, mushrooms, potatoes, spinach and radishes for sale at places such as farmers markets where the produce has not been washed or exposed to any kind of post-harvest treatment such as chlorination (Johnston *et al.*, 2006; Matthews, 2006).

11.3.4 Salmonella

Like *E. coli, Salmonella* is also a pathogen of the family *Enterobacteriaceae*. These bacteria are Gram-negative and rod-shaped. The genus comprises five pathogenic strains namely *S.* Typhimurium*, S. enteritidis, S.* Heidelberg*, S.* Saint-Paul and *S.* Montevideo (Francis *et al.*, 1999)*. Salmonella* is a very resistant pathogen and it has a wide survival range. It has adapted well to survive outside intestine, particularly at water activities between 0.43 and 0.52 (Maciorowski *et al*., 2007) and at very low oxygen concentrations (Francis *et al.*, 1999) making it a very effective human pathogen (Maciorowski *et al*., 2007). *Salmonella* grows optimally in warm temperatures between 35° and 43°C while its growth is substantially retarded at 15°C and generally prevented at 7°C. It was assumed for a long time that the low pH of fruit juices had a preventative effect on the survival of *Salmonella* until an outbreak was traced back to unpasteurised orange juice (Johnston *et al.*, 2006). This pathogen alone is annually responsible for 1.3 million outbreaks of foodborne illnesses as well as for being the second biggest cause of diarrhoea, both in the USA.

Salmonella is usually carried by animals such as pigs or poultry or insects and is passed on to humans when meats, eggs or milk from these animals are consumed when they are undercooked (Johnston *et al.*, 2006). Alternatively, non-animal products that have made contact with faeces of these infected animals as a result of animals grazing over the crops or fertilisation with manure can also carry *Salmonella* (Maciorowski *et al*., 2007).

Since contamination with this pathogen is of animal origin there is an extremely wide range of fresh products on which it has been found. According to Flowers *et al.* (1992) the occurrence of Salmonellosis in America increased greatly over the last two decades, and average between 740 000 to 5 300 000 incidents per year. According to Beuchat (1996), *Salmonella* has been isolated from raw vegetables from countries such as USA, Canada, Sweden and Finland (Table 5). Salmonellosis has been linked to tomatoes, lettuce, seed sprouts, cantaloupe, watermelon, apple juice and orange juice (Wood *et al.*, 1991; Hedberg *et al.*, 1999; Burnett & Beuchat, 2001). In a reported outbreak associated with diced tomatoes, *S. baildon,* a serotype rarely implicated in human salmonellosis, was isolated from patients in geographically separate areas of the USA (Weissinger *et al.,* 2000). Other MPFs that have tested positive for *Salmonella* include bean sprouts, leafy vegetables (Francis *et al.*, 1999), carrots, melons, strawberries, mung bean sprouts, alfalfa seeds, lettuce (Francis *et al.*, 1999; Johnston *et al.*, 2006), salad greens, mixed raw vegetables, chilli, cilantro, parsley, artichoke, cabbage, cardoon, cauliflower, celery, aubergine, endive, fennel (Francis *et al.*, 1999; Johnston *et al.*, 2006), green onions, spinach, beet leaves and tomatoes (Johnston *et al.*, 2006; Korsten & Zagory, 2006). Of these products, pre-cut melon in salad bars has been associated with several major *Salmonella* outbreaks, of which one outbreak was thought to have affected up to 25 000 people (Johnston *et al.*, 2006). Commonest invasive and non-invasive non-typhoidal *Salmonella* serotypes reported per province by GERMS-SA per month in South Africa for 2010 (n = 1 384) including the audit reports (NICD, 2011) are summarised in Table 6.

Table 6. Commonest invasive and non-invasive non-typhoidal *Salmonella* serotypes reported in South Africa for 2010 (n = 1 384). These include the audit reports (NICD, 2011).

Province	**Serotype**				
	Enteritidis	**Heidelberg**	**Infantis**	**Isangi**	**Typhimurium**
Eastern Cape	32	3	1	32	85
Free State	15	0	3	0	32
Gauteng	337	15	27	18	295
KwaZulu-Natal	79	5	4	33	72
Limpopo	7	1	0	7	7
Mpumalanga	21	14	5	0	32
Northern Cape	4	0	0	1	14
North West	14	1	0	1	14
Western Cape	49	6	4	2	92
South Africa	558	45	44	94	643

11.3.5 Faecal streptococci and enterococci

The genera *Streptococcus* and *Enterococcus* are Gram-positive, spherical, non-spore forming, facultative anaerobic, catalase negative and homofermentative microbe. Species such *S. pyogenes* and *S. pneumoniae* are known human pathogens (Hardie & Whiley, 1997). Although there are no reports of faecal streptococci food-borne outbreaks, Turantas (2002) isolated faecal streptococcus from 41 (75%) frozen vegetables out of 55 frozen vegetables. This result is in agreement with the findings of Insalata *et al.* (1969) who recovered faecal streptococci from frozen vegetables. Vegetables irrigated with waste water were also reported to contain equal number of "*S. faecium*" (*Enterococcus faecium*) and "*S. faecalis*" (*Enterococcus faecalis*) but "*S. faecium*" survived the effect of chlorination better than "*S. faecalis*" (Sadovski & Ayala, 1980).

11.3.6 Shigella

Shigella is another widespread foodborne pathogen of the family *Enterobacteriaceae*. The four species, namely *S. sonnei, S. boydii, S. dysenteriae and S. flexneri*, are pathogenic. Shigellas are regarded as rather fragile organisms which do not survive well outside their natural habitats. However, some stains are capable of survival below pH 6 and *S. sonnei* can survive at low temperatures as well as at low oxygen concentrations (Gil & Selma, 2006). *Shigella* has a very low infectious dose, making even a low level of contamination dangerous for the consumer (Gil & Selma, 2006). Virulence occurs only at temperatures around 37°C and secretion of an exotoxin takes place. In view of the fact that the infective dose for the genus *Shigella* varies between 10^1 and 10^4 cells per person, and infection occurs through faecally contaminated water or food (Smith & Buchanan, 1992), great care should be taken when irrigating minimally processed foods with water of questionable microbial quality. It has been reported that 10 cfu have been found to initiate infection in susceptible individuals (Stine, 2004).

Shigella does not occur naturally in soil, water or on produce but is transmitted via the faecal-oral route. Its faecal origin makes it an indicator of faecal contamination, and this contamination could have come from faecally contaminated water or bad worker hygiene. It has been estimated to have caused more than 166 million illnesses throughout the world in 1999 alone (Johnston *et al.*, 2006). Transmissions of this organism usually occurs by person-to-person, but several outbreaks have been reported due to the consumption of contaminated water and foods, particularly raw vegetables (Stine, 2004). There are also reports that sliced fresh vegetables and fruits, including watermelon, papaya and jicama can support the growth of all species of *Shigella*.

Foodborne outbreaks of the disease are usually linked to the use of raw, contaminated products in salads or foods that have not been properly cooked before consumption. Symptoms of *Shigella* infections can be relatively serious and outbreaks to have been traced back to Shigella contamination on raw baby corns, green onions, lettuce (Gil & Selma, 2006; Johnston *et al.*, 2006), melon, celery and parsley. Lettuce contaminated with *S. sonnei*, from Texas, USA and green onions imported from Mexico caused serious outbreaks of gastroenteritis as a result of consumption of the raw vegetables contaminated with *Shigella* sp (Beuchat, 1996; Harris *et al.*, 2003). Where water is contaminated with faeces of animal origin, this pathogen may be present (Savichtcheva & Okabe, 2006). Brackett (1999) considers *Shigella* species to be a very serious threat to human health in cases where fresh produce is irrigated with contaminated water and then consumed raw.

In South Africa *Shigella* infections are largely due to water-borne outbreaks. Higher isolation rates in January through to May 2010 were seen as potentially a surveillance artefact, due to heightened awareness of food and water borne diseases prior to the World Cup and increased testing of symptomatic patients (NICD, 2011).

11.3.7 Staphylococcus

There are currently 27 species and several subspecies of the genus *Staphylococcus* but enterotoxin production is principally associated with *S. aureus, S. intermedius* and *S. hvicus*. Although *S. aureus* is associated with food handlers, it has been isolated from vegetables but so far there has been no reported outbreak due to consumption of vegetables contaminated with *S. aureus* (Harris *et al.,* 2003). However, vegetable-associated outbreaks due to *Staphylococcus* could occur under conditions that favour the growth of the organisms and subsequent toxin production. The number of *Staphylococcus aureus* bacteraemia reported to the GERMS-SA from July through December 2010 was 506 (Table 7) while an additional 280 cases (36%) were identified during an audit (total number of cases available for analysis was 786) (NICD, 2011).

Table 7. Number of *Staphylococcus aureus* cases reported to GERMS-SA sentinel sites by provinces in South Africa from July- December 2010 (n = 786) (including audit cases) (NICD, 2011)

Province	***S. aureus***
Free State	40
Gauteng	510
KwaZulu-Natal	26
Limpopo	3
Western Cape	207
Total	786

11.3.8 Vibrio

Historically cholera has been one of the diseases most feared by mankind. It is endemic to the Indian subcontinent where it is estimated to have killed more than 20 million people during the 20th century. The genus *Vibrio* includes at least three species that are known as human pathogens: *Vibrio cholerae* that is the etiological agent in cholera; *V. parahaemolyticus* that is often found in seafood and seawater, and *Vibrio vulnificus* that causes septicaemia (Kaysner *et al.*, 1992). These organisms can be described as Gram-negative, curved, motile rods that do not form spores. Most *Vibrio* species can ferment glucose without the formation of gas and are oxidase and catalase positive.

Vibrio parahaemolyticus infections are usually associated with raw fish and seafood, and pose a serious threat to the health of especially Japanese citizens, seeing as they eat most of their fish raw (Kaysner *et al.*, 1992). Breaks in the cold chain or unsatisfactory hygiene practices are given as other possible reasons for foodborne outbreaks (Kaysner *et al.*, 1992).

Most cholera patients contract the disease via the faecal-oral route through ingestion of contaminated water, or eating minimally processed or raw vegetables that was either irrigated with contaminated water, or fertilized using contaminated faeces. Foodborne outbreaks of the disease are also associated with raw or undercooked seafood (Van Elfen, 2001). Vast amounts of the organism are isolated from the excreta of infected individuals (Kaysner *et al.*, 1992) and animals (Hurst *et al.*, 2002). If these excreta were to contaminate irrigation water, consumers are at great risk of contracting the disease (Brackett, 1999).

11.3.9 Yersinia

This rod-shaped organism is a member of the family *Enterobacteriaceae.* The genus is Gram-negative, oxidase-negative, facultative anaerobic and is able to ferment substrates to obtain necessary nutrients (Schiemann & Wauters, 1992). Three species of *Yersinia* are known to be human pathogens: *Y. pestis*, which is the organism responsible for plague, *Yersinia pseudotuberculosis*, which is not normally linked to foodborne diseases, and *Y. enterocolitica* which is more often linked to foodborne outbreaks than the other two species.

Although the organism is not usually associated with food, evidence exists that it is present in the faeces of animals (Hurst *et al.*, 2002). If animals are allowed to graze near rivers or other surface-water bodies, their excreta can easily end up in water that may be destined for the irrigation of minimally processed foods. There have not been reported outbreaks of food-borne illness due to contamination of vegetables with *Yersinia* (Schiemann & Wauters, 1992). The organism has also been isolated from several raw vegetables (Harris *et al.,* 2003). In a survey done on 58 samples of grated carrots in France, 27% of the samples were contaminated with *Y. enterocolitica* serotypes and 7% of the 27% contained *Y. enterocolitica* serotypes pathogenic to humans (Harris *et al.,* 2003).

Seeing that the organism is a psychrotroph, it can survive and multiply at refrigerator temperatures without difficulty. This is important if you consider that fresh fruit and vegetables are usually kept at low temperatures to prolong their shelf-life. Its biochemical activity is visibly higher at 25° than 35°C, in that it gives a positive result for the Voges-Proskauer test only at 25°C and the organism only exhibits motility at the lower temperature (Schiemann & Wauters, 1992).

11.3.10 Spore-forming bacteria

Endospores of members of the genera *Bacillus and Clostridium (B. cereus*, *Cl. botulinum* and *Cl. perfringens)* can contaminate vegetables. When processed and packaged under favourable conditions, i.e. minimally processed products modified atmosphere packaged, their spores may germinate and pose a possible health hazard (Harris *et al.,* 2003). Cabbage and sliced onions are able to support the growth of *C. botulinum*. Mixed seed sprouts caused an outbreak due to *B. cereus,* while salad contaminated with *C. perfringens* was also associated with an outbreak (Harris *et al.,* 2003)

Bacillus cereus is found widely as it occurs naturally in the soil as well as on plants. *B. cereus* is a spore-former which means that extra care must be taken to store products testing positive for it under the correct storage conditions in order to prevent the spore from resuming their vegetative state (Johnston *et al.*, 2006). *B. cereus* has been detected on alfalfa, mung bean, wheat sprouts and broccoli (Johnston *et al.*, 2006). An outbreak of food poisoning due to contamination of mixed seed sprouts have also been shown to be caused by *B. cereus* (Harris *et al.*, 2003).

The two members of the genus *Clostridium* that are of major pathogenic concern are *Cl. perfringens* and *Cl. botulinum*. Cl. perfringens is commonly found in the faeces of both humans and animals (Johnston et al, 2006), both of which are found abundantly in local river systems. *Cl. perfringens* is also found in soil and dust but faeces are the most common source. Thus far, findings of *Cl. perfringens* in the food industry have been fairly limited to mixed raw vegetables (Johnston *et al.*, 2006). However, mixed seed sprouts and salad contaminated with *C. perfringens* was also associated with an outbreak (Harris *et al.*, 2003).

Clostridium botulinum is a Gram-positive, rod-shaped, endospore-forming obligate anaerobe (Francis *et al.*, 1999) and needs a temperature above 5°C to grow (Gil & Selma, 2006). Therefore strict temperature control throughout storage is imperative (Gil & Selma, 2006). It is the bacteria responsible for botulism and while some *Cl. botulinum* strains need external proteases to activate the neurotoxin; others can activate the neurotoxin themselves, increasing their pathogen city further. It is known for its acid-tolerant properties and is usually associated with fermenting silage, wheat and hay but it is also commonly found in soil and thus can be housed in the sediment of a river or in soil after irrigation (Johnston *et al.*, 2006).

Contamination of produce with *Cl. botulinum* is thought to happen when soil is disturbed or moved in such a way that it lands on the produce (Maciorowski *et al*., 2007). It is also associated with the intestinal tracts of fish and the gills and viscera of shellfish (Johnston *et al.*, 2006). It is commonly only seen as a threat in canned foods but the increase in popularity to store MPFs under MAP has created ideal growth and survival conditions for the pathogen and thus a rise in cases related to MPFs has occurred. Fresh produce that have been carriers of the toxin are cabbage, asparagus, broccoli, tomatoes, lettuce and melons (Francis *et al.*, 1999). The neurotoxigenic *C. botulinum* is the etiological agent for a condition known as botulism (Kautter *et al.*, 1992). These anaerobic bacilli are often isolated from improperly processed canned foods, where it proliferates and produces a deadly neurotoxin, botulin. Although this foodborne infection is rare it claims many lives when outbreaks occur (Kautter *et al.*, 1992).

11.3.11 Plesiomonas

The only species in this genus, *Plesiomonas shigelloides*, is an opportunistic enteropathogen commonly associated with illness after ingestion of raw seafood (Smith & Buchanan, 1992). Motility, facultative oxygen needs and the presence of oxidase and catalase are some characteristics of these Gram-negative rod-shaped organisms (Smith & Buchanan, 1992). The organism thrives in water, whether it be fresh or ocean waters, and therefore can be considered a potential pathogen occurring on irrigated produce worldwide (Brackett, 1999).

11.4 Parasites

11.4.1 Cryptosporidium

Cryptosporidium is an obligate, intracellular, eukaryotic protozoa from the phylum Apicomplexa. The first cases of cryptosporidiosis were reported in 1976 and cryptosporidiosis infections have now been reported in over 90 countries and six continents (Fayer *et al*., 2000). *Cryptosporidium* spp. infect both human and animals and over 152 species of mammals have been reported to be infected with *Cryptosporidium* (Fayer *et al.,* 2000). *Cryptosporidium hominis* is the species that causes most human cryptosporidiosis and *Cryptosporidium parvum* is typically found in ruminant but can also infects humans (Dawson, 2005). Other species of *Cryptosporidium* have also been reported to infect humans and zoonotic transmission from animal to human is thus possible (MMWR, 2010).

Cryptosporidium has a two-stage life cycle consisting of a reproductive stage and an environmentally resistant oocyst stage. Oocysts are spore-like survival structures, resistant to environmental stresses and commonly used disinfectant such as chlorine (Moriarty *et al*., 2005). The great resistance of *Cryptosporidium* oocysts to chemical disinfectants is attributed to their oocysts walls which are made of a double layer of protein-lipid-carbohydrate matrix (Templeton *et al*., 2004). Oocysts are spherical in shape, measure between 4 to 6 µm and contain four sporozoites. Amylopectin is the polysaccharide found in coccidian protozoa and provide energy for excystation and penetration of host cells (Harris *et al*., 2003). Small amounts of amylopectin are also used during the dormancy stage and *Cryptosporidium* oocysts have been found to be unable to infect host one the amylopectin store has been depleted (Vetterling & Doran, 1969).

Oocysts are the stage transmitted from an infected host to a susceptible host by the faecal oral route (Fayer *et al.,* 2000). Oocysts can be transmitted from person to person, from animal to human, through drinking contaminated water or eating contaminated food and from swimming in contaminated water (Fayer *et al.,* 2000; MMWR, 2010). Once ingested, oocysts release the reproductive stage: the sporozoites. Sporozoites attach themselves to the intestinal epithelium and multiply, damaging the mucous membrane of the intestinal lining and causing diarrhoea (Dawson, 2005). New oocysts are then formed and shed in the faeces of infected host. *Cryptosporidium* has a low infective dose and the ingestion of as few as 10 oocysts has been shown to cause infections. The infective dose varies from species to species but the average infective dose has been calculated to be 87 oocysts (Okhuysen *et al*., 1999)**.** The incubation period varies between 3-7 days (Tzipori & Ward, 2002). *Cryptosporidium* infections are usually self-limiting in healthy individuals but can become chronic and life threatening in individuals with weakened immune systems (Lane & Lloyd, 2002; Dawson, 2005). Population especially at risk of infections are children, malnourished persons, and individuals with compromised immunity including AIDS patient, transplant recipient, patients receiving chemotherapy for cancer, institutionalized patients and patients with immunosuppressive infectious diseases (Fayer *et al.,* 2000). Up to 2002, no drug therapy had been found effective against *Cryptosporidium* infections. Treatment for cryptosporidiosis is mainly symptomatic and supportive (Fayer *et al.,* 2000). In 2002, Nitazoxanide was the first and only broad spectrum antiparasitic drug approved for use in the USA for the treatment of cryptosporidiosis (MMWR, 2010).

11.4.2 Giardia

Giardia lamblia is a waterborne, flagellated protozoan from the order Diplomonadida and was first discovered in 1681 by Antoine Van Leeuwenhoek (Lane & Lloyd, 2002). The *Giardia* species infecting humans are *G. intestinalis* and *G. duodenalis* sometimes called *G. lamblia*. *Giardia lamblia* is endemic throughout the world with a high incidence in the tropics and subtropics and infects humans, mammals, reptile and birds (Lane & Lloyd, 2002). Giardiasis is now recognized as a traveller's disease worldwide, it is one of the most common source of intestinal infection in the developed world as well as a serious cause of infection in developing countries (Stevens, 1982; Swaminathan *et al*., 2009).

Giardia lamblia has a two stage life cycle and alternates between trophozoite and cysts. The trophozoite is the vegetative reproductive stage. It is pear shape and 9.5 to 21 µm long by 5 to 15 µm wide, has four pairs of flagella and two nuclei. The trophozoite has ventral sucking disk made of microtubule which help with attachment to the intestinal mucosal layer of its host (Wolfe, 1992). Cysts are the survival stage and the infectious form that is found in the environment (Lane & Lloyd, 2002). They are ovoid, measure 8 to 12 µm long by 7 to 10 µm wide and contain four nuclei. Cysts have a though hyaline cyst wall which makes them resistant to chlorination and they can survive for several months in cold surface water (Lane & Lloyd, 2002).

Infection occurs when cysts are ingested with contaminated food or water. Cysts pass through the stomach undamaged and excyst once they reach the duodenum where the alkaline pH is favourable for the growth of the trophozoites. Two trophozoites are released from each mature cyst. Some trophozoites then attach themselves to the intestinal villi with the help of the sucking disks while others swim freely in the duodenum and ileum (Lane & Lloyd, 2002). Trophozoites replicate asexually by binary fission inside the intestine, damaging the epithelial cells and causing the symptoms of giardiasis. New cysts are then formed which are discharged in stools of infected hosts and then transmitted to new host through food or water (Thompson, 2000). Humans are the main reservoir of *Giardia* but animals can carry species of *Giardia* similar to those infecting humans (Wolfe, 1992). Calves are also a big reservoir of *Giardia* with high incidence of infection worldwide. *Giardia duodenalis* which includes *G. lamblia,* naturally infects humans, beavers, coyotes, cattle, cats and dogs (Wolfe, 1992). Species of *Giardia* originating from animals have been reported to be infectious to humans as well (Wolfe, 1992). Shedding of high numbers of cyst in the environment by livestock is thus a public health concern as this could lead to zoonotic infections (Lane & Lloyd, 2002).

Giardiasis infection occurs by faecal-oral contamination with cysts or indirectly through consumption of contaminated food or water. Ingestion of 100 or more cysts is required to ensure infections in human but as few as 10 cysts have resulted in infections in volunteers. This is due to the fact that a single ingested cyst will divide and quickly multiply to infectious levels (Lane & Lloyds, 2002). Traveller's often become infected by drinking contaminated water from ground or surface water. The incubation time varies between 9 to 15 days and the symptoms of the disease are variable but include nausea, low fever, watery diarrhoea and malabsorption (Wolfe, 1992; Thompson, 2000**)**. The acute stage last between 3 to 4 days and usually clears spontaneously in individuals with healthy immune system but chronic infection may develop and may last two or more years or even become fatal in individuals with compromised immunity (Wolfe, 1992). However, around 13% of infected adults and up to 50% of infected children remain asymptomatic (Wolfe, 1992). Several drugs are available to treat the infection; however, in South Africa the use of metronidazole is recommended (NDH, 1998).

11.4.3 Water contamination with Cryptosporidium and Giardia

Cryptosporidium and *Giardia* have mainly been associated with waterborne outbreaks. *Cryptosporidium* oocysts are ubiquitous in surface water worldwide (WHO, 2008) and have been found in untreated surface water (Ong *et al*., 1996), ground water (D'Antonio *et al*., 1985), treated drinking water (D'Antonio *et al.*, 1985), recreational water and even bottle water (Franco & Neto, 2002). In South Africa, the presence of *Cryptosporidium* and *Giardia* in surface water was investigated by Kfir *et al*. (1995). They found 30% of samples positive for *Giardia* cysts while 25% of samples tested positive for both *Giardia* and *Cryptosporidium* (Kfir *et al.,* 1995).

The first confirmed documented waterborne outbreak of cryptosporidiosis occurred in 1984, in Texas and was caused by unfiltered ground water supply (D'Antonio *et al.*, 1985). The number of reported cases of cryptosporidiosis has been increasing ever since and the Center of disease control in the United State has reported an increased from 6 479 cryptosporidiosis cases in 2006 to 10 500 cases in 2008 (MMWR, 2010). On the other hand, giardiasis cases are on a slight decline and 19 140 cases of giardiasis were reported in the United State during 2008 while 19 238 cases were reported in 2006. More cases of cryptosporidiosis were reported in summer than in winter. This was mainly attributed to the increase in recreational water-associated outbreak due to *Cryptosporidium* oocysts' resistance to chlorine (MMWR, 2010). Recreational water-associated outbreaks of giardiasis are also well documented and *Giardia* was found responsible for 3.7% of recreational water-associated gastroenteritis outbreaks in the United State between 1997 and 2006 (MMWR, 2010).

Humans and animals act as reservoir of *Cryptosporidium* and *Giardia*. In a study carried out in a Durban hospital, South Africa, in 1986, *Cryptosporidium* was the second most common organism detected among children admitted with diarrhoea (Van den Enden, 1986). In Limpopo, a *Cryptosporidium* incidence of 18% was observed in school children and hospital patients (Samie *et al*., 2006). It has been estimated that 32% of AIDS patient have suffered from cryptosporidiosis as some stage in their life (Hunter & Nichols, 2002 according to Moore *et al.*, 2007). Cattle and calves are big reservoirs of the parasites with high prevalence of *Giardia and Cryptosporidium* having been reported (Olson *et al.*, 1997; Lefay *et al*., 1999). Furthermore, a higher *Cryptosporidium* sero-prevalence has been observed among dairy farmers compared to other farmers which suggest zoonotic transmission from cattle to human (Fayer *et al*., 2004).

Large numbers of cysts or oocysts are shed in faeces of infected hosts. As much as 10^{10} *Cryptosporidium* oocysts or 10^{9} *Giardia* cysts can be released daily from host during acute or chronic infection and excretion of infectious *Cryptosporidium* oocysts can carry on up to 50 days after diarrhoea has stopped while shedding of infectious *Giardia* cysts can carry on for months (Tzipori & Ward, 2002; MMWR, 2010). In South Africa, water sources located near farms or informal settlements are thus more likely to become contaminated with *Cryptosporidium* oocysts and/or *Giardia* cysts. Water sources can become contaminated through run off water from infected field or poorly sanitized settlements and direct contamination by infected animal and human faeces is also likely to occur. Rivers downstream of cattle farms have indeed been found to contain higher levels of *Cryptosporidium* oocysts and *Giardia* cysts than water upstream of the farms (Ong *et al*., 1996).

The many outbreaks associated with drinking water confirm that purification system can fail to eliminate *Cryptosporidium* oocysts and *Giardia* cysts (Fayer *et al*., 2000). Badly designed conventional water treatment using sand filtration and chlorine systems may not remove all oocysts or cysts. A typical example of water contamination is the Milwaukee outbreak in 1993. It was caused by *Cryptosporidium* oocysts passing through one of the drinking water treatment facilities and resulted in more than 400 000 ill people and several deaths (Bailey *et al*., 2004). This was the largest documented water-borne outbreak in United State history. More recently, *Cryptosporidium* oocysts were found in water supplies in Ireland in 2007. The water came from a lake and then underwent treatments with coagulation and/or filtration. The heavy rainfall are suspected to have increased pathogen loads in the lake due to run off from farm and *Cryptosporidium* oocysts were not completely eliminated by the water treatment facilities (Pelly *et al*., 2007). *Giardia* has also been associated with drinking water outbreak and was responsible for 10.6% of drinking water-associated outbreaks in the United State between 1997 and 2006 (MMWR, 2010). Recently, *Giardia* has been involved in drinking water associated outbreaks in the USA in 2007 and in Norway in 2004 (Daly *et al*., 2010; Robertson *et al*., 2006).

11.4.4 Food-borne outbreaks of Cryptosporidiosis and Giardiasis

Although outbreaks of food-related cryptosporidiosis and giardiasis (Table 8) are less reported than water-borne infections, oocysts and cysts are able to survive in wet or moist contaminated food (Schlundt *et al*., 2004). *Cryptosporidium parvum* was found to be the causative agent of 1699 food-borne infection cases in England and Wales between 1996 and 2000 (Adak *et al*., 2005). In the USA *Cryptosporidium* was the fourth most causative agent of food-borne infection in 2004, leading to 637 infections (CDC, 2006). Food associated outbreak of giardiasis are rarer than cryptosporidiosis foodborne outbreaks (MMRW, 2010). *Giardia* was found responsible for 18 foodborne outbreaks in the European Union during 2006 (EFSA, 2006) while the CDC reported 16 outbreaks in the USA between 1998 and 2007 (MMRW, 2010).

Cryptosporidium oocysts have been found on the surface of raw vegetables in various countries (Monge & Chinchilla, 1996; Robertson & Gjerde, 2001). According to Chaidez *et al.* (2005) contaminated irrigation water is one of the major sources of contamination for fresh produce. It was found in a recent study by Macarisin *et al.* (2010) that *C. parvum* oocysts were able to adhere to spinach plants after contact with contaminated water and were also able to infiltrate the plant through the stomata opening. This was the first study showing attachment and internalization of oocysts on vegetables. Fresh produce irrigated with water contaminated with *Cryptosporidium* oocysts could thus become contaminated. The physical structure of the fruit or vegetable's surface seems to influence the amount of oocysts or cysts retained on the product. The presence of hairy structures on the surface of the product might increase the amount of cysts or oocysts retained on the fruit or vegetable (Armon *et al*., 2002; Kniel *et al*., 2002).

Table 8. Reported food-borne outbreaks of Cryptosporidiosis and Giardiasis

Product	Year	Country	Organism	Infected cases	Reference
Unpasteurised milk	2001	Australia	*Cryptosporidium* spp.	8	Harper *et al.*, 2002
Salad bar: whole carrots, grated carrots, red peppers	2005	Denmark	*Cryptosporidium* spp.	99	Ethelberg *et al.*, 2009
Salad bar	2008	Finland	*Cryptosporidium* spp.	72	Ponka *et al.*, 2009
Parsley	2008	Sweden	*Cryptosporidium* spp.	21	Insulander *et al.*, 2008
Sliced raw vegetable	1990	USA	*Giardia lamblia*	26	Mintz *et al.*, 1993
Cold noodle salad	1985	USA	*Giardia lamblia*	16	Petersen *et al.*, 1988
Fruit salad	1986	USA	*Giardia lamblia*	9	Porter *et al.*, 1990
Ice	1990	USA	*Giardia lamblia*	28	Quick *et al.*, 1992

Fruits and vegetables can also become contaminated with oocysts or cysts during production when farm workers work with soiled hands or when contaminated fertilisers are used (Fayer *et al.*, 2000). The presence of cattle near vegetable field has also been linked to the contamination of vegetable with *Cryptosporidium* (Rzezutka *et al.*, 2010). Flies could be another possible source of contamination of fruits and vegetables as viable *Cryptosporidium* oocysts and *Giardia* cysts have been recovered from flies by Szostakowska *et al.* (2004). Oocysts are able to survive in the soil for up to 150 days (WRC, 2007) and *Giardia* cysts can survive for more than 2 months in cool condition (Adam, 1991). The cool and moist surface of fruits and vegetables thus provides suitable conditions for the survival of *Cryptosporidium* oocysts and *Giardia* cysts. The multiple possible sources of contamination indicate that sufficient viable oocysts could contaminate fresh produce and be ingested by susceptible host thus being of public health concern.

11.5 Viral pathogens

11.5.1 Background

Viral contamination of wastewater, recreational water, drinking water, irrigation water, ground and surface water has been reported in many studies (Barnes & Taylor, 2004; Rutjes *et al.*, 2005). The microbiological water quality is usually assessed using bacterial indicators such as faecal coliforms and *Escherichia coli* (Lee & Kim, 2002; Brassard *et al.*, 2005). Several researchers have reported the presence of enteric viruses in treated water meeting the recommended standards of coliform bacteria *(*Lee & Kim, 2002; Koopmans & Duizer, 2004; Brassard *et al.*, 2005; van Heerden *et al.*, 2005). This indicates the absence of a significant relationship between the presence of indicator bacterial and viral pathogens and therefore bacterial indicators alone are inadequate predictors of the presence of viral pathogens (Fuhrman *et al.*, 2005). Water treatment systems aiming at reducing the risk of bacterial contamination; have been historically found to be more or less adequate in eliminating resistant enteric viruses (Brassard *et al.*, 2005). It is, therefore, important to develop simple and efficient diagnostic tools to detect enteric viruses in water even if they are present at a very low concentration (Brassard *et al.*, 2005).

A large number of viruses found in the human intestinal tract are potential pollutants and three disease categories are associated with food- and waterborne viruses, namely: gastroenteritis caused by HRV, HuCV which include the NoV and the SaV, HAstVs and the enteric adenoviruses; hepatitis caused by the faecally transmitted hepatitis viruses, namely HAV and HEV; and other severe illnesses such as myocarditis caused by enteroviruses which include polioviruses, coxsackie A and B viruses, echoviruses and enteroviruses 68-71 (Koopmans *et al.*, 2002; Koopmans & Duizer, 2004; Butot *et al.*, 2007; Taylor, 2011).

Fruits and vegetables are traded around the world, originating from areas where sunlight permits growth but where water quality is not assured (Carter, 2005). Several foods have been implicated in viral outbreaks including fruits and vegetables, sliced deli meats, shellfish, and hand prepared foods such as salads and sandwiches (Koopmans *et al.*, 2002; Jean *et al.*, 2004). Though infected food handlers are identified as a common source of contamination of ready-to-eat and prepared food (Barrabeig *et al.*, 2010; Tuan Zainazor *et al.*, 2010), other major routes of contamination of fruits and vegetables includes: contamination of food in their growing area before harvest by coming into contact with inadequately treated sewage or sewage polluted water, during processing, storage, distribution or final preparation either directly from infected individual or by contact with a contaminated environment (Seymour & Appleton, 2002). Though foodborne disease associated with viruses is a well-documented phenomenon, foods are rarely tested for viral contamination (Leggitt & Jaykus, 2000). Foods served raw (fruit and vegetables) have been implicated in outbreaks because they are more vulnerable to contamination. Unfortunately, most of fresh produce washing systems are designed to remove gross contamination (such as dirt, insects, and foreign matter) and they are reported to less successful at removing microbial contamination (Croci *et al.*, 2002; Seymour & Appleton, 2002).

There is very little data on the presence of HAV, HuCV and HAstV in food sources and domestic, agricultural and recreational water sources in SA (Marx *et al.*, 1998). This is partly due to the lack of infrastructure for the detection and recording of such infections (Grabow, 1996). Escalating demands and pollution of already limited water sources, particularly in rural and developing communities, may even elevate risks (Barnes & Taylor, 2004, Venter *et al.*, 2007). Common-source viral foodborne outbreaks, such as SRSV-associated gastroenteritis, have been described in SA (Taylor *et al.,* 1993), but to date, no food and waterborne outbreaks of hepatitis A or HAstV have been documented due to the relative underreporting of these diseases. Hepatitis A virus, HAstV and HRV have been detected in raw and treated water sources in SA (Marx *et al.*, 1998; Grabow *et al.*, 2001; Taylor *et al.*, 2001; van Zyl *et al.*, 2004, 2006) confirming the potential risk of waterborne transmission of these viruses, and the risk of contracting HAV from selected water sources has been quantified (Venter *et al.*, 2007). In view of these recent findings, improved strategies to prevent the contamination of foods with HAV, HuCV and HAstV via faecally contaminated irrigation and processing water, and food handlers are needed in SA. On-going research is therefore needed to detect and identify the viral pathogens in food and water to facilitate correct management procedures to reduce the burden of food- and waterborne disease. In addition, more effective techniques for the recovery and detection of these viruses in food and water will be beneficial (Keddy, 1998).

11.5.2 Health impact of viruses

Food and waterborne viruses are an important cause of illnesses worldwide (Table 9) (Koopmans *et al.*, 2003; Richards, 2005). However, due to the prevalence of many asymptomatic or mild infections (Marx, 1997), underreporting (Parashar & Monroe, 2001) and the fact that the health effects of waterborne disease can be non-specific (Meinhardt, 2006) the true clinical and economic impact of food- and waterborne HAV, HuCV and HAstV infections may be underestimated. In addition person-to-person and foodborne routes of transmission may overlap (Carter, 2005) which further impacts on the estimation of foodborne illness. The WHO estimates that 70% of diarrhoeal episodes are caused by biologically contaminated food (Satcher, 2000) and the Center for Disease Control and Prevention (CDC) estimates that in the USA that there are 76 million cases, 325 000 hospitalisations and 5 000 deaths associated with foodborne disease annually (Acheson, 2001; Bresee *et al.*, 2002; Jones *et al.*, 2006). There is no reason to believe that risks of food and waterborne disease in SA are any different from those in the rest of the world.

Table 9. Most important food and waterborne viruses and the associated clinical syndrome

Likelihood of food and waterborne	**Gastroenteritis**	**Hepatitis**	**Other**
Common	Norovirus	Hepatitis A virus	
Occasionally	Enteric adenovirus Rotavirus Sapovirus Astrovirus Coronovirus Aichivirus	Hepatitis E virus	Enterovirus

11.5.3 Food and waterborne viruses

Hepatitis A virus (Mead *et al.*, 1999) and HuCVs, especially NoVs (Parashar & Monroe, 2001; Koopmans *et al.*, 2002; Koopmans & Duizer, 2004) are leading the causes of foodborne illness (Butot *et al.*, 2007) (Table 9), and HAstVs are increasingly being identified as important water- and foodborne viruses (Glass *et al.*, 1996; Ferrari & Torres, 1998; Walter & Mitchell, 2000). Most of these food- and waterborne viruses are non-enveloped and are resistant to heat, disinfection and pH changes. Unlike bacteria, viruses are strict intracellular parasites and they cannot replicate in food and water (Koopmans & Duizer, 2004). This means that the concentration of virus in contaminated food or water will not increase during processing (Koopmans *et al.*, 2002). Infection via contaminated food or water therefore depends on viral stability, amounts of virus shed by infected person, method of processing of water or food, dose needed to produce infection, and susceptibility of the host (Koopmans *et al.*, 2002).

Unfortunately to consumers, food or water contaminated with viruses will look, smell and taste normal (Koopmans & Duizer, 2004). Many enteric viruses are extremely infectious, only a small amount of these viruses is usually sufficient to infect a human host. Currently, the infectious dose for NoV and HAV is estimated to be around 10-100 infectious viral particles (Guévremont *et al.*, 2006). These viruses are shed in large numbers in the acute phase of disease thus increasing the risk of faecal contamination of food and water sources (Brassard *et al.*, 2005).

Many of the foodborne viruses cannot be isolated or detected by conventional routine laboratory techniques and molecular methods for the detection of HAV, HuCV and HAstV have, until recently, had limited applicability in food and environmental virology (Richards, 1999). The use of rapid and sensitive molecular detection and characterisation methods has proved effective in tracing sources of infection and transmission routes (Dowell *et al.*, 1995; Lappalainen *et al.*, 2001). Recent studies in the Dept. of Medical Virology, University of Pretoria/NHLS have characterised identical HAstV strains in wastewater samples and stool specimens from hospitalised patients thus identifying a potential reservoir or source of infection (Nadan *et al.*, 2003). Thus the characterisation of HAV, HuCVs, HAstVs and HRVs isolates from food and water sources and comparison to clinical isolates will provide valuable information as to the potential role of food and water as source of human enteric viral infection in SA.

11.5.4 Noroviruses

The Family *Caliciviridae* is divided into four genera: *Vesivirus* and *Lagosvirus* which are associated with veterinary infections, and *Norovirus* (formerly called Norwalk-like viruses [NLV]) and *Sapovirus* (formerly called Sapporo-like viruses [SLV]), which cause human infections (Chiba *et al.*, 2000, Moreno-Espinosa *et al.*, 2004, Martínez *et al.*, 2006; Zheng *et al.*, 2006; Scipioni *et al.*, 2008). An additional three genera have been proposed for inclusion within the family Caliciviridae: i) *Becovirus* or *Nabovirus*, to accommodate a unique bovine enteric calicivirus (Farkas *et al.*, 2008; Scipioni *et al.*, 2008), ii) *Valovirus* to accommodate the St-Valérien-like viruses isolated from pigs (L'Homme *et al.*, 2009), and iii) *Recovirus* for the novel calicivirus which was isolated from the stools of rhesus macaques (Farkas *et al.*, 2008). Based on molecular characterization of complete capsid gene sequences, the NoV genus is divided into five genogroups: GI to GV. Genogroup I and II can further be divided into subtypes or genotypes. Strains GI, GII and GIV are associated with human infection, and GIII and GV strains are found in cows and mice (Martínez *et al.*, 2006). Certain genotypes are commonly associated with specific routes of infection (Atmar, 2010) and since 2001, GII.4 NoVs have been associated with the majority of outbreaks worldwide (CDCP, 2011). Sapoviruses are also classified into five genogroups based on the capsid gene: GI, II, IV, and V which infect humans, and GIII which infect pigs (Wang *et al.*, 2006).

In both developing and industrialized countries, NoVs have been found to be the most important cause of nonbacterial acute gastroenteritis in all ages (Moreno-Espinosa *et al.*, 2004). Longitudinal studies performed in the Netherlands and Finland have shown that the clinical picture of gastroenteritis is similar for both NoVs and SaVs (Richards, 2005). They have incubation period of about 24-48 h, with the disease lasting for three to four days. Noroviruses and SaVs differ epidemiologically as NoVs cause illness in of all age groups, whereas the SaVs predominately cause illness in children (Koopmans & Duizer, 2004). The syndromes associated with NoV gastroenteritis includes non-bloody diarrhoea (lacks mucus but may be loose and watery), vomiting, nausea, abdominal cramps, fever, and malaise. Diarrhoea occurs more typically in adults, whereas vomiting occurs more frequently than does diarrhoea in children. Though the symptoms are similar, vomiting occurs more commonly with NoVs and diarrhoea more commonly with SaVs (Moreno-Espinosa *et al.*, 2004). Starting from the incubation period, virus is shed from stool and vomit for up to 10 days, and possibly longer (Koopmans *et al.*, 2002). Death associated with NoV outbreaks have been reported (Koopmans *et al.*, 2002, Thornton *et al.*, 2004). Although a winter seasonal peak has been suggested, both NoV and SaV infections occur year-round. It has been established that many different types of NoV are co-circulating in the general population, causing sporadic cases and outbreaks (Koopmans & Duizer, 2004). Transmission of NoV and SaV can occur by two routes, faecal-oral and aerosol formation following projectile vomiting (Souza *et al.*, 2006). They are transmitted directly by person-to-person contact or indirectly via contamination food, water or fomites (Souza *et al.*, 2006). Infected food handlers play an important role in the transmission of these viruses on the so called ready to eat foods (Souza *et al.*, 2006;).

Noroviruses are resistant to low pH and heat treatment (30 min at 60ºC). The virus can still remain infectious after 30 min in the presence of 0.5-1 mg of free chlorine per litre, meaning that the virus is quite resistant to chlorine. The virus can, however be inactivated at higher concentration of >2 mg free chlorine per litre (Koopmans & Duizer, 2004). Control depends on hygienic measures, particularly those aimed at reducing the spread by the faecal-oral route. These include proper management of food, water supplies, sewage, and individual measures such as hand washing and correct disinfection and disposal of contaminated material (Moreno-Espinosa *et al.*, 2004; Patel *et al.*, 2009). To control food-handler-associated transmission strict personal hygiene and proper disinfection of surfaces is required. In addition food-handlers should be excluded from work during the acute phase and 48-72 h after recovery from an episode of NoV-associated gastroenteritis (Patel *et al.*, 2009).

11.5.5 Hepatitis A Virus

Hepatitis A virus belongs to Family *Picornavidae* and is the sole member of the genus *Hepatovirus* (Carter, 2005; Richards, 2005; Spradling *et al.*, 2009). Hepatitis A virus is further divided into six genotypes, with genotypes I, II, and III found in humans, while genotypes IV, V, and VI were recovered from simians. Genotypes I to III are further classified into subtypes, IA, IB, IIA, IIB, IIIA, and IIIB (Pintó *et al.*, 2010). Genotype I is the most common human type worldwide, particularly IA, with genotype III representing the majority of the remaining human strains (Hollinger & Emerson, 2007; Pintó *et al.*, 2010). Genotypes IIA and IIB have been rarely reported (Jothikumar *et al.*, 2005; Sánchez *et al.*, 2007). Clusters of strains within genotypes and sub genotypes dominate in certain geographical regions (Robertson *et al.*, 1992; Hollinger & Emerson, 2007).

Hepatitis A virus has an incubation period of 15 to 45 days and viruses are present in the blood and faeces a few days after exposure and before the onset of clinical symptoms (Richards, 2005; Spradling *et al.*, 2009). Large numbers of virions are excreted in the faeces for several days before and after the onset of jaundice. Hepatitis A is characterized by nausea, vomiting, anorexia, malaise, myalgia, fever, and abdominal pain in the upper right quadrant. Since the severity of the disease correlates with age and demographics, children often experience asymptomatic illness, whereas non-previously exposed adults develop symptomatic infection and have a greater chance of hepatic failure and other complications such as cholestatic and relapsing hepatitis A (Richards, 2005; Spradling *et al.*, 2009). Endemnicity of HAV infection varies worldwide according to regional hygienic standard, with the highest prevalence of infection occurs in regions with lowest socioeconomic levels (Nainan *et al.*, 2006). The main route of transmission of HAV is by the faecal-oral route, more often from person-to-person. Outbreaks of hepatitis A are well documented in crowded situations such as institutions, schools, prisons, and in the military forces where common source epidemics evolve rapidly (Koopmans *et al.*, 2002; Spradling *et al.*, 2009). Ingestion of contaminated food and water is also an important route of infection and contaminated raw produce reaching the food service establishments is recognized as an important source of HAV transmission (Cuthbert, 2001; Nainan *et al.*, 2006). Shellfish has been implicated in a number of other outbreaks of hepatitis A (Sánchez *et al.*, 2007; Pintó *et al.*, 2010). In the USA outbreaks of hepatitis A have been associated with consumption of green onions (Dentinger *et al.*, 2001; Wheeler *et al.*, 2005), strawberries (Hutin *et al.*, 1999) and lettuce (Rosenblum *et al.*, 1990; Cuthbert, 2001). The ingestion of raw blueberries were identified as the cause of an outbreak of hepatitis A in New Zealand (Calder *et al.*, 2003) and raspberries have been linked to outbreaks of hepatitis A (Croci *et al.*, 2008). Three deaths were reported from a recent common source outbreak of hepatitis A where green onions were identified as the vehicle of transmission (Wheeler *et al.*, 2005). Waterborne outbreaks of hepatitis A have been reported (Morse *et al.*, 1972; Bloch *et al.*, 1990; Bosch *et al.*, 1991), but since the introduction of effective drinking water treatment waterborne outbreaks of hepatitis A are less common (Fiore, 2004; Pintó & Saiz, 2007).

Hepatitis A virus is resistant to various physicochemical agents and may survive for days and even weeks in water, shellfish, marine sediments and soil and so pose a potential threat to public health (Feinstone & Gust, 1997; Cuthbert, 2001; Collier & Oxford, 2006). Hepatitis A virus was found to survive for up to 9 days on lettuce, 7 days on fennel and 4 days on carrots (Croci *et al.*, 2002). This is due to the stability of HAV which is directly related to the virion structure. Hepatitis A virus has been found to survive elevated temperatures of up to 60°C for one hour, 25°C for several weeks and years at -20°C (Feinstone & Gust, 1997; Koopmans *et al.*, 2002). At temperatures greater than 60°C, HAV rapidly loses its infectivity if present in an isotonic solution and inactivation is virtually instant at temperatures of 85°C and higher (Gust & Feinstone, 1988; Zachoval & Deinhardt, 1993). Faecal suspensions of HAV are more resistant to heat than cell culture derived viruses. This may be due to small particles of faecal material clustering around the virus, shielding it from direct heat (Gust & Feinstone, 1988). In faecal material, HAV does not lose any infectivity after treatment for two hours at 60°C. Only a 100-fold loss in titre was recorded when incubated for seven days at 37°C or four weeks at 4°C (Gust & Feinstone, 1988). HAV has also remained viable in pasteurised milk products (Gust & Feinstone, 1988). Dairy foods with high fat content, such as cream and milk, need an exposure time of at least 30 s at 85°C to cause a 5-log reduction in the HAV titre (Bidawid *et al.*, 2000). Foodborne outbreaks of hepatitis A have been linked to the consumption of both raw and cooked shellfish harvested from water contaminated with human sewage (Bidawid *et al.*, 2000; Mullendore *et al.*, 2001). For the internal temperature of shellfish to reach 100°C, four to six minutes steaming is required (Bidawid *et al.*, 2000). Steaming or just heating the shellfish to open the shell is insufficient to inactivate any HAV present (Gust & Feinstone, 1988; Abad *et al.*, 1997). HAV survives long on nonporous environmental surfaces. This emphasizes the need for careful handling of any potentially infectious specimens or samples (Feinstone and Gust, 1997). HAV is stable at pH values as low as pH 3 (Hollinger, 1985; Gust & Feinstone, 1988) and survival of at pH values as low as pH 1 has been reported (King *et al.*, 2000; Koopmans *et al.*, 2002). HAV can be inactivated by treatment chlorine (10-15 $mg.L^{-1}$ residual chlorine concentration for 30 min) (Hollinger, 1985) or chlorine-containing compounds such as sodium hypochlorite at concentrations of 10 $mg.L^{-1}$ for 15 min at 20°C (Grabow *et al.*, 1983; Hollinger, 1985). Treating HAV preparations with 0.5-1.5 mg free residual chlorine per L for one hour at pH 7 reduces HAV infectivity (Gust & Feinstone, 1988). To inactivate any infectious HAV in water, treatment with 2.0-2.5 mg of free-residual chlorine per L of water for 15 min should be considered (Hollinger & Ticehurst, 1996; Feinstone & Gust, 1997).

Although the availability of vaccine which provides a long-term protection against HAV infection has potentially reduced disease incidence, good personal hygiene, hygiene procedure and correct disposal of contaminated material still play important role in the control of infection. The prevention can also depend mainly in the awareness of all food handlers about transmission of these viruses (Collier & Oxford, 2006)

11.5.6 Recovery and detection of viruses from food and water

The analysis of food and water for enteric viruses is a complex two stage process. As viral particles present in food and water are presumed to be low in number the first step is to apply efficient recovery and concentration procedures (Köster *et al.,* 2003; Wyn-Jones, 2007; Mattison & Bidawid, 2009). Thereafter a range of isolation and/or detection methods may be applied (Wyn-Jones & Sellwood, 2001; Wyn-Jones, 2007; Bosch *et al.*, 2011). There are, however, no standard procedures for the recovery and detection of viruses in water (Verheyen *et al.,* 2009) and food samples (Mattison & Bidawid, 2009).

Viral recovery from water samples: Important criteria to be considered when concentrating viruses from water are: i) the technique should be easy to complete in a short time; ii) have high virus recovery rate; iii) should concentrate large range of viruses; iv) be less costly; v) be capable of processing large volumes of water; and vi) be repeatable within a laboratory. For this reason, different methods have been used for the recovery of viruses from water in different studies. Based on the different properties of the viruses four main principal approaches are used for viral recovery and concentration. Each method has its own variations, advantages and disadvantages.

Concentration based on ionic charge: Due to the smaller size of viral particles, mechanical filtration is often not possible and adsorption-elution methods are then employed (Fong & Lipp, 2005). This method involves manipulation of charges on the virus surface, using pH changes to maximise their adsorption to charged filters (Fong & Lipp, 2005). Different types of filters and filtration methods, such as cartridge filters (electropositive and electronegative), glass fibre filters, glass wool filters, vortex flow filtration, tangential flow filtration and acid flow flocculation have been used for the recovery of viruses (Venter, 2004; Wyn-Jones, 2007). The glass-wool adsorption elution method has proved to be an efficient for the recovery of viruses from large volumes of water (Grabow *et al*., 1996, 2004; Lambertini *et al.*, 2008). The benefits of this method is the ease of use, there is no pre-treatment of the water sample required, and the cost of the equipment to be used is minimal which allow successful detection of enteric viruses from water sample in the field (EPA, 2000). Depending on the quality of water, the columns of glass have been proved to be suitable for the routine recovery of enteric viruses from large volumes of water at efficiencies of 30-50% (Grabow *et al*., 2004). In order to elute the virus from the trap, a relatively mild pH 9.5 beef extract-glycine buffer can be used (EPA, 2000). The recovered viruses are usually further concentrated and purified to reduce the final volume of samples to one or two millilitres for processing using secondary concentration procedures such as polyethylene glycol (PEG) precipitation, organic flocculation and centrifugal ultrafiltration (Venter, 2004; Fong & Lipp, 2005). In the PEG precipitation method, viral particles are precipitated by addition of 0.5 M sodium chloride (NaCl) and 7% PEG to beef extract with constant stirring which is followed by centrifugation. The virus pellet can then be resuspended in an appropriate buffer (Fong & Lipp, 2005, Wyn-Jones, 2007).

Concentration by entrapment: Ultrafiltration has been applied to concentrate multiple types of viruses in large volumes (~100 L) of water by physical retention based on virus and pore size, with the most commonly used filter being the hollow fibre ultrafilters (Wyn-Jones, 2007). Advantages of ultrafiltration include high recovery efficiencies and viruses are not exposed to pH extremes or other unfavourable conditions which may affect their viability (Wyn-Jones, 2007; Wu *et al.*, 2011). Although it is possible to adsorb viruses efficiently with this protocol, factors that limits the field application include the fact that the membranes clog rapidly which implies that the volumes of water that can be processed are restricted and equipment set-up is still not readily portable (Wyn-Jones, 2007; Wu *et al.*, 2011).

Concentration by ultracentrifugation: This method is commonly referred to as “catch-all” method concentrating sample based on the time and applied gravitational force (Wyn-Jones, 2007). This method however, is limited to small volumes of water. Different centrifugal separation methods such as continuous flow system are also commercially available (Wyn-Jones, 2007).

Concentration by immuno-affinity and other methods: Immuno-affinity methods involve incubation of magnetic beads that are coated with specific antibodies for a target organism, in a mixture of the sample suspension (Wyn-Jones, 2007). The efficiency of the reaction relies on the specificity and affinity of the commercially available monoclonal antibodies and on the turbidity of the water sample. The technique has been used to isolate a number of different organisms from water samples. Although the technique is simple and fast, the technique is only useful for a small volume of water, which makes it prohibitive for routine monitoring (Wyn-Jones, 2007; Mattison & Bidawid, 2009). The are several other methods that can also be used in the primary concentration of water samples such as hydro-extraction with hydroscopic solids, iron oxide flocculation, two-phase separation and freeze-drying (Wyn-Jones, 2007).

Viral recovery from food samples: The method of virus recovery and concentration from food samples is dependent on the food matrix and eluant. Several methods for the recovery of viruses from fresh produce by rinsing and secondary concentration have been described (Shan *et al.*, 2005; Butot *et al.*, 2007; Mattison & Bidawid, 2009; Bosch *et al.*, 2011). Centrifugal ultrafiltration concentration, based on a molecular weight cut-off is often used for secondary concentration and no manipulation of the sample is required (Fong & Lipp, 2005).

11.5.7 Viral detection

Once viruses have been recovered from the food matrix or water sample the viral nucleic acid is extracted and purified from the recovered concentrate. A number of commercial kits have proved to be reliable but differences have been noted depending on the virus or the matrix to be analysed (Bosch *et al.*, 2011). Automated nucleic acid extraction platforms, e.g. MagNA Pure LC instrument (Roche Diagnostics GmbH, Mannheim, Germany), have also successfully been used for the nucleic acid extraction of viruses recovered from water and food sources.

Viral isolation in cell culture is the “gold standard” for the detection and quantification of infectious enteric viruses from food and environmental samples (Mattison & Bidawid, 2009; Bosch *et al.*, 2011). However cell culture for detection of enteric viruses in water and food has numerous shortcomings (Fong & Lipp, 2005). Appropriate and susceptible cell culture systems are not available for all enteric viruses (Fong & Lipp, 2005) and in the case of NoV, efforts to isolate the virus in cell culture have been unsuccessful (Duizer *et al.*, 2004; Goyal, 2006), while HAV does not cause a CPE (Dotzauer, 2008). Concentrates and extracts from food and water samples may also be cytotoxic to the cell cultures (Goyal, 2006). Viral culture is also costly, labour intensive and time consuming, taking several days or weeks to be conclusive which makes it unsuitable for routine analysis of enteric viruses from environmental samples (Fong & Lipp, 2005; Rutjes *et al.*, 2005; Goyal, 2006). Cell cultures are however used to in conjunction with molecular methods, e.g. integrated cell culture-PCR/RT-PCR assays, for the selective detection of infectious viruses. In these assays the target nucleic acid is increased with a consequent increase in sensitivity of the immunological or molecular detection method (Goyal, 2006; Bosch *et al.*, 2011).

Molecular techniques such as reverse transcriptase-polymerase chain reaction (RT-PCR) offers the best alternative sensitive and specific methods for the detection of enteric viruses from environmental samples, but these assays are affected by inhibitory substances co-extracted with the viral nucleic acid (Butot *et al.*, 2007). Currently, PCR and RT-PCR is considered to be the gold standard for virus detection (Bosch *et al.*, 2008). Further improvements and advancements in technology lead to the development of real-time RT-PCR assays, which some authors claim it to be the new gold standard for the quantification of enteric viruses (El-Senousy *et al.*, 2007). Real-time RT-PCR has the advantage over conventional RT-PCR in that it is less time consuming, more sensitive and human error is reduced in that the post-amplification interpretation of results is automated. In addition contamination is reduced since post-amplification handling of samples is avoided (Kleiboeker, 2004; Manojkumar *et al.*, 2006). Probes used in a real-time RT-PCR assay are highly specific and can be labelled with different coloured fluorophores, allowing for the presence and detection of more than one probe in a single reaction. This has facilitated the detection of several pathogens simultaneously in a multiplex real-time RT-PCR reaction. Although multiplex real-time RT-PCR assays have a reduced sensitivity compared to monoplex real-time RT-PCRs they are still however considered efficient enough to detect targeted pathogens and was recommended for the use for routine surveillance of clinical specimens and food samples for viruses (Jokela *et al.*, 2005; Kou *et al.*, 2008).

11.5.8 Quality control

As enteric viruses are present in low numbers in food and water samples effective quality assurance and quality control procedures are required to exclude false positive or false negative results (Pintó & Bosch, 2008; Lauri & Mariani, 2009; Bosch *et al.*, 2011). False negative results may result from poor virus recovery, inefficient nucleic acid extraction and inhibitory substances in the water or on the food matrix, while false positive results could be due to cross-contamination. It is therefore important to monitor the efficiency or accuracy of the critical steps in the recovery and detection assays for the generation of reliable and comparable results which are acceptable to the regulatory authorities (Sánchez *et al.*, 2007; Bosch *et al.*, 2011). Due to some technical difficulties in the environmental virology, it is therefore important to ensure consistency of the efficiency of the optimised procedure. In order to ensure the quality of sample processing, it is therefore important to include sample processing controls (Pintó & Bosch, 2008; Mattison & Bidawid, 2009; Pintó *et al.*, 2009). The processing control should have similar features to target virus, it must not be associated or naturally present in the water or food sample. The control must be added to the sample prior to sample processing, as this should be co-extracted and co-concentrated with the target virus and detected from the same extract (Mattison & Bidawid, 2009). Feline calicivirus (Mattison *et al.*, 2009) and mengovirus (Pintó *et al.*, 2009) have been proposed as candidate process controls for controlling the whole process of sample analysis. The *rt* PCR/RT-PCR is affected by the presence of inhibitory compounds which are co-extracted during the nucleic acid isolation procedure (Sánchez *et al.*, 2007). The *rt* PCR/RT-PCR should therefore also include an internal control (IC), which will distinguish a truly negative result from false negative results due to the presence of PCR/RT-PCR inhibitors (Rutjes *et al.*, 2005). The following controls should also be included in each of the individual real-time RT-PCR runs: i) a negative

RNA extraction control to monitor for cross-contamination; ii) a negative RT-PCR control, usually sterile distilled water, to control for contamination in the real-time RT-PCR reagents, and iii) a positive real-time RT-PCR control to control for the quality of the real-time RT-PCR reagents (Atmar, 2006; Bosch *et al.*, 2011).

12. SOURCES OF CONTAMINATION

Contamination of fruits and vegetables can be divided into pre-harvest and post-harvest contamination (Beuchat and Ryu, 1997; Beuchat, 2002, Berger *et al.*, 2010). Potential pre-harvest sources of microorganisms include soil, faeces, irrigation water, water used to apply fungicides and insecticides, dust, aerosols, insects, inadequately composted manure and other biosolids, wild and domestic animals, fomites, and human handling (Beuchat, 2006; Stopforth *et al.*, 2010). Even seeds are a potential source of pre-harvest contamination as demonstrated recently by an outbreak of severe and lethal STEC (*E. coli* O104:H4) infections due to the consumption of contaminated seeds and sprouts produced thereof in Europe (BfR, 2011; EFSA, 2011). Post-harvest sources include faeces, human handling, harvesting equipment, transport containers, wild and domestic animals, insects, dusts, rinse water, ice, transport vehicles and processing equipment (Beuchat & Ryu, 1997; Beuchat, 2002; Matthews, 2006; Erickson, 2010).

In recent times the call of the public has been to produce minimally processed foods (MPFs) that have been produced without harmful or chemical-loaded pesticides and fertilisers and the alternative fertiliser of choice has become manure, an approach implemented by many “organic” farmers. Ironically it is only for conventional fertilisers that the microbiological safety can be assured and the use of them is, in fact, a much less risky one than with manure. Interestingly, the low prevalence rate of *E. coli* in fresh produce recently analysed in Germany was considered to be due to some degree to the fact that vegetables are mostly fertilized with synthetic fertilizers and not manure (Schwaiger *et al.*, 2011). Manure is likely to be loaded with bacteria present in human and animal faeces and the risk of contamination runs high (Berger *et al.*, 2010; Byappanahalli & Ishii, 2011). Biosolids, or sewage sludge as it is traditionally referred to, are what remains after the water portion of sewage has been removed for treatment (Bihn and Gravani, 2006). This has been used as a fertiliser or added to nutritious slurries for crops. However, this has been recognised to be a substance that is potentially heavily loaded with pathogens and has therefore been outlawed by the British Retail Consortium (BRC) (Coetzer, 2006) and, ideally, should not be used without appropriate sanitization (Arthurson, 2008). A study of soil and domestic animal faeces in the USA indicated that *Listeria* sp. is more often present in fields fertilised with manure during July to September than other months (MacGowan *et al.*, 1994; Beuchat & Ryu, 1997). Wild birds and animals can also be sources responsible for the distribution of *L. monocytogenes* to fruits and vegetables because 23% of samples collected from wild life feeding grounds were positive for *L. monocytogenes* (Weiss & Seeliger, 1975).

While animal faeces is a problematic contaminant that is very difficult to control, contamination of rivers and water systems by human faeces is an enormous problem that is on the increase as most South African farmers rely on local river systems to provide them with water for irrigation and they simply pump this water directly from the river onto the crops (Barnes, 2003). In recent years an increase in urbanisation has led to fast-growing informal settlements that have not been equipped with appropriate sanitation facilities. Many of these settlements are situated close to natural water resources such as lakes or rivers and thus this water becomes the obvious dumping ground of the generated waste. Until these communities are provided with appropriate sanitation facilities of sufficient capacity to prevent river contamination and are trained to use them properly, little change for the better can be expected and these communities have no option but to continue dumping their waste into nearby river systems. While informal settlements are definitely to be blamed for some of the pollution and dumping, they are, by far not the only guilty party. It was reported by Barnes (2003) that some wineries in the vicinity just downstream of Kayamandi (Western Cape, South Africa) were dumping cellar and production effluents into the rivers. According to Barnes (2003), this could also include farm sewage waste and the combination of these depositions can result in increased fermentation and nutrient availability in the rivers which alters the acidity and survival conditions of the water, thereby allowing organisms which would not normally be able to survive in river water to grow and multiply. Where the problem really comes in is with the way that this situation affects the environment which is in turn affecting those who rely on the quality and safety of environmental resources for their livelihood. Due to no alternative suitable resource, farmers are forced to continue using river water for irrigation. Already in 2003 Barnes stated that pollution was reaching a climax and concerns were being voiced that should the pollution levels continue rising, an increase in the occurrence of food-related outbreaks could be expected.

In many parts of South Africa river water is used mainly without any treatment (DWAF, 1996b) and some of the water is faecally polluted. In addition, river water receives the majority of the nations treated sewage, consequently irrigation water may be contaminated to a greater or lesser extent with sewage, and microorganisms causing diseases may find their way onto fresh fruits and vegetables (WHO, 2002). Some of the pollution in the rivers that is not as a direct runoff from rural settlements is industrial waste that is released directly into the rivers or public water systems. This wastewater is known for its concentrations of heavy metals but also for its high microbial load (Barnes & Taylor, 2004). In many cases, this waste has also been found to contain untreated sewage in combination with the other waste probably in order to save money on water treatment costs. In wastewater from a factory producing baker's yeast (generally considered to be a harmless food product) no less than 53 different microbial species were found with *Acinetobacter*, *Clostridium*, *Staphylococcus* and *E. coli* O157:H7 present amongst others. While some of the species found were yeasts being produced in the factory, others were pathogenic, toxic and even deadly (Van Der Merwe & Britz, 1994).

Microbial pollution in rivers is starting to have a serious impact on the health of humans and poses a great potential threat to their safety, especially in view of the more susceptible members of the population such as infants and immune compromised citizens for whom a microbiologically safe diet has been recommended to avoid infections (Lund & O'Brien, 2011). While most studies seem to treat waterborne bacteria as neutral inclusions, it has been found that they are generally associated more with sediment and it is during times of rapid water flow or storms that the sediment becomes churned up and the bacteria are re-suspended in the water. However, according to Ouattara *et al.* (2011), the *E. coli* concentration established for sediments of rivers of the Scheldt watershed (range $2.1 \times 10^2 - 3.3 \times 10^5$ cfu.g^{-1}) is only having a limited impact on the *E. coli* burden of the corresponding water column (range $1.4 \times 10^3 - 4.6 \times 10^5$ cfu.100 mL^{-1}) via re-suspension. Bacteria have the ability to adhere strongly to both abiotic and biotic surfaces and for this reason are usually found in clusters attached to a piece of sediment, rather than as individual cells. This adherence is so strong that it is thought by some to be irreversible (Jamieson *et al.*, 2005).

When considering the current downward trend in water quality in South Africa, it is clear that the situation will never improve without a control body or regulatory agency being instated to control the quality and quantity of non-point-source polluted water that is released into natural water systems which are to be drawn from for irrigation (Dinar & Xepapadeas, 1998).

While fruits and vegetables are in the field, water is suspected to be a major threat for product contamination (Krtinić *et al.*, 2010; Berger *et al.*, 2010). The produce is exposed to water during both irrigation and application of pesticides and the water used for these purposes can be drawn from rivers, streams, open ditches or canals, lakes or ponds, or reservoirs. Alternatively municipal water can be used but the quality of this water should not be relied on since in many cases shortcuts are taken during treatment processes and for different reasons the results are not questioned and the water is allowed to be used (Johnston *et al.*, 2006).

Minimally processed foods (MPFs) as well as products consumed raw are naturally colonised by a variety of saprophytic, mostly Gram-negative bacteria, even when they are produced under ideal conditions. However, the presence of pathogens of human origin has started being detected on them in routine testing which indicates the beginning of a large problem (Legnani & Leoni, 2004; Beuchat, 2006; Erickson, 2010).

A source of contamination that is often forgotten but is important is the land itself (Coetzer, 2006). In some cases, farms have been acquired without knowledge of its previous purpose and if it was used for animals, or was even loaded heavily with manure, then the reservoir that has built up in the soil can potentially contaminate the produce. In the case of farms positioned near rivers, the land use upstream is also important for the safety of the plot. For example, during times of flooding, contaminants that are carried by the river from various sources upstream can be washed onto land that the river does not usually reach and result in unexpected and irreversible contamination. It is ideal that land should have previously also been used for the production of produce for human consumption. Contaminated surrounding plots can cause problems if they are sloped towards the crop as the runoff from rainwater can result in pathogens being washed from the one field into the other (Coetzer, 2006).

Contamination of the produce can also take place post-harvest and vehicles of transmission include harvesting equipment, packing house, unhygienic workers, processing plants and pests (in the field or post-harvest) (Matthews, 2006; Erickson, 2010). In the packing house, transmission of pathogens through practices such as washing can occur if the water is not properly disinfected, filtered or replaced on a regular basis. Guidelines recommend that potable water be used for post-harvest washing (Coetzer, 2006). It is important that producers acknowledge the role that the origin and also the treatment of the irrigation water can play in the final safety of the end product (Legnani & Leoni, 2004).

The introduction of pathogens into warehouses, cold-rooms or other storage facilities is a dangerous occurrence since this can simply lead to further post-harvest cross-contamination. There are many machinery components as well as small crevices in which these organisms can be harboured and cannot easily be gotten rid of. Especially the ability of potential pathogens to form biofilms that are less susceptible to biocidal activity and disinfection measures is a reason for concern (Kumar & Anand, 1998). Contaminated machinery is also a serious vehicle of transmission and it is thus of the utmost importance that such circumstances prevented in the first case, rather than being left with the problem to solve (Korsten & Zagory, 2006). It is too risky to the safety of the produce to introduce contaminants onto a MPF so late in its growth cycle and so close to sale and consumption (Francis *et al.*, 1999).

13. IRRIGATION WATER AND PATHOGEN TRANSFER

Though direct evidence of foodborne illness due to contamination of edible horticultural commodities during commercial production is limited, compelling epidemiological evidence involving these crops has implicated specific production practices (Lynch *et al.*, 2009). Already in 1987 in a survey done by Garcia *et al.* (1987), out of 181 irrigation samples and 859 vegetables irrigated with the same water source in Spain, *Salmonella* Typhimurium*; Salmonella* Kapemba*; Salmonella* London and *Salmonella* Blockley were the isolated serotypes. Similarly, contaminated irrigation water was the suspected source of pathogens in the case of *Salmonella* infections due to the use of mung bean sprouts (Mohle-Boetani *et al.*, 2009), tomatoes (Greene *et al.*, 2008) as well as an enterotoxigenic *E. coli* outbreak due to the use of fresh basil (Pakalniskiene *et al.*, 2009).

The use of animal waste or manure; faecal contaminated agricultural water for irrigation or pesticide/ crop management application; and farm labour personal hygiene, leads to direct contamination (Bracket, 1999; Beuchat, 2006). Brackett (1999) also suggested that only clean, potable water should be used for irrigation of fruits and vegetables after planting. However, this approach fails to take into account many aspects of water availability, water conservation programs, irrigation method, geographic diversity, crop diversity, temporal factors, and the significant difficulty inherent in water monitoring for microbial content during production (FDA, 2001).

The microbial quality of agricultural water is critical because poor quality water can introduce pathogens into produce during pre- and post-harvest (Table 10). Because of this problem, indirect or direct contamination of produce from water or water aerosols of persistent pathogens on harvested vegetables has been long recognized as a potential hazard (FDA/CFSAN, 2001; WHO, 2003).

Beuchat and Ryu (1997) clearly showed that irrigation and surface run-off water can be sources of pathogenic microorganisms that contaminate fruits and vegetables in the field. Irrigation water containing raw sewage or improperly treated agricultural water and effluents from sewage treatment plants may contain a host of viral and bacterial pathogens (Heaton & Jones, 2008). Steel *et al.* (2005) carried out a survey on 500 hundred irrigation water samples used for production of fruit and vegetables in Canada and found out about 25% of the samples to be contaminated with faecal *E. coli* and faecal streptococci. More recently, Tiefenthaler *et al.* (2011) reported that mean levels of *E. coli* and enterococci in storm water runoff from different land use sites in California (USA) were typically exceeding 1 000 MPN per 100 mL.

The transfer of foodborne pathogenic microorganisms from irrigation water to fruits and vegetables is dependent on the irrigation technique and on the nature of the produce (NACMCF, 1999). Spray irrigation could be expected to increase the risk of contamination in comparison to drip irrigation or flooding because leafy vegetables provide large contact surfaces for water and for the attachment of microorganisms (ECSCF, 2002; Doyle & Erickson, 2008). In addition, once present on the surface of plants, bacteria such as *Salmonella* spp. might enter the plant via stomata and thus become internalized as was demonstrated for lettuce (Kroupitski *et al.*, 2009). Consequently, there is a guideline in Florida, USA, that states that reclaimed water is not allowed for direct contact application methods (i.e. spray irrigation) used on crops that will not be peeled, skinned, cooked, or thermally processed (FDEP, 1999). It is recommended that indirect contact methods, ridge and furrow, drip, or subsurface irrigation, for products such as salad crops are used. In the US spray irrigation resulted in a greater number of lettuce plants' testing positive for *E. coli* O157:H7 at harvest following a single exposure to the pathogen. Similarly in Nigeria lettuce and carrots were positive for *Salmonella* spp., *Vibrio* spp. or *E. coli* following irrigation with water that tested positive with the same pathogens (Matthews, 2006).

Zhou *et al.* (2005) investigated strawberries for the presence of *E. coli,* irrigated either by trickle irrigation with well water, trickle irrigation with surface water and overhead irrigation with surface water. They found that *E. coli* was present in approximately 50% of the surface water samples at levels of 0.07-2.45 log cfu per 100 mL, but *E. coli* was not found on fruit treated with the surface water. *E. coli*, 1.17-2.64 log cfu/g, was found on 3 out of 5 samples of the fruit that had been irrigated with well or municipal water. Irrigation water met the Canadian Water Quality Guideline maximum of 100 *E. coli* per 100 mL.

Table 10. Sources of pathogenic microorganisms from fresh fruit and vegetables (Beuchat, 1996, 2006).

Pre-harvest	**Post-harvest**
Faeces Soil Irrigation water Water used to apply fungicides, insecticides Green or inadequately composted manure Air (dust, aerosols) Wild and domestic animals Poultry and reptiles) Insects Human handling	Faeces Human handling (workers, consumers) Harvesting equipment Transport containers (field to packing shed) Wild and domestic animals (including fowl and reptiles) Insects Air (dust) Wash and rinse water Sorting, packing, cutting, further processing equipment Ice Transport vehicles Improper storage (temperature, physical environment) Improper packaging (new packaging technologies) Cross-contamination (foods in storage, preparation, display areas) Improper display temperature Improper handling after wholesale or retail purchase

Contaminated irrigation water, surface run-off waters and the use of sewage as a fertilizer can also be sources of pathogenic microorganisms that contaminate fruits and vegetables in the field (Beuchat & Ryu 1997; Ibenyassine *et al.,* 2006; Lynch *et al.*, 2009). MacGowan *et al.* (1994) found 84-100% of sewage samples to be contaminated with *L. monocytogenes* or *L. innocua* during a two year sampling period. *Salmonella* was isolated from more than 50% of irrigation water samples contaminated with raw sewage or primary treated chlorinated effluents (Wang *et al.*, 1996).

Pre-harvest sources may also contribute to post-harvest contamination of vegetables (Beuchat & Ryu, 1997). Johnston *et al.* (2005) carried out a survey on the microbiological quality of fresh produce and concluded that every step from production to consumption may predispose produce to microbial contamination and each of these steps need to be included in a food safety programme to ensure safety. For instance workers handling vegetables from the time of harvest through to packaging and processing, even in the home might act as sources of transmission of pathogens (Beuchat & Ryu, 1997).

Different researchers have evaluated the presence or persistence of pathogens conveyed to crops by spray irrigation, irrigation aerosols of sewage effluent (Teltsch & Katznelson, 1978; Garcia *et al.,* 1987) or drip irrigation (Sadovski *et al.*, 1978). It was found that detection varied and depended upon the level and nature of environmental stress. Detection was correlated to population densities of target pathogens in the source water and spatial orientation relative to the point source. The level of organic matter in the water affected survival of pathogens.

Polluted irrigation water and contaminated manure have been implicated in the outbreaks of enterohemorrhagic *E. coli* O157:H7 infections. The infections were associated with lettuce and other leaf crops such as spinach or parsley and they are occurring with increasing frequency (Islam *et al.*, 2004a; Berger *et al.*, 2010). Similarly, outbreaks caused by serovars of *Salmonella enterica* related to the consumption of fresh produce such as tomatoes (Greene *et al.*, 2008), rocket leaves (Nygǻrd *et al.*, 2008), or jalapeno pepper (Behravesh *et al.*, 2011) are probably due to the pre- or post-harvest use of microbiologically contaminated water. In a recent study, Erickson *et al.* (2010) detected GFP labelled *E. coli* O157:H7 isolates at levels exceeding 2 log cfu per g even 14 days after lettuce and spinach leaves had been contaminated using a spray mist approach. Interestingly, in this study internalization of *E. coli* O157:H7 took only place at levels of 6.4 log cfu applied per lettuce leave but not at 4.4 log cfu per lettuce leave. Based on recent experimental studies using *E. coli* spiked water for spray irrigation of spinach, Wood *et al.* (2010) concluded that a 6 day period between the last irrigation and harvest can reduce the risk of *E. coli* survival on spinach. It has been found that *Salmonella* became undetectable on effluent-irrigated lettuce five days after irrigation was terminated while *E. coli* indicator strains persisted (Vazda *et al.*, 1991). However, Islam *et al.* (2003, 2004b, c) showed that *Salmonella* Typhimurium applied to carrots or parsley and lettuce via contaminated water can be detected on the plant for several months.

14. INFECTIOUS DOSES OF THE MAIN WATER, SOIL AND PRODUCE PATHOGENS

The infective dose of bacterial pathogens can be grouped into one of three categories namely high, medium and low. In Table 11 a summary is given of some the main pathogens that can be found in water along with the respective infective dose category.

In recent times however, analyses of river water samples have revealed indications of disturbingly high levels of pathogens in these rivers. Results such as these are what have brought about such concern regarding the carryover of these pathogens to MPFs through the use of it for irrigation. Levels of *E. coli as* high have 10 800 000 cfu.100 mL^{-1} in the Plankenburg River, 11 190 cfu.100 mL^{-1} in the Berg River, and 61 310 cfu.100 mL^{-1} in Franschhoek River (Britz, 2005). All of these figures exceed infectious doses and accidental ingestion of this water, even if it is diluted, could lead to serious infections among the population. Some viruses only need one organism to cause an infection and bacteria that are to infect intestinally can need as few as 10 colony forming units, but often up to 1 000 or more are needed as an infectious dose (the amount needed to cause infection in 50 per cent of individuals) (Velusamy *et al*., 2010).

While some microbes infect the host immediately, others infect on a cumulative basis and thus the infection only shows after a long period of time (Legnani & Leoni, 2004). Pathogens can be transported by healthy carriers and thus infections are spread when the host comes into contact with these carriers. These carriers can include contaminated seafood or water, inhalation of aerosols that are contaminated or consumption of raw food products that were irrigated with contaminated water.

While in most cases the numbers that are ingested at one time are too low to cause concern (Legnani & Leoni, 2004), the suspected high levels of contamination in rivers that pollution is dumped into – both by industries and informal settlements – and could pose a health problem (Matthews, 2006). Another problem with pathogens is that they have the ability to reproduce both on the product itself and in the host. This makes pathogens more difficult to deal with since it becomes difficult to predict potential load on a product but it is also very difficult to predict the degree of multiplication inside the host, should the host become infected. The risk of infection by a pathogen can also not be determined simply by quantifying its presence, because this also depends on the invasiveness of each particular pathogen, as well as the strength of the immune system of each individual (Matthews, 2006).

Table 11. Infective dose of major pathogens responsible for foodborne illness (Velusamy *et al*., 2010).

Bacteria	**Infective dose** (no of organisms)
Campylobacter jejuni	400-500
Escherichia coli	<10
Salmonella spp.	15-20
Shigella spp.	<10
Vibrio cholerae	<100
Listeria monocytogenes	<1 000
Bacillus cerues	<1 000 000 per gram
Yersinia enterocolitica	unknown
Clostridium botulinum	< nano grams
Clostridium perfringens	>10 000 000

While a single MPF or those eaten in small amounts would require a high load of certain pathogens to cause infection, most products carry loads low enough that they are below the infectious dose and therefore do not result in infection. However, the global increase in consumption of MPFs along with a concurrent increase in contamination being found has led to a greater total number of infectious organisms being ingested and thus there are more cases occurring where the infectious dose is being consumed (Matthews, 2006).

The nature of waterborne pathogens is that they form large clusters together around pieces of sediment (Flint, 1987), making the determination of their precise amounts in a water sample almost impossible. Ingestion of one of these clusters also poses a much higher risk of infection since the number of colonies in the cluster is very likely to exceed the infectious dose of the pathogen (Jamieson *et al.*, 2005).

The infectious doses of pathogens are lowered in poorer or developing countries such as South Africa where a large percentage of the exposed population is immunocompromised due to malnourishment, youth, old age, diseased or suffering from HIV/AIDS. This factor further increases the importance of minimising the contamination loads on MPFs as it placed a large portion of the population in a position where it has a much higher risk of infection. In contrast to bacteria, the infectious dose of enteric viruses is low, e.g. <10 viral particles for NoV (Teunis *et al.*, 2008). This, together with the stability of enteric viruses in the environment, facilitates their epidemic potential.

15. REMOVAL OF PATHOGENS FROM FRESH FRUIT AND VEGETABLES

Survival and multiplication of pathogens on and in vegetables is determined by the pathogen, the vegetable, environmental conditions and storage conditions (Harris *et al.,* 2003). Survival and growth of food-borne pathogens on vegetables are possible once the protective epidermal barriers are broken as a result of punctures, bruising or by degradation by plant pathogens (Jay, 1992, 1997). Various microbes like *C. botulinum, L. monocytogenes* and *Y. enterocolitica* have been shown to be able to multiply on the surface of cut melons, shredded lettuce, chopped parsley and chopped tomatoes (Harris *et al.,* 2003). According to Harris *et al.* (2003), it will be impossible for pathogens to grow on the uninjured outer surface of vegetables due to the protective character of the plant's natural barriers but *E. coli* O157:H7 has been reported to grow on the uninjured outer surface of watermelon and cantaloupe rinds. Once there is an injury on the outer surface of the vegetable or fruit, food-borne pathogens, like *E. coli, Salmonella, Listeria* from pre-harvest or post-harvest sources, are likely gain entrance and multiply within the cells of the fruit and vegetables (Harris *et al.*, 2003)

Most processors and consumers have assumed that washing and sanitizing fresh fruits and vegetables will reduce the microbial load. However, published efficacy data indicate that these methods cannot reduce microbial populations on produce by more than 90-99% (Gerald & Perkin, 2003), while such population reductions are useful and not to be minimized, they are insufficient to assure microbiological safety. Conventional washing technology was developed primarily to remove soil from produce, not microorganisms, even with newer sanitizing agents such as chlorine dioxide; ozone, and peroxyacetic acid, improvements in efficacy have been with shortcomings such as the inability of chlorine dioxide to reduce the population of *E. coli* O157:H7 on inoculated apples. Alternatives to chlorine were limited in their ability to kill bacteria when realistic inoculation and treatment conditions were used (Sapers, 2001). Because of these limitations, it is preferable, wherever possible, to avoid contamination of fruits and vegetables by following good agricultural and manufacturing practices rather than depend on decontamination (Sapers, 2001).

Factors that limit the efficacy of washing are; contamination conditions, interval between contamination, attachment in inaccessible sites, biofilms and internalization of bacteria within produce. According to Sapers (2001), *Salmonella* sp. survived washing to a greater extent when attached to cut surfaces of apple and green pepper than on unbroken external surface. Fresh produce like apples, pears, cherries, grapes, potatoes, carrots and lettuce were reported to often have punctures, cuts or splits providing space for attachment and internalization of food-borne pathogens (Sapers, 2001). *E. coli* was also reported to grow in wounds on apples and it was difficult to kill after it was established within the wounds and punctures.

According to Barmore (1995), there is no effective substitute for chlorine for washing fruits and vegetables. It is routinely used as a sanitizer in wash, spray and flume waters used in the fresh fruit and vegetable industry (Beuchat & Ryu, 1997; Beuchat, 1998; Hagenmaier & Baker, 1998; Seymour & Appelton, 2001). Antimicrobial activity depends on the amount of free available chlorine in water that comes in contact with microbial cells. Mazollier (1988) studied the effect of chlorine concentration on aerobic microorganisms and faecal coliforms on leafy salad greens. Total counts were markedly reduced with increased concentrations of chlorine up to 50 ppm, but a further increase in concentration up to 200 ppm did not have a substantial additional effect.

The effectiveness of treatment with water containing up to 200 ug.mL^{-1} chlorine in reducing numbers of naturally occurring microorganisms and pathogenic bacteria is minimal often not exceeding 2 logs on lettuce (Adams *et al.*, 1989; Beuchat & Brackett, 1990; Beuchat *et al.*, 1998; Beuchat, 1999; Weissinger *et al.*, 2000) and tomatoes (Beuchat *et al.*, 1998; Weissinger *et al.*, 2000). Several other researchers have emphasized that chlorine cannot be relied on to eliminate pathogenic microorganisms such as *L. monocytogenes* (Nguyen-the & Carlin, 1994; Beuchat & Ryu, 1997; Hagenmaier & Baker, 1998). Another problem of using chlorine is its residual by-products that may be generated, such trihalomethanes, in the waste water (Simpson *et al.,* 2000).

The hydrophobic cutin, diverse surface morphologies and abrasions in the epidermis of fruits and vegetables limit the efficacy of sanitizers (Burnett & Beuchat, 2001). The inaccessibility of chlorine to the microbial cells in the crevices, pockets and natural openings in the skin of the fruits and vegetables contributes to the overall lack of effectiveness of chlorine in killing pathogens (Lund, 1983).

Use of electrolyzed water as a sanitizing agent is a type of chlorination. Electrolysis of water containing a small amount of sodium chloride generates a highly acidic hypochlorous acid solution containing 10-100 ppm available chlorine and it was effective in reducing pathogens in apple and lettuce leaves (Sapers, 2001), but reaction of chlorine with organic residues can result in the formation of potentially mutagenic or carcinogenic reaction products (Hidaka *et al.*, 1992). This is a cause for concern since some restrictions in the use of chlorine might eventually be implemented by regulatory agencies. A number of alternatives to chlorine such as chlorine dioxide, ozone, and peroxyacetic acid have been examined (Sapers, 2001).

A potential replacement for chlorine as a sanitizer is ozone (Graham *et al.*, 2004). In 2001, the FDA approved the use of gaseous and aqueous ozone for application as an antimicrobial agent for foods (FDA, 2001). Garcia *et al.* (2003) determined the effectiveness of using ozone and in combination with chlorine as a sanitizer in the treatment of minimally processed lettuce. They found that lettuce treated with chlorine, ozone or a combination had a shelf life of 16, 20, or 25 days respectively, indicating that chlorine-ozone combinations may have beneficial effects on shelf life and quality of lettuce salads as well as on the water used for rinsing or cleaning the lettuce. However, ozone treatment was ineffective in reducing decay of pears and food-borne pathogens (Sapers, 2001).

Application of UV irradiation to river water for irrigation of celery was effective in reducing total coliforms and non-pathogenic *Escherichia coli* but had no effect on food-borne pathogens like *Salmonella* and *Listeria* (Robinson & Adams, 1978). Other sanitizing agents that have been used for produce are peroxyacetic acid (which was recommended for treatment of process water), hydrogen peroxide which is Generally Recognised as Safe (GRAS) for some food applications but it has not yet been approved as anti-microbial wash for produce (Sapers, 2001). Novel sanitising applications include vacuum infiltration and vapour-phase treatments and surface pasteurization (Sapers, 2001).

16. TREATMENT TECHNOLOGIES FOR IRRIGATION WATER TREATMENT

16.1 Background

Several risks have been identified when polluted water is used for crop irrigation. Risks can be short-term and range in seriousness, depending on the potential contact with humans, animals and the environment (e.g. microbial pathogens). Long-term impacts could arise from continued use of polluted water (e.g. chemical effects on soil) (Toze, 2006). Based on the evidence of microbial pollution in rivers used for irrigation in South Africa and the potential risk of causing disease in consumers, the pollution either needs to be prevented at source or treated at the point of use. Of primary concern during such treatment, is the reduction of pathogens in the irrigation water intended for use on food crops (WHO, 2006).

Reducing the health risk of using polluted/contaminated irrigation water can be achieved by implementing the health protection measures as described by the *WHO Guidelines for the Safe Use of Wastewater, Excreta and Greywater, Volume II, Wastewater Use in Agriculture* (WHO, 2006) or combinations of these measures: crop restriction; wastewater application technique (i.e. irrigation mode);

pathogen die-off between last irrigation and consumption; food preparation measures; human exposure control; and water treatment.

In the WHO guidelines, a pathogen reduction of 6-7 log units is used as the performance target for unrestricted irrigation to achieve the tolerable additional disease burden of ≤ 10^{-6} DALY per person per year. In these guidelines restricted irrigation is defined as the use of water to grow crops that are not eaten raw by humans, while unrestricted irrigation is defined as the use of clean water/treated wastewater to grow crops that are normally eaten raw (WHO, 2006). For labour intensive restricted irrigation, a pathogen reduction of 4 log units is used as the performance target for unrestricted irrigation to achieve the tolerable additional disease burden of ≤10^{-6} DALY per person per year. For highly mechanized agriculture, wastewater treatment to 10^{5}-10^{6} *E.coli* per 100 mL is required, i.e. a pathogen reduction of 3 log units (WHO, 2006).

"Disability adjusted life years" (DALYs) – are a measure of the health of a population or burden of disease due to a specific disease or risk factor. DALYs attempt to measure the time lost because of disability or death from a disease compared with a long life free of disability in the absence of the disease. DALYs are calculated by adding the years of life lost to premature death to the years lived with a disability. Years of life lost are calculated from age-specific mortality rates and the standard life expectancies of a given population. Years lived with a disability are calculated from the number of cases multiplied by the average duration of the disease and a severity factor ranging from 1 (death) to 0 (perfect health) based on the disease (e.g. watery diarrhoea has a severity factor ranging from 0.09 to 0.12, depending on age group). DALYs are an important tool for comparing health outcomes, because they account not only for acute health effects but also for delayed and chronic effects – including morbidity and mortality. When risk is described in DALYs, different health outcomes (e.g. cancer vs. giardiasis) can be compared and risk management decisions can be prioritized" (WHO, 2006).

The definition of DALY's can be clarified by means of an example: for carcinogenic chemicals in drinking-water, for instance, the WHO guideline values have been set at a 10^{-5} upper-bound excess risk (WHO, 2004). This means that there would be a maximum of one excess case of cancer per 100 000 of the population ingesting drinking-water that contained the chemical at the guideline concentration over a lifetime. The disease burden associated with this level of risk and adjusted for the severity of the illness is approximately 1 x 10^{-6} DALY (1 µDALY) per person per year (WHO, 2004). This level of disease burden can be compared with a mild but more frequent illness, such as self-limiting diarrhoea caused by a microbial pathogen. The estimated disease burden associated with mild diarrhoea (e.g. with a case fatality rate of approximately 1 x 10^{-5}) at an annual disease risk of 1 in 1000 (10^{-3}) (1 in 10 lifetime risk) is also about 1 x 10^{-6} DALY (1 µDALY) per person per year (WHO, 2004). Although the first five health protection measures are preventative actions rather than treatment *per se*, a brief overview of these measures will be given.

16.2 Crop restriction

Crop restriction implies growing plants that are not eaten directly by humans or are always processed or cooked prior to consumption (Carr *et al.*, 2004). Crop restriction can be used to grow 1) non-food crops ; 2) food crops that are processed before consumption (wheat); and 3) food crops that have to be cooked (potatoes, rice). These crops may, however, still be very profitable (WHO, 2006). As mentioned previously, the health-based target of $\leq 10^{-6}$ DALY per fieldworker per year can usually be met with a 2 or 3 log unit reduction (WHO, 2006).

Implementation of the crop restriction in existing irrigation areas may not always be that simple, as farmers might be resistant to change their current crops. In terms of health risks, the field workers are at the greatest risk, especially if spray or sprinkler irrigation is practiced. Nearby residents also need to be protected from aerosol drift, and thus buffer zones are often necessary. Consumers are at the lowest risk as these foods normally undergo further processing or cooking before consumption.

Another drawback of crop restriction is that of legal administration to monitor and control compliance. Paramount is that only water that meets the requirements for unrestricted irrigation is used (Shuval *et al.*, 1986) to irrigate food crops that are to be eaten uncooked. Restricted irrigation is feasible where (Mara and Cairncross, 1989): a law-abiding community and/or strong law enforcement exists; a public body controls allocation of the irrigation water and has the legal authority to enforce crop restrictions; there is adequate demand for the crops allowed under crop restriction, and where they produce a reasonable profit; and there is little market pressure in favour of excluded crops. (FAO, 1997; WHO, 2006)

For crop restriction to be successful, both the authorities and the farmers must be aware of the risk status of the river and the risks to consumers and fieldworkers.

16.3 Irrigation mode

The type of irrigation mode can have an effect on the health risks to farm workers, consumers and nearby residents. Spray and sprinkler irrigation carries with it the highest risk of spreading contamination through the produced aerosols. Choice of irrigation mode directly affects the risk, but also has certain practical and financial constraints. The effect of irrigation mode on health risks is summarised in Table 12.

16.4 Pathogen die-off before consumption

It has been shown that excreted pathogens, including bacteria, viruses, helminths and protozoa can survive for extended periods in soil and on food crops (Strauss, 1996; WHO, 2006) (Table 13). Survival times are dependent on a variety of environmental factors (humidity, soil content, temperature, pH, sunlight) as well as produce specific factors (foliage/plant type and competitive flora) (FAO, 1997). Pathogen reduction is more rapid in hot, dry weather (when approximately 2 log units per day can be achieved) compared to cool or wet weather (approximately 0.5 log units per day) (WHO, 2006), where low temperatures prolong the survival times. This has serious consequences for the consumer of crops which have been refrigerated after harvest.

Crops which are eaten raw such as salad crops, and especially root crops such as radish and onions or ones which grow close to the soil (lettuce, zucchini) pose the highest risk to the consumer. Certain crops are also more prone to contamination than others, such as onions (WHO, 2006), zucchini (Armon *et al.*, 2002) and lettuce (Solomon *et al.*, 2002). Pathogens are often protected from exposure to radiation or wash-off by rain by surface properties (e.g. hairs, stickiness, crevices and rough surfaces, etc.).Certain crops also retain more of the irrigation water than others. It has been estimated that lettuce retain 10.8 mL of irrigation water, whereas cucumbers only retain 0.36 mL. Stine *et al.* (2005) reported that lettuce and cantaloupe surfaces retained pathogens from irrigation water while smooth bell peppers did not. Helminth eggs can remain viable on crop surfaces for up to two months, although few survive beyond approximately 30 days (Strauss, 1996).

16.5 Food preparation measures

Effective education of local food handlers, people preparing food for consumption (homes, restaurants, food kiosks, etc.) and food processors and packers is essential in creating awareness of the need and importance of food preparation measures. The importance and methods of effectively washing produce with water or disinfectant and/or detergent should be emphasized in such programmes. These measures can be effective if properly applied (WHO, 2006):

- vigorous washing of rough-surfaced salad crops (e.g. lettuce, parsley) – 1 log unit reduction;
- washing of smooth-surfaced salad crops (e.g. cucumbers, tomatoes) – 2 log unit reduction;
- washing in disinfectant solution and rinsing with tap water – 1-2 log unit reduction;
- peeling fruits and vegetables – 2 log unit reduction;
- cooking vegetables – essentially complete reduction (5-6 log units).

People preparing food crops for consumption are best protected by exposure control techniques such as rigorous personal and domestic hygiene, frequent hand washing with soap, the use of separate areas for food preparation and subsequent handling of washed, disinfected and cooked food (Carr *et al.,* 2004; WHO, 2006).

16.6 Human exposure control

Agricultural fieldworkers are at the highest risk of being infected. These risks can be minimized, or even eliminated, by the use of less-contaminating irrigation methods and the use of protective clothing, especially gloves and shoes (in extreme case face-masks). Maintenance of strict personal and domestic hygiene standards and possibly immunizations can also reduce the health risks associated with contaminated irrigation water (WHO, 2006). Fieldworkers should have easy access to proper sanitation facilities, adequate and safe water for drinking and hygienic purposes. Pack sheds and markets should also provide safe water for washing and “freshening” of produce (Carr *et al.*, 2004).

16.7 Water treatment

Most treatment technologies available for the purification of irrigation water, are processes originally designed for treatment of secondary wastewater from wastewater treatment plants (WWTP). These technologies can, however, also be applied to reduce the microbial load of water intended for irrigation, which is the main aim of any health protection measure, including water treatment. Ranges of pathogen removal by various processes is summarized in Table 14 (WHO, 2006). Treatment processes can be classified into low-rate biological systems and high-rate processes (WHO, 2006).

Table 12. Effect of irrigation mode on risks associated with use of polluted irrigation water (WHO, 2006).

Irrigation mode	Factors affecting choice	Precautions for heavily polluted water
Flood	Lowest cost Exact levelling not required	Thorough protection for fieldworkers, crop handlers and consumers
Furrow	Low cost Levelling may be needed	Protection for fieldworkers, possibly for crop handlers and consumers
Spray and sprinkler	Medium water use efficiency Levelling not required	Some crops, especially tree fruits, are prone to more contamination Minimum 50-100 m from houses and roads
	Advanced sprinklers that reduce crop contamination and potential contamination have been developed that can reduce exposure to pathogens by 1 log unit	New technologies reduce spray drift and may be able to reduce crop contamination by better targeting
Subsurface and localized (drip, trickle and bubbler)	High cost High water use efficiency Higher yields Potential for significant reduction of crop contamination Localized and subsurface irrigation can substantially reduce exposure to pathogens by 2-6 log units	Localized irrigation: selection of non-clogging emitters; filtration to prevent clogging of emitters

Table 13. Survival of various organisms on crops and in soil at 20-30°C (WHO, 2006).

Organism	Survival times (d)	
	Crops	**Soils**
Viruses		
Enteroviruses	<60, usually <15	<100, usually <20
Bacteria		
Thermotolerant coliforms	<30, usually <15	<70, usually <20
Salmonella spp.	<30, usually <15	<70, usually <20
Shigella spp.	<10, usually <5	ND
V.cholerae	<5, usually <2	<20, usually <10
Protozoa		
E.histolytica cysts	<10 usually <2	<20, usually <10
Cryptosporidium oocysts	<3, usually <2	<150, usually <75
Helminths		
Ascaris eggs	<60, usually <20	Years
Tapeworm eggs	<60, usually <30	Many months

ND, no data

16.7.1 Stabilization ponds

Stabilization ponds are usually shallow basins in which natural factors such as sunlight, temperature, sedimentation, and biodegradation are used to treat water. Stabilization pond systems usually consist off anaerobic, facultative and maturation ponds linked in series (Droste, 1997; Bitton, 1999). Stabilization ponds are more effective in warmer climates. Other advantages include: effective pathogen reductions, low costs of construction, operation and maintenance, little sludge production and minimal electricity requirements. Disadvantages include: algal growth interfering with irrigation, large land areas required, high evaporation rates in dry, arid climates and the fact that stabilization pond effluents can only be used for irrigation during the irrigation season (Mara & Pearson, 1998; WHO, 2006; Kehl *et al*., 2009). Pathogen reduction efficiencies are summarized in Tables 13 and 14.

16.7.2 Water storage and treatment reservoirs

These can be used to overcome the problem of having to dispose of effluent from stabilization ponds during the non-irrigation periods of the year. Storage of these effluents in reservoirs allows the whole year's stabilization pond effluent to be used for irrigation together with a reduction in pathogens (Droste, 1997; Mara & Pearson, 1998; Faby *et al.*, 1999; WHO, 2006; Mancini *et al.*, 2007). Advantages and disadvantages of storage and treatment reservoirs are very similar to those of stabilization ponds, and this can be seen form the similar pathogen reduction efficiencies (Table 14). Mancini *et al.* (2007) showed that high pathogen removal efficiencies of up to 5 log units could be achieved in a storage reservoir operated in the batch-mode with a 31 d mean retention time.

16.7.3 Constructed wetlands

Constructed wetlands are natural wastewater treatment systems that combine biological, chemical and physical treatment processes (Crites, 1994). Constructed wetlands are secondary or tertiary treatments, normally used for polishing semi-treated effluents, but their main purpose is to artificially recreate the filtering capacity of natural wetlands (Droste, 1997). Some pathogen reduction does occur (Table 11), but often it is inconsistent (Bitton, 1999; WHO, 2006; Redder *et al*., 2010). Wetland systems have the following advantages compared with conventional treatment methods: utilisation of natural processes; simple construction; simple operation – they can be established and operated by untrained personnel; lower operating costs; low maintenance requirements; they are robust and stable – being able to withstand a wide range of operating conditions; little excess sludge production; they are environmentally acceptable; and offer considerable potential for conservation of wildlife (WRC, 1993; Haberl, 1999; Konnerup *et al*., 2009).

Disadvantages of wetland systems are: slow rate of operation; land area requirements (Ayaz and Akça, 2000), overloading, surface flooding and media clogging of the subsurface systems are common occurrences resulting in stagnant water and reduced efficiency (De Gueldre *et al.*, 2000; Schutes, 2001; Turon *et al*., 2009), may facilitate vector breeding (i.e. mosquitoes) (WHO, 2006).

16.7.4 High-rate processes

High-rate treatment processes are usually expensive, labour intensive systems involving complex infrastructure and operating procedures. These technologies usually consist of primary and secondary treatments, with tertiary treatments an option (Droste, 1997; Bitton, 1999, WHO 2006). High-rate processes often involve the removal of nitrogen, phosphorous and organic matter, which could be beneficial to irrigation. Therefore, low-rate biological processes are often more appropriate, especially in developing countries. Low-rate systems have lower costs, better pathogen removal efficiencies and simplicity of operation and maintenance (WHO, 2006).

Table 14. Log unit reduction of pathogens achievable by various treatment processes (WHO, 2006).

Treatment process	**Log unit pathogen removals**			
	Viruses	**Bacteria**	**Protozoan oocysts**	**Helminth eggs**
Low-rate biological				
Waste stabilization ponds	1-4	1-6	1-4	1-3
Wastewater storage + treatment reservoirs	1-4	1-6	1-4	1-3
Constructed wetlands	1-2	0.5-3	0.5-2	1-3
High-rate processes				
Primary treatment				
Primary sedimentation	0-1	0-1	0-1	0-<1
Chemically enhanced primary treatment	1-2	1-2	1-2	1-3
Secondary treatment				
Activated sludge + secondary sedimentation	0-2	1-2	0-1	1-<2
Trickling filters + secondary sedimentation	0-2	1-2	0-1	1-2
Aerated lagoon + settling pond	1-2	1-2	0-1	1-3
Tertiary treatment				
Coagulation/flocculation	1-3	0-1	1-3	2
High-rate granular or slow-rate sand filtration	1-3	0-3	0-3	1-3
Dual-media filtration	1-3	0-1	1-3	2-3
Membranes	2.5->6	3.5->6	>6	>3
Disinfection				
Chlorination (free chlorine)	1-3	2-6	0-0.15	0-<1
Ozonation	3-6	2-6	1-2	0-2
Ultraviolet radiation	1->3	2->4	>3	0

A treatment plant using sedimentation as the major treatment is commonly referred to as a primary treatment plant. In cases where a biological treatment is incorporated, they are termed secondary treatments. The use of physical-chemical treatments is referred to as advanced or tertiary treatment (Droste, 1997; WHO, 2006).

16.7.5 Primary treatment

Primary treatment usually takes place in sedimentation tanks (2-6 h) and pathogen removal is minimal (<1 log unit). Advantages are the low cost and simplicity of the technology (WHO, 2006). The pathogen reduction efficiency can be increased by including coagulation/flocculation upstream of the sedimentation step or by using filtration downstream of sedimentation (Droste, 1997). Coagulation and flocculation is brought about by the addition of certain chemicals (e.g. lime or ferric chloride, and sometimes high-molecular mass anionic polymer). These processes can be combined with polishing sand filters, which improve the removal of suspended solids, helminth eggs and virus particles. Further advantages such as low area requirements and agricultural suitability of the effluent are sometimes negated by higher sludge production, the need to treat the sludge to inactivate pathogens and the need to use chemicals (WHO, 2006). Pathogen reduction efficiencies are summarized in Table 13.

16.7.6 Secondary/biological treatment

Secondary treatment systems are biological processes which usually follow primary treatment, and also involve a separation of solids and liquids (Droste, 1997; Bitton, 1999; Faby *et al.*, 1999). Secondary treatments are primarily designed to remove BOD, suspended solids and nutrients like nitrogen and phosphorous, some pathogen removal (up to 2 logs)(Table 13) can take place (WHO, 2006). Secondary treatment usually involves an aerobic reaction stage followed by secondary clarification or sedimentation to remove, concentrate or recycle the produced biomass (Droste, 1997). The suspended solids concentration efficiency also affects the pathogen removal efficiency. The aerobic biological processes can be either suspended-growth (activated sludge, aerated lagoons, oxidation ditches) or fixed-film processes, such as trickling filters or rotating biological contactors. The advantages of secondary treatment processes are that the technology is widely available and well understood, the performance of these systems can be optimized for pathogen reduction and in the case of aerated lagoons, no primary sedimentation is required. Aerate lagoons are also somewhat less costly than other high-rate processes. Some disadvantages, however, are their high cost and complexity (except aerated lagoons), the need for trained staff and electricity requirements. Furthermore, large volumes of sludge are produced, which must be disposed of and treated to inactivate pathogens (WHO, 2006).

16.7.7 Tertiary treatment

These are treatments which are included after primary and secondary treatment steps. Tertiary treatments can consist of additional solids removal by flocculation, coagulation and sedimentation and/or granular medium filtration and disinfection:

- *Coagulation, flocculation and sedimentation* usually involves the addition of chemicals to secondary effluents, causing very small particles to combine or aggregate. The larger particles then settle out during sedimentation. Viruses and bacteria often attach to particulates and are removed in this manner (Droste, 1997; WHO, 2006; Zheng *et al*., 2011). Examples of the chemicals used include: ferric chloride, ferrous chloride, aluminium trisulphate and calcium oxide (Bitton, 1999; WHO, 2006). Pathogen reduction efficiencies are normally increased at a low additional cost. Sludge production is, however, increased and this also needs to be treated to inactivate pathogens. The pathogen reduction efficiencies are summarized in Table 13;
- *Filtration* of various types are also an effective means of reducing pathogens in water. Filtration through sand or other porous media is normally carried out after secondary treatment processes (Droste, 1997). Both particulate material and pathogens are removed during filtration, with the effectiveness being dependant on the operating conditions. The most commonly used filtration techniques are high-rate granular, slow sand and dual-media filtration. Filtration can also be preceded by coagulation to further improve efficiency (Bitton, 1999; WHO, 2006; Shirasaki *et al*., 2010). Sand filtration, usually in combination with secondary treatments, has been extensively employed as a means to improve the quality of water for irrigation (Rose *et al.*, 1996; Hamoda *et al.*, 2004; Petala *et al.*, 2006; WHO, 2006; Wand *et al.*, 2007; Bauer *et al*., 2011). Filtration technologies are well understood and low additional costs are involved in their

incorporation into the treatment process. Their use does, however, require careful management to optimize efficiency. Viral and bacterial removal is generally low (Table 13).

- *Disinfection* of water by the use of chlorine (and chlorine dioxide), ozone and ultraviolet (UV) irradiation can be very effective in reducing pathogens (Bitton, 1999; WRC, 2003). The effectiveness of these disinfectants is largely dependent on factors such as type of disinfectant, pH, temperature, water quality, type of pathogen, suspended solids and organic material (Bitton, 1999; WRC, 2003; WHO, 2006). Generally speaking, chlorine is more effective against bacteria and viruses, while ozone and UV are effective against bacteria, viruses and some protozoa (WHO, 2006). Although chlorination is a low cost and well understood technology, it requires pre-treatment to be efficient and exhibits lower efficiency against protozoa and helminths. Chlorine is also a hazardous chemical and produces disinfection by-products (Lopez-Galvez *et al*., 2012). Ozone use is more expensive and needs to be generated on-site, is less effective where organic matter is present in high concentrations. Ozone is also less effective against helminths and protozoa. UV radiation is advantageous as the costs are lower and no by-products are formed. High suspended solids concentrations and biofilm formation can hamper efficiency. Disinfection treatments and combination treatments (especially ozone and UV) have been shown to be capable of reducing different pathogens by between 0 and 6 log units (Rose *et al.*, 1996; Lazarova *et al.*, 1998; Liberti & Notarnicola, 1999; Gotor *et al.*, 2001; Xu *et al.*, 2002; Petala *et al.*, 2006; WHO, 2006; Sharrer & Summerfelt, 2007; Olmez & Kretzschmar, 2009).
- *Membranes* operating in the ultrafiltration and microfiltration range (20-500 nm pore sizes) have also been shown to be very effective in removing nearly all pathogens from water (>6 log unit reductions)(Gotor *et al.*, 2001; Al-Shammiri *et al.*, 2005; Oron *et al.*, 2006; Wisniewski, 2007; Arnal *et al*., 2009). The operation of membrane systems is expensive and complex and the main concerns are membrane fouling and the fact that the retained sludge material needs to treat to inactivate pathogens (Droste, 1997, Bitton, 1999; WHO, 2006). Membranes can also be combined with biological processes in the form of submerged filters for separation of biomass and liquid (Droste, 1997; Bitton, 1999; Delgado *et al.*, 2002; Lawrence *et al.*, 2002; Pollice *et al.*, 2004; Lopez *et al.*, 2006; Wisniewski, 2007). Synthetic layered double hydroxide nanocomposites have also been shown to be useful in the removal of bacteria and viruses form water. Viral and bacterial adsorption efficiencies of ≥99% have been achieved at viral concentrations up to 9.1 x 10^6 plaque forming units.L^{-1} and bacterial concentrations up to 2.6 x 10^{10} cfu.L^{-1} (Jin *et al.*, 2007).

16.8 Summary

Although health protection measures exist to reduce the number of pathogens (Table 15) that end up on food crops, combinations of these measures or various water treatment techniques need to be implemented to increase the level of food safety of these crops to sufficient levels (>7 log unit reductions)(Table 15). Selection of these treatment technologies is not easy, and is dependent on factors such as the level of contamination (chemical, organic and pathogens) in the water, cost, irrigation mode, crop type, environment, fieldworker and consumer education. Prevention of river/irrigation water pollution would be the ultimate solution, but in the interim cost-effective treatment techniques for irrigation water will be necessary. Conventional treatment methods (stabilization ponds, storage reservoirs, slow-sand filtration, etc.) have been shown to be effective, but the inclusion/use of increasingly cost-effective technologies such as ozone and UV exhibit potential.

Table 15. Pathogen reductions achievable by different health protection measures (WHO, 2006).

Control measure	**Pathogen reduction (log units)**	**Descriptions**
Wastewater treatment	1-6	Pathogen reduction achieved by combinations of protection measures and/or wastewater treatments.
Localized (drip) irrigation (low-growing crops)	2	Root crops such as lettuce that grow just above, but partially in contact with the soil.
Localized (drip) irrigation (high-growing crops)	4	Crops such as tomatoes which are trellised.
Spray control (spray irrigation)	1	Use of micro-irrigation, anemometer-controlled direction-switching sprinklers.
Spray zone (spray irrigation)	1	Buffer zone of 50-100 m to protect residents.
Pathogen die-off	0.5-2 per d	Die-off on crop surfaces between last irrigation and consumption
Produce washing with water	1	Washing with clean water
Produce disinfection	2	Washing with weak disinfectant solution and rinsing with water
Produce peeling	2	Fruits and root crops
Produce cooking	6-7	Immersion in boiling/close-to-boiling water until food is cooked

17. GENERAL CONCLUSIONS

A safe and abundant supply of water is not only of paramount importance to everyone but it is inextricably linked to every industry in our country. The country's water custodian is the Department of Water Affairs and Forestry and they are responsible for the challenge to manage the water resources and provide safe water.

Water use in South Africa is dominated by agricultural irrigation which accounts for >60% of all water used. Over the last few years it has been brought to light by various local research institutions that many of the South African rivers that are drawn from for agricultural irrigation purposes are carrying extraordinarily high pathogenic loads and some of the produce irrigated by this water are minimally processed foodstuffs or products that are consumed raw.

Waterborne pathogens not only degrade the environment, but enter the food chain as well. Some of the pathogens have the ability to adhere to or even penetrate vegetables and fruit and can infect humans and farm animals. The risk of disease transmission is especially high when the foodstuff is eaten raw or just lightly cooked. Some pathogens transmitted from fodder crops that infect slaughter animals can infect humans in turn. Agricultural workers coming into contact with contaminated irrigation water can become infected and spread the diseases further.

If a susceptible person becomes infected by contaminated water or food and subsequently infects others by person-to-person contact, then the initial involvement of water may be unsuspected. This leads to underestimation of the health impact of the original pollution. The burden of disease caused by contaminated water has been seriously under-investigated and thus under-estimated in South Africa. The connection between the source of the outbreak and the eventual sufferers are almost never investigated. The health services are bearing a disproportionate burden in coping with these cases of illness and death.

No irrigation water contaminated by untreated or poorly treated faecal waste is risk-free. All such sources of water contain harmful disease-causing organisms and have the potential to make people ill; it is only the concentration of such organisms that varies for different sources of such water. The risks of using such water to produce edible crops should be weighed up against the crises of poor hygiene and hunger.

Apart from the threat to the health of consumers, large outbreaks of associated illnesses would damage the trust of the public, thereby affecting consumer confidence in the local produce as well as the sales of all similar products. These outbreaks could also result in legal challenges that could potentially lead to loss of entry into lucrative export markets as well as possible rejection by the local market. Such consequences would be disastrous for South African agriculture considering that this sector is one of the largest employers of labour in the country and rapidly increasing in economic importance.

Evidence of poor quality irrigation water as well as potential health risks emanating from the use of such water are setbacks that South Africa can ill afford in the present world economic climate. The social consequences of increased risk of disease is equally devastating, especially to the substantial proportion of the population living in poverty.

The ultimate solution to the irrigation water problem is properly and reliably treating water pollution at the source. An even better solution is to prevent the pollution. This is easier said than done! There are so many political, financial, social and resource problems, as well as water shortages and environmental limitations that must be taken into consideration that short-term solutions have become a huge challenge. The sad fact is that many of our rivers are heavily contaminated and must be seen as the direct source of contaminated irrigation water. It is no longer acceptable or even ethical for the various tiers of authority to maintain that they are unaware of the extent and seriousness of the problem of contaminated surface waters. What are they doing to solve the problem? There have already been many local examples of health problems directly caused by contaminated water. These occurrences “seem” to have been managed by a combination of denial and disregard for the consequences.

There is an urgent need for research into the microbial water quality used for irrigation during food production in South Africa and the risks posed by such water. This includes potential pathogens and other compounds impacting on health. The regular monitoring of such disease-causing constituents in irrigation water is very infrequent at present except in a few cases for large-scale producers. This means that opinions expressed about the microbiological safety and suitability of irrigation water do not rest on actual assessments of real risk.

It is easy to say that South African legislation pertaining to foodstuffs clearly state that it is the obligation of the producer to sell a product that is safe. Unfortunately many food producers are not aware of the risks posed by the use of polluted water for irrigation purposes, not only to themselves and their workers but also to those further along the marketing chain, namely the many consumers.

It is all too easy to pass the responsibility on to the consumer by posting warnings that produce should be properly handled before consumption. Increasing awareness of proper food handling processes is a slow process and the public cannot be relied on to wash or cook fruits and vegetables sufficiently to destroy any pathogens that may be present. It is, therefore, the responsibility of the agricultural industry to investigate the likely link between contaminated irrigation water and the carryover of the pathogens onto the produce that it is irrigated with so that the necessary adjustments and treatments can be made to deliver a safe, raw product can be sold to the consumer. The health risks associated with the use of contaminated irrigation water on agricultural products is thus of increasing concern.

18. REFERENCES

Abad, F.X., Pinto, R.S., Gajardo, R. & Bosch, A. (1997). Viruses in mussels: Public health implications and depuration. *Journal of Food Protection,* 6, 677-681.

Acheson, D.W.K. (2001). Foodborne diseases update: Current trends in foodborne diseases. Internet Document – medscape.com/Medscape/ journal 2001/v04.n10/mid1017.ache/.

Adak, G.K. Meakins, S.M., Yip, H., Lopman, B.A. & O'Brien, J.J. (2005). Disease risks from foods, England and Wales, 1996-2000. *Emerging Infectious Diseases,* 11 (3), 365-372.

Adams, M.R., Hartley, A.D. & Cox, L.J. (1989). Factors affecting the efficacy of washing procedures used in the production of prepared salads. *Food Microbiology*, 6(2), 69-77.

Adams, R.D. (1991). The biology of *Giardia* spp. *Microbiological Reviews,* 55, 706-732.

Al-Shammiri, M., Al-Saffar, A., Bohamad, S. & Ahmed, M. (2005). Waste water quality and reuse in irrigation in Kuwait using microfiltration technology in treatment. *Desalination*, 185, 213-225.

Altekruse, S.F. & Swerdlow, D.L. (1996). The changing epidemiology of foodborne diseases. *American Journal of Medical Science, 311*, 23-29.

Alzamora, S.M., Lopez-Malo, A. & Tapla, M.S. (2000). Overview In: *Minimally Processed Fruits and Vegetables: Fundamental Aspects and Applications* (Edited by Alzamora, S.M., Tapia M.S. & Lopez-Malo, A.). p1-9. Galthersburg, Md: Aspen.

Armon, R., Gold, D., Brodsky, M. & Oron, G. (2002). Surface and subsurface irrigation with effluents of different qualities and presence of *Crytosporidium* oocysts in soil and on crops. *Water Science & Technology*, 46(3), 115-122.

Arnal, J.M., Garcia-Fayos, B., Verdu, G. & Lora, J. (2009). Ultrafiltration as an alternative membrane technology to obtain safe drinking water from surface water: 10 years of experience on the scope of the AQUAPOT project. *Desalination*, 248, 34-41.

Arthurson, V. (2008). Proper sanitization of sewage sludge: a critical issue for a sustainable society. *Applied and Environmental Microbiology*, 74, 5267-5275.

Atmar, R.L. (2006). Molecular methods of virus detection in foods. In: *Viruses in Foods*. (Edited by S.M. Goyal). p121-150. New York: Springer Science and Business Media.

Atmar, R.L. (2010). Noroviruses: State of the art. *Food and Environmental Virology,* 2, 117-126.

Ayaz, S.Ç. & Akça, I. (2000). Treatment of wastewater by constructed wetlands in small settlements. *Water Science & Technology*, 41(1), 69-72.

Backeberg, G.R. & Odendaal, P.E. (1998). Water for Agriculture: a Future Prospective. *Proceedings of the 39th General Meeting of the Fertilizer Society of South Africa.* Pilanesberg. p49-61.

Backeberg, G.R. (1996). Presidential Address: Constitutional change and natural resource policy reform in South Africa. *Agrekon*, 35(4), 160-169.

Backeberg, G.R. (2006). Reform of user charges, market pricing and management of water: problem or opportunity for irrigated agriculture. *Irrigation & Drainage*, 55(1), 1-12.

Backeberg, G.R., Bembridge, T.J., Bennie, A.T.P., Groenewald, J.A., Hammes, P.S., Pullen, R.A. & Thompson, H. (1996). *Policy Proposal for Irrigated Agriculture in South Africa – Discussion Paper.* WRC Report KV96/96. Water Research Commission, Pretoria.

Bailey, I.W., Jarmey-Swan, C., Venter, S.N. & Du Preez, M. (2004). Occurrence and source of *Cryptosporidium* and *Giardia* in catchment areas and wastewater works. Water Research Commission report. p2. Water Research Commission, Pretoria.

Barmore, C.R. (1995). Chlorination, are there alternatives? *Cutting Edge,* Spring, 4-5.

Barnes, J.M. & Taylor, M.B. (2004). Health risk assessment in connection with the use of microbiologically contaminated source waters for irrigation. March 2004 WRC Report No 1226/1/04, ISBN 1-7005-010-8. Water Research Commission, Pretoria.

Barnes, J.M. (2003). *The Impact of Water Pollution from Formal and Informal Urban Developments along the Plankenbrug River on Water Quality and Health Risk*. PhD Dissertation, University of Stellenbosch, South Africa.

Barrabeig, I., Rovira, A., Buesa, J., Bartolomé, R., Pintó, R., Prellezo, H. & Domínguez, A. (2010). Foodborne norovirus outbreak: the role of an asymptomatic food handler. *BMC Infectious Diseases,* 10, 269.

Bartram. J., Fewtrell, L. & Stenström, T-A. (2001). Harmonised assessment of risk and risk management for water-related infectious disease: an overview. Chapter 1 In: *Water Quality – Guidelines, Standards and Health: Assessment of risk and risk management for water-related infectious disease.* World Health Organization. p1-16. IWA Publishing: London.

Bauer, R., Dizer, H., Graeber, I., Rozenwinkel, K-H. & López-Pila, J.M. (2011). Removal of bacterial fecal indicators, coliphages and enteric adenoviruses from waters with high fecal pollution by slow sand filtration. *Water Research*, 45, 439-452.

Beans, N.H., Goulding, J.S., Daniel, M.T. & Angelo, F.J. (1997). Surveillance for foodborne disease outbreaks-United States, 1997-1992. *Journal of Food Protection,* 60, 1265-1286.

Behravesh, C.B., Mody, R.K., Jungk, J., Gaul, L., Redd, J.T., Chen, S., Cosgrove, S., Hedican, E., Sweat, D., Chávez-Hauser, L., Snow, S.L., Hanson, H., Nguyen, T.A., Sodha, S.V., Boore, A.L. (2011). 2008 Outbreak of *Salmonella* Saintpaul infections associated with raw produce. *New England Journal of Medicine*, 364, 918-927.

Berger, C.N., Sodha, S.V., Shaw, R.K., Griffin, P.M., Pink, D., Hand, P. & Frankel, G. (2010) Fresh fruit and vegetables as vehicles for the transmission of human pathogens. *Environmental Microbiology*, 12, 2385-2397.

Beuchat, L.R. & Brackett, R.E. (1990). Survival and growth of *Listeria monocytogenes* on lettuce as influenced by shredding, chlorine treatment, modified atmosphere packaging and temperature. *Journal of Food Science, 55*, 755-758.

Beuchat, L.R. & Ryu, J. (1997). Produce handling and processing practices. *Emerging Infectious Diseases,* 3(4), 1-9 and 459-465.

Beuchat, L.R. (1986). *Food Safety Issues: Surface decontamination of Fruits and Vegetables Eaten Raw: A review.* Food Safety issue: Food Safety Unit: World Health Organisation. WHO/FSF/FOS98.2. p42-65.

Beuchat, L.R. (1996). Pathogenic microorganisms associated with fresh produce. *Journal of Food Protection,* 59, 204-206.

Beuchat, L.R. (1998). *Surface Decontamination of Fruits and Vegetables Eaten Raw. A Review.* Food Safety Unit, World Health Organization. WHO/FSF/FOS/98.2.

Beuchat, L.R. (1999). Survival of Enterohemorrhagic Escherichia coli O157:H7 in bovine feces applied to lettuce and the effectiveness of chlorinated water as a disinfectant. *Journal of Food Protection*, 62(8), 845-9.

Beuchat, L.R. (2002). Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables. *Microbes and Infection,* 4, 413-423.

Beuchat, L.R. (2006). Vectors and conditions for preharvest contamination of fruits and vegetables with pathogens causing enteric diseases. *British Food Journal,* 108, 38-53.

Beuchat, L.R., Nail, B.V., Alder, B.B. & Clavero, M.R. (1998). Efficacy of spray application of chlorinated water in killing pathogenic bacteria on raw apples, tomatoes, and lettuce. *Journal of Food Protection,* 61, 1305-1311.

BFAP (Bureau for Food and Agricultural Policy) (2011). The South African Agricultural Baseline. Pretoria and Stellenbosch, South Africa.

BfR (Bundesinstitut für Risikobewertung) (2011). Relevance of sprouts and germ buds as well as seeds for sprout production in the current EHEC O104:H4 outbreak event in May and June 2011. p1-34. Internet Document – www.bfr.bund.de/cm/349/ .

Bidawid, S., Farber, J.M., Sattar, S.A. & Hayward, S. (2000). Heat inactivation of hepatitis A virus in dairy foods. *Journal of Food Protection*, 63, 522-528.

Bihn, E.A. & Gravani, R.B. (2006). Role of Good Agricultural Practices in fruit and vegetable safety. In: *Microbiology of Fresh Produce* (Edited by K.R. Matthews). p21-28. Washington DC: ASM Press.

Bitton, G. (1980). Viruses in human food. In: *Introduction to Environmental Virology* (Edited by Bitton, G.). 1st edition. p273-383. New York: John Wiley and Sons.

Bitton, G. (1999). *Wastewater Microbiology*. p211-371. New York: John Wiley & Sons, Inc, USA.

Bloch, A.B., Stramer, S.L., Smith, J.D., Margolis, H.S., Fields, H.A., McKinley, T.W., Gerba, C.P., Maynard, J.E. & Sikes, R.K. (1990). Recovery of hepatitis A virus from a water supply responsible for a common source of outbreak of hepatitis A. *American Journal of Public Health* 80, 428-430.

Bosch, A., Sánchez, G., Abbaszadegan, M., Carducci, A., Guix, S., Le Guyader, F.S., Netshikweta, R., Pintó, R.M., van der Poel, W.H.M., Rutjes, S., Sano, D., Taylor, M.B., van Zyl, W.B., Rodríguez-Lázaro, D., Kovač, K. & Sellwood, J. (2011). Analytical methods for virus detection in water and food. *Food Analytical Methods,* 4, 4-12.

Bosch, A., Guix, S., Sano, D. & Pintó, R.M. (2008). New tools for study and direct surveillance of viral pathogens in water. *Current Opinion in Biotechnology,* 19, 295-301.

Bosch, A., Lecena, F., Díez, J.M., Gajardo, R., Blasi, M. & Jofre, J. (1991). Waterborne viruses associated with hepatitis outbreak. *Journal American Water Works Association,* 83, 80-83.
Brackett, R.E. (1999). Incidence, contributing factors, and control of bacterial pathogens in produce. *Postharvest Biology and Technology*, 15, 305-311.
Bradshaw, D. & Nannan, N. (2004). Health Status. Chapter 4. In: South African Health Review 2003/4, 45-53.
Brassard, J., Seyer, K., Houde, A., Simard, C. & Trottier, Y-L. (2005). Concentration and detection of hepatitis A virus and rotavius in spring water samples by reverse transcription – PCR. *Journal of Virological Methods*, 123,163-169.
Bresee, J.S., Widdowson, M-A., Monroe, S.S. & Glass, R.I. (2002). Foodborne viral gastroenteritis: Challenges and opportunities. *Clinical Infectious Diseases*, 35, 748-753.
Britz, T.J. (2005). Impact of polluted irrigation water on agricultural products. Paper presented at the Imbizo of the Cape Winelands District Municipality. April 2005, Wellington, Western Cape Province.
Bruhn, C. (2006). Consumer handling of fresh produce from supermarket to table. In: *Microbial Hazard Identification in Fresh Fruits and Vegetables* (Edited by J. James). p95-109. New Jersey: John Wiley & Sons.
Buchanan, R.L. (2006). Spinach outbreak as part of broader concerns about produce safety – A FDA perspective. IAFP Rapid Response Symposium. Arlington, Virginia, USA, October 6, 2006.
Burmaster, D.E. & Anderson, P.D. (1994). Principles of good practice for use of Monte Carlo techniques in human health and ecological risk assessment. *Risk Analysis,* 14(4), 477-481.
Burnett, S.L. & Beuchat, L.R. (2001). Food-Borne pathogens: Human pathogens associated with raw produce and unpasteurized juices and difficulties in decontamination*. Journal of Industrial Microbiology and Biotechnology, 27*, 104-110.
Butot, S., Putallaz, T. & Sánchez, G. (2007). Procedure for the rapid concentration and detection of enteric viruses from berries and vegetables. *Applied and Environmental Microbiology,* 73, 186-192.
Byappanahalli, M.N. & Ishii, S. (2011). Environmental sources of fecal bacteria. In: *The Fecal Bacteria* (Edited by Sadowsky, M.J. & Whitman, R.L.) p93-110. Washington DC: ASM Press.
Calder, L., Simmons, G., Thornley, C., Taylor, P., Pritchard, K., Greening, G. & Bishop, J. (2003). An outbreak of hepatitis A associated with consumption of raw blueberries. *Epidemiology and Infection* 131, 745-751.
Calvin, L., (2006). The economics of a produce outbreak: Spinach and *E. coli* O157:H7. IAFP Rapid Response Symposium. Arlington, Virginia, USA, October 6, 2006.
Calvin, L., Avendaño, B. & Schwentesius, R. (2006). The economics of food safety and produce: The case of green onions and Hepatitis A outbreaks. In: *Microbial Hazard Identification in Fresh Fruits and Vegetables* (Edited by J. James). p95-109. New Jersey: John Wiley & Sons.
Carden, K., Armtage, N., Winter, K., Sichone. & Rivett, U. (2007). *Understanding the Use and Disposal of Greywater in the Non-Sewered Areas in South Africa*. WRC Report 1524/1/07. Water Research Commission, Pretoria.
Carr, R.M., Blumenthal, U.J. & Mara, D.D. (2004). Health Guidelines for the Use of Wastewater in Agriculture: Developing Realistic Guidelines. In: *Wastewater Use in Irrigated Agriculture, Confronting the Livelihood and Environmental Realities* (Edited by C.A. Scott, N.I. Faruqui & L. Raschid-Sally). p41-58. Oxfordshire: CABI Publishing.
Carter MJ. (2005). Enterically infecting viruses: pathogenicity, transmission and significance for food and waterborne infection. *Journal of Applied Microbiology,* 98,1354-1380.
CDC (Centers for Disease Control and Prevention) (2006). FoodNet 2006 Surveillance Report. Atlanta: U.S. Department of Health and Human Services.
CDC (Centers for Disease Control and Prevention) (2011). Updated norovirus outbreak management and diseases prevention guidelines. *Morbidity and Mortality Weekly Report,* 60, 1-14.
Chadha, M.L. & Oluoch, M.O. (2003). Home-based vegetable gardens and other strategies to overcome micronutrient malnutrition in developing countries. *Food, Nutrition and Agriculture,* 32, 17-21.
Chaidez, C., Soto, M., Gortares, P. & Mena, K. (2005). Occurrence of *Cryptosporidium* and *Giardia* in irrigation water and its impact on the fresh produce industry. *International Journal of Environmental Health Research,* 15, 339-345.
Chaturvedi, M.C. (2000). Water for food and rural development. *Water International*, 25, 40-53.
Chiba, S., Nakata, S., Numata-Kinoshita, K. & Honman, S. (2000). Sapporo Virus: History and recent findings. *The Journal of Infectious Diseases*, 181, S303-308.
Coetzer, E. (2006). Microbiological risk in produce from the field to packing. In: *Microbial Hazard Identification in Fresh Fruits* and *Vegetables* (Edited by J. James). p76-92. New Jersey: John Wiley & Sons.
Collier, L. & Oxford, J. (2006). *Human Virology*, 3rd edition. p100-101. Oxford: Oxford University Press Inc.
Cowling, R. (1991). Option for rural land use in Southern Africa; an ecological perspective. In: *A harvest of discontent: the land question in South Africa.* (Edited by M. de Klerk.). Cape Town: Idara.

Crites, R.W. (1994). Design criteria and practice for constructed wetland. In: *Wetland Systems in Water Pollution Control,* (Edited by Bavor, H.J. & Mitchell, D.S.). *Water Science & Technology,* 29(4), 1-6.

Croci, L., de Medici, D., Scalfaro, C., Fiore, A. & Toti, L. (2002). The survival of hepatitis A virus in fresh produce. *International Journal of Food Microbiology*, 73, 29-34.

Croci, L., Dubois, E., Cook, N., de Medici, D., Schultz, A.C., China, B., Rutjes, S.A., Hoorfar, J. & Van der Poel W.H.M. (2008). Current methods for extraction and concentration of enteric viruses from fresh fruit and vegetables; towards international standard. *Food Analytical Methods* 1, 73-84.

Cuthbert, J.A. (2001). Hepatitis A: Old and new. *Clinical Microbiology Reviews*, 14, 38-58.

D'Antonio, R.G., Winn, R.E., Taylor, J.P., Gustafson, T.L., Current, W.L., Rhodes, M.M., Gary, G.W. & Zajac, R.A. (1985). A waterborne outbreak of Cryptosporidiosis in normal hosts. *Annals of Internal Medicine,* 103, 886-888.

DAFF (Department of Agriculture, Forestry and Fisheries) (2011a). Abstract of Agricultural Statistics 2010/11. Department of Agriculture, Forestry and Fisheries, Pretoria, South Africa.

DAFF (Department of Agriculture, Forestry and Fisheries) (2011b). Trends in the Agricultural Sector. Internet Document – www.daff.gov.za. Pretoria, South Africa.

DAFF (Department of Agriculture, Forestry and Fisheries) (2011c). Crops and Markets, First quarter, Vol 92, No 947, 1-11. Internet Document – www.daff.gov.za. Pretoria, South Africa.

DAFF (Department of Agriculture, Forestry and Fisheries) (2011d). Economic Review of South African agriculture. Internet Document – www.daff.gov.za. Pretoria, South Africa.

Dally, E.R., Roy, S.J., Blaney, D.D., Manning, J.S., Hill, V.R., Xiao, L. & Stull, J.W. (2010). Outbreak of giardiasis associated with a community drinking-water source. *Epidemiology of Infections,* 138, 491-500.

Dawson, D. (2005). Foodborne protozoan parasites. *International Journal of Food Microbiology,* 103, 207-227.

De Gueldre, G., Thoeye, C., Vandaele, S. & Van Eygen, B. (2000). Small wastewater treatment plants in Flanders (Belgium): Standard approach and experiences with constructed reed beds. *Water Science & Technology*, 41(1), 57-63.

De Roever, C. (1998). Microbiological safety of evaluations and recommendations on fresh produce. *Food Control, 9*, 321-347.

DEAT (Department of Environmental Affairs and Tourism) (2006). South Africa Environment Outlook. A report on the state of the environment. Department of Environmental Affairs and Tourism, Pretoria. p371.

Delgado, S., Díaz, F., Villaroel, R., Vera, L., Díaz, R. & Elmaleh, S. (2002). Influence of biologically treated wastewater quality on filtration through hollow-fibre membrane. *Desalination*, 146, 459-462.

Deregnier, D.P., Cole, L., Schupp, D.G. & Erlandsen, S.L. (1989). Viability of Gi*ardia* cysts suspended in lake, river, and tap water. *Applied and Environmental Microbiology,* 55, 1223-1229.

Desmarais, T.R., Solo-Gabriele, H.M. & Palmer, C.J. (2002). Influence of soil on fecal indicator organisms in a tidally influenced subtropical environment. *Applied and Environmental Microbiology,* 68, 1168-1172.

Dinar, A. & Xepapadeas, A. (1998). Regulating water quantity and quality in irrigated agriculture. *Journal of Environmental Management*, 54, 273-289.

Dlamini, D.J.M. & Schulze, R.E. (2005). Water poverty within a context of climate change: A case study at meso-scale in the Thukela Catchment. *In*: *Climate Change and Water Resources in Southern Africa: Studies on Scenarios, Impacts, Vulnerabilities and Adaptation (Edited by* Schulze, R.E.). Water Research Commission, Pretoria, RSA, WRC Report 1430/1/05. Chapter 24, p387-394.

Dotzauer, A.(2008). Hepatitis A Virus. In: *Encyclopedia of Virology*, 3rd ed (Edited by B.W.J. Mahy & M.V. van Regenmortel). p343-350. Slovenia: Academic Press.

Dowell, S.F., Groves, C. & Kirkland, K.B. (1995). A multistate outbreak of oyster-associated gastroenteritis: implications for interstate tracing of contaminated shellfish. *Journal of Infectious Diseases*, 171, 1497-1503.

Doyle, M.P. & Erickson, M.C. (2008). Summer meeting 2007 – the problems with fresh produce: an overview. *Journal of Applied Microbiology*, 105, 317-330.

Droste, R.L. (1997). *Theory and Practice of Water and Wastewater Treatment*. p285-751. New York: John Wiley & Sons, Inc, USA.

Duizer, E., Schwab, K.J., Neill, F.H., Atmar, R., Koopmans, M.P.G. & Estes, M.K. (2004). Laboratory efforts to cultivate noroviruses. *Journal of General Virology*, 85, 79-87.

DWAF (Department of Water Affairs and Forestry) (1996a). South African Water Quality Guidelines (second edition). Volume 1: Domestic Water Use. Produced by the CSIR Environmental Services: Pretoria. Printed by: The Government Printer, Pretoria.

DWAF (Department of Water Affairs and Forestry) (1996b). South African Water Quality Guidelines (second edition). Volume 4: Agricultural Use: Irrigation. Produced by the CSIR Environmental Services: Pretoria. Printed by: The Government Printer, Pretoria.

ECSCF (European Commission Scientific Committee on Food) (2002). Risk profile on the microbiological contamination of fruits and vegetables eaten raw. European Commission Scientific Committee on Food. SCF/CS/FMH/SURF/Final. 29 April 2002.

EFSA (European Food Safety Authority) (2006). The Community summary report on trends and sources of Zoonoses, Zoonotic agents. Antimicrobial resistance and Foodborne outbreaks in the European Union in 2006. Internet Document – www.efsa.europa.eu/EFSA/efsa. Accessed 17 August 2008.

EFSA (European Food Safety Authority) (2011). Tracing seeds, in particular fenugreek (*Trigonella foenum-graecum*) seeds, in relation to the Shiga toxin-producing *E. coli* (STEC) O104:H4 2011 outbreaks in Germany and France. EFSA Technical Report, p1-34. European Food Safety Authority (EFSA), Parma, Italy.

Egli, T., Köster, W. & Meile, L. (2002). Editorial: Pathogenic microbes in water and food: changes and challenges. *FEMS Microbiology Reviews*, 26, 111-112.

El-Senousy, W.M., Guix, S., Abid, I., Pintó, R.M. & Bosch, A. (2007). Removal of astrovirus from water and sewage treatment plants, evaluated by a competitive reverse transcription-PCR. *Applied and Environmental Microbiology* 73, 164-167.

Emmerson, A.M. (2001). Emerging waterborne infections in health-care settings. *Emerging Infectious Diseases*, 7, 272-276.

EPA (Environmental Protection Agency) (2000). *Optimisation of a New Method for Detection of Viruses in Groundwater.* National Groundwater and Contained Land Centre Project NC/99/40/2000. Environmental Protection Agency, New York: USA.

Erickson, M.C. (2010). Microbial risks associated with cabbage, carrots, celery, onions, and deli salads made with these produce items. *Comprehensive Reviews in Food Science and Food Safety*, 9, 602-619.

Erickson, M.C., Liao, J., Payton, A.S., Riley, D.G., Webb, C.C., Davey, L.E., Kimbrel, S., Ma, L., Zhang, G., Flitcroft, I., Doyle, M.P. & Beuchat, L.R. (2010). Preharvest internalization of *Escherichia coli* O157:H7 into lettuce leaves, as affected by insect and physical damage. *Journal of Food Protection*, 73, 1809-1816.

Ethelberg, S., Lisby, M., Vestergaard, L.S., Enemark, H.L., Olsen, K.E.P., Stensvold, C.R., Nielsen, H.V., Porsbo, L.J., Plesner, A.-M. & Molbak, K. (2009). A foodborne outbreak of *Cryptosporidium hominis* infection. *Epidemiology and Infection*, 32*,* 1-9.

EWTSIM (European Work Team on Sustainable Irrigation Management) (2005). Committee of the ICID, Frankfort, Germany.

Faber, M. & Benadé, A.J.S. (2003). Integrated home-gardening and community-based growth monitoring activities to alleviate vitamin A deficiency in a rural village in South Africa. *Food, Nutrition and Agriculture,* 32, 24-32.

Faby, J.A., Brissaud, F. & Bontoux, J. (1999). Wastewater reuse in France: water quality standards and wastewater treatment technologies. *Water Science & Technology*, 40(4-5), 237-42.

FAO (Food and Agricultural Organization) (1997). *Quality Control of Wastewater for Irrigated Crop Production*. Rome: Food and Agriculture Organization of the United Nations, Italy.

FAO (Food and Agricultural Organization) (2004). Key statistics of Food and Agricultural External Trade. Internet Document – Internet Document – www.fao.org/es/ess/toptrade/trade.asp.

FAO (Food and Agricultural Organization) (2004). Key statistics of Food and Agricultural External Trade. Internet Document – Internet Document – www.fao.org/es/ess/toptrade/trade.asp.

FAO (Food and Agricultural Organization) (2005). AQUASTAT. Country profile South Africa. FAO, Rome, Internet Document – Internet Document – www.fao.org/ag/agl/aglw/aquastat/countries/index.stm.

FAO (Food and Agricultural Organization) (2005). AQUASTAT. Country profile South Africa. FAO, Rome, Internet Document – www.fao.org/ag/agl/aglw/aquastat/countries/index.stm.

FAO/WHO (2006). The use of microbiological risk assessment outputs to develop practical risk management strategies: Metrics to improve food safety. A joint FAO/WHO Expert Meeting Report, Kiel Germany, 3-7 April.

Farkas, T., Sestak, K., Wei, C. & Jiang, X. (2008). Characterization of a rhesus monkey calicivirus representing a new genus of Caliciviridae. *Journal of Virology* 82, 5408-5416.

Faruqui, N. (2001). Trip report (December 2001). Senegal. Cities feeding people. International Development Research Centre (IDRC), Ottawa, Canada, p10-15.

Faruqui, N.I., Scott, C.A. & Raschid-Sally, L. (2004). Confronting the realities of wastewater use in irrigated agriculture: Lessons learned and recommendations. In: *Wastewater Use in Irrigated Agriculture, Confronting the Livelihood and Environmental Realities* (Edited by C.A. Scott., N.I. Faruqui & L. Raschid-Sally). p173-185. Oxfordshire: CABI Publishing.

Fayer, R., Dubey, J.P. & Lindsay, D.S. (2004). Zoonotic protozoa: from land to sea. *Trends in Parasitology,* 20, 531-536.

Fayer, R., Gamble, H.R., Lichtenfels, J.R. & Bier, J.W. (1992). Waterborne and Foodborne Parasites. In: *Compendium of Methods for the Microbiological Examination of Foods* (Edited by Vanderzant, C. & Splittstoesser, D.F.). p789-809. Washington DC: American Public Health Association.

Fayer, R., Morgan, U. & Upton, S.J. (2000). Epidemiology of *Cryptosporidium*: transmission, detection and identification. *International Journal for Parasitology,* 30, 1305-1322.

FDA (Food and Drug Administration). (2001). Secondary direct food additives permitted in food for human consumption. *Federal Register,* 66(123), 33929-33930.

FDA/CFSAN (2001). Production practices as risk factors in microbial food safety of fresh and fresh-cut produce in analysis and evaluation of preventive control measures for the control and reduction/elimination of microbial hazards on fresh and fresh-cut produce. US Food and Drug Administration, Center for Food Safety and Applied Nutrition, September 30, 2001.

FDEP (Florida Department of Environmental Protection) (1999). Reuse of reclaimed water and land application. Tallahassee: Florida Department of Environmental Protection. p62-610, F.A.C.

Feinstone, S.M. & Gust, G.I. (1997). Hepatitis A virus. In: *Clinical Virology.* (Edited by Richman, D.D., Whitley, R.J. & Hayden, F.G.). p1049-1072. New York: Churchill Livingstone Inc.

Ferrari, C.K. & Torres, E.A. (1998). Viral contamination of food products: a poorly understood public health problem. *Reviews Panam Salud Publications*, 3, 359-366.

Fiore, A. (2004). Hepatitis A transmission by food. *Clinical Infectious Diseases,* 38, 705-715.

Flint, K.P. (1987). The long-term survival of *Escherichia coli* in river water. *Journal of Applied Bacteriology,* 63(3), 261-270.

Flowers, R.S., D'Aoust, J.Y., Andrews, W.H. & Bailey, J.S. (1992). *Salmonella*. In: *Compendium of Methods for the Microbiological Examination of Foods* (Edited by Vanderzant, C. & Splittstoesser, D.F.). p371-422. Washington DC: American Public Health Association.

Fong, T-T. & Lipp, E.K. (2005). Enteric virus of humans and animals in aquatic environments: Health risks, detection, and potential water quality assessment tools. *Microbiology and Molecular Biology Reviews*, 69, 357-371.

Francis, G.A., Thomas, C. & O'Beirne, D. (1999). The microbiological safety of minimally processed vegetables. *International Journal of Food Science and Technology*, 34, 1-22.

Franco, R.M.B & Neto, R.C. (2002). Occurrence of Cryptosporidial oocysts and *Giardia* cysts in bottled mineral water commercialized in the city of Campinas, State of Sao Paulo, Brazil. *Memorias do Instituto Oswaldo Cruz,* 97, 205-207.

Fuhrman, J.A., Liang, X. & Noble, R.T. (2005). Rapid detection of enteroviruses in small volume of natural waters by real-time quantitative reverse transcriptase PCR. *Applied and Environmental Microbiology*, 71, 4523-4530.

Garcia, A., Mount, J.R. & Davidson, P.M. (2003). Ozone and chlorine treatments of minimally processed lettuce. *Journal of Food Science,* 68(9), 2747-2751.

Garcia-Villanova, R.B., Cueto, E.A. & Bolanos, M.J. (1987). A comparative study of strains of salmonella isolated from irrigation waters, vegetables and human infections. *Journal of Epidemiology of Infection,* 98, 271-276.

GCIS (Government Communication and Information Services) (2011). South Africa Yearbook 2010/11. Department of Government Communication and Information Services, Pretoria.

Gerald, B.L. & Perkin, J.E. (2003). Position of the American Dietetic Association: food and water safety. *Journal of the American Dietetic Association,* 103(9), 1203-1218.

Germs, W., Coetzee, M.S., Van Rensburg, L. & Maboeta, M.S. (2004). A preliminary assessment of the chemical and microbial water of the Chunies river – Limpopo. *Water SA*, *30(2)*, 267.

Gil, M.I. & Selma, M.V. (2006). Overview of hazards in fresh-cut produce production: control and management of food safety hazards. In: *Microbial Hazard Identification in Fresh Fruits and Vegetables* (edited by J. James). p95-109. New Jersey: John Wiley & Sons.

Gilbert, R.J., Louvois, J., Donovan, T., Little, C., Nye, K., Ribero, C., Richards, J., Roberts, D. & Boolton, F.J. (2000). Guidelines for the microbiological quality of some ready-to-eat food sampled at the point of sale. *Common Disease Pubic Health*, 3(3), 163-167.

Glass, R.I., Noel, J., Mitchell, D., Herrmann, J.E., Blacklow, N.R., Pickering, L.K., Dennehy, P., Ruiz-Palacios, G., de Guerrero, M.L. & Monroe, S.S. (1996). The changing epidemiology of astrovirus-associated gastroenteritis: a review. *Archives of Virology*, 2 [Suppl], 287-300.

Gotor, A.G., Baez, S.O.P., Espinoza, C.A. & Bachir, S.I. (2001). Membrane processes for the recovery and reuse of wastewater in agriculture. *Desalination*, 137, 187-192.

Goyal, S.M. (2006). Methods of virus detection in foods. In: *Viruses in Foods.* (Edited by S.M. Goyal). p101-119. New York: Springer Science and Business Media.

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

Grabow, W.O.K., Gauss-Müller, V., Prozesky, O.W. & Deinhardt, F. (1983). Inactivation of hepatitis A virus and indicator organisms in water by free chlorine residuals. *Applied and Environmental Microbiology*, 46, 619-624.

Grabow, W.O.K., Taylor, M.B. & de Villiers, J.C. (2001). New methods for the detection of viruses call for review of drinking water quality guidelines. *Water Science & Technology*, 43, 1-8.

Grabow, W.O.K., Taylor, M.B. & Ehlers, M.M. (2004). Assessment of the risk of infection associated with viruses in South African drinking water supplies. WRC Report No. 1164/1/04 ISBN 1 77005-170-8. Water Research Commission, Pretoria.

Grabow, W.O.K., Taylor, M.B. & Wolfaardt. M. (1996). Research on human viruses in diffuse effluents and related water environments. WRC Report No. 496/1/96 ISBN 186845 243 3. Water Research Commission, Pretoria.

Graham, J.L., Striebich, R., Patterson, C.L., Radha Krishnan, E. & R. C. Haught, R.C. (2004). MTBE oxidation byproducts from the treatment of surface waters by ozonation and UV-ozonation. *Chemosphere*, 54(7), 1011-1016.

Greene, S.K., Daly, E.R., Talbot, E.A., Demma, L.J., Holzbauer, S., Patel, N.J., Hill, T.A., Walderhaug, M.O., Hoekstra, R.M., Lynch, M.F. & Painter, J.A. (2008). Recurrent multistate outbreak of Salmonella Newport associated with tomatoes from contaminated fields, 2005. *Epidemiology Infection*, 136, 157-165.

Griffin, D.W., Lipp, E.K., Mclaughlin, M.R. & Rose, J.B. (2001). Marine recreation and public health microbiology: quest for the ideal indicator. *BioScience* 51, 817-825.

Guévremont, E,. Brassard, J., Houde, A., Simard, C. & Trottier, Y-L. (2006). Development of an extraction and concentration procedure and comparison of RT-PCR primer systems for the detection of hepatitis A virus and norovirus GII in green onions. *Journal of Virological Methods*, 134, 130-135.

Gust, I.D. & Feinstone, S.M. (1988). *Hepatitis A*. Boca Raton: CRC Press, Inc.

Gust, I.D. & Feinstone, S.M. (1998). *Hepatitis A*. Boca Raton: CRC Press, Inc.

Haas, C. & Eisenberg, J.N.S. (2001). Risk Assessment. Chapter 8. In: *Water Quality – Guidelines, Standards and Health: Assessment of Risk and Risk Management for Water-related Infectious Disease – Foreword (Edited by* Fewtrell, L. & Bartram, J.). p161-183. IWA Publishing: London.

Haas, C.N. (1996). How to average microbial densities to characterise risk. *Water Research* 30(4), 1036-1038.

Haberl, R. (1999). Constructed wetlands: A chance to solve wastewater problems in developing countries. *Water Science & Technology*, 40(3), 11-17.

Hagenmaier, R.D. & Baker, R.A. (1998). Microbial population of shredded carrot in modified atmosphere packaging as related to irradiation treatment. *Journal of Food Science,* 63(1), 162-164.

Hamilton, A.J., Stagnitti, F.S., Premier, R. & Boland, A.M. (2006a). Is the risk of illness through consuming vegetables irrigated with reclaimed wastewater different for different population groups? *Water Science & Technology,* 54(11-12), 379-861.

Hamilton, A.J., Stagnitti, F.S., Premier, R., Boland, A.M. & Hale, G. (2006b). Quantitative Microbial risk assessment models for consumption of raw vegetables irrigated with reclaimed water. *Applied and Environmental Microbiology,* 72(5), 3284-3290

Hamoda, M.F., Al-Ghusain, I. & Al-Mutairi, N.Z. (2004). *Desalination*, **164**, 203-211.

Hardie, J.M. & Whiley, R.A. (1997). Classification and overview of the genera *Streptococcus* and *Enterococcus*. *Journal of Applied Microbiology (Symposium Supplement),* 83, 1S-11S.

Harper, C.M., Cowell, N.A., Adams, B.C., Langley, A.J. & Wohlwen, T.D. (2002). Outbreak of *Cryptosporidium* linked to drinking unpasteurised milk. *Communicable Disease Intelligence,* 26, 449-450.

Harris, J.R., Adrian, M. & Petry, F. (2004). Amylopectin: a major component of the residual body in *Cryptosporidium parvum* oocysts. *Parasitology,* 128, 269-282.

Harris, L.J., Farber, J.N., Beuchat, L.R., Parish, M.E., Suslow, T.V., Garrett, E.H. & Busta, F.F. (2003). Outbreaks associated with fresh produce: Incidence, growth and survival of pathogens in fresh and fresh- cut produce. *Comprehensive Reviews in Food Science and Food Safety, 2*, 78-141.

Harwood, V.J., Levine, A.D., Scott, T.M., Chivukula, V., Lukasik, J., Farrah, S.R. & Rose, J.B. (2005). Validity of the indicator organism paradigm for pathogen reduction in reclaimed water and public health protection. *Applied and Environmental Microbiology,* 71, 3163-3170.

Havelaar, A.H. & Melse, J.M. (2001) Quantifying Public Health Risks in the WHO Guidelines for Drinking Water Quality: A burden of disease approach. RIVM Report 734301022/2003. Rijksinstituut voor Volksgezondheid en Milieu (National Institute for Public Health and the Environment), Bilthoven, The Netherlands.

Havelaar, A.H., Van Olphen, M. & Drost, Y.C. (1993). F-Specific RNA bacteriophages are adequate model organisms for enteric viruses in fresh water. *Applied and Environmental Microbiology,* 59, 2956-2962.

Health Systems Trust. (2007). Health Statistics database. Continually updated from the best available formal data sources. Internet Document – www.hst.org.za/healthstats.

Heaton, J.C, & Jones, K. (2008). Microbial contamination of fruit and vegetables and the behaviour of enteropathogens in the phyllosphere: a review. *Journal of Applied Microbiology*, 104, 613-626.

Hedberg, C.W., MacDonald, K.L. & Osterholm, MT. (1999). Changing epidemiology of food-borne disease: a Minnesota perspective. *Clinical Infection Disease, 18*, 671-682.

Hidaka, T., Kirigaya, T., Kamijo, M., Kikawa, H., Kawamura, T. & Kawauchi, S. (1992). Disappearance of residual chlorine and formation of chloroform in vegetables treated with sodium hypochlorite. *Journal of the Food Hygienic Society of Japan*, 33(3), 267-273.

Hollinger, B. & Ticehurst, J. (1996). Hepatitis A virus. In: *Fields Virology*. (edited by Fields, B.N., Knipe, D.M. & Howley, P.M.) 3rd edition. p735-782. Philadelphia: Lippincott, Raven.

Hollinger, F.B. & Emerson, S.U. (2007). Hepatitis A virus. In: *Fields Virology.* 5th ed. (Edited by D.M. Knipe, D.E. Griffin, R.A. Lamb, S.E. Straus, P.M. Howley, M.A. Martin, B. Roizman). p911-947. Philadelphia: Lippincott Williams & Wilkins.

Hollinger, F.B. (1985). Hepatitis A virus. In: *Viral Hepatitis: Biological and clinical features, specific diagnosis, and prophylaxis* (edited by Hollinger, F.B., Melnick, J.L. & Robinson, W.S.). p11-18. New York: Raven Press Books.

Horman, A., Rimhanen-Finne, R., Maunula, L., Von Bonsdorff, C., Torvela, N., Heikinheimo, A. & Hanninen, M. (2004). *Campylobacter* spp., *Giardia* spp., *Cryptosporidium* spp., noroviruses, and indicator organisms in surface water in south-western Finland, 2000-2001. *Applied and Environmental Microbiology,* 70, 87-95.

Hurst, C.J., Crawford, R.L., Knudsen, G.R., McInerney, M.J. & Stetzenbach, L.D. (2002). Manual of Environmental Microbiology, 2nd ed. Washington, DC: ASM Press.

Ibenyassine, K., Aitmhand, R., Karamoko, Y., Cohen, N. & Ennaji M.M. (2006). Use of repetitive DNA sequences to determine the persistence of enteropathogenic Esacherichia coli in vegetables and in soil grown in fields treated with contacted irrigation water. *Letters in Applied Microbiology,* 43(5), 528-533.

Insulander, M., De Jong, B. & Svenungsson, S. (2008). A food-borne outbreak of cryptosporidiosis among guests and staff at hotel restaurant in Stockholm County, Sweden, September 2008. *Eurosurveillance,* 13, 1-2.

Insulata, W.F., Witzeman, J.S. & Sunya, F.C. (1969). Faecal Streptococci in industrially processed foods: An incidence study. *Food Technology,* 10, 1316-1318.

Islam, M., Doyle, M.P., Phatak, S.C., Millner, P. & Jiang, X. (2004a). Persistence of enterohemorrhagic *Escherichia coli* O157:H7 in soil and on leaf lettuce and parsley grown in fields treated with contaminated manure composts or irrigation water. *Journal of Food Protection,* 67, 1365-1370.

Islam, M., Doyle, M.P., Phatak, S.C., Milner, P. & Jiang, X. (2003). Survival of *Escherichia coli* in soil and on carrots and onions grown in fields treated with contaminated manure composts or irrigation water. *Food Microbiology*, *22*, 63-70.

Islam, M., Morgan, J., Doyle, M.P., Phatak, S.C., Millner, P. & Jiang, X. (2004b). Fate of *Salmonella enterica* serovar Typhimurium on carrots and radishes grown in fields treated with contaminated manure composts or irrigation water. *Applied and Environmental Microbiology*, 70, 2497-2502.

Islam, M., Morgan, J., Doyle, M.P., Phatak, S.C., Millner, P. & Jiang, X. (2004c). Persistence of *Salmonella enterica* serovar Typhimurium on lettuce and parsley and in soils on which they were grown in fields treated with contaminated manure composts or irrigation water. *Foodborne Pathogens and Disease*, 1, 27-35.

James, J. (2006). Overview of microbiological hazards in fresh fruit and vegetable operations. In: *Microbial Hazard Identification in Fresh Fruits and Vegetables* (edited by J. James). New Jersey: John Wiley & Sons.

Jamieson, R., Gordon, R., Joy, D. & Lee, H. (2004). Assessing microbial pollution of rural surface waters: A review of current watershed scale modelling approaches. *Agricultural Water Management*, 70, 1-17.

Jamieson, R., Joy, D.M., Lee, H., Kostaschuk, R. & Gordon, R. (2005). Transport and deposition of sediment-associated *Escherichia coli* in natural streams. *Water Research*, 39, 2665-2675.

Jay, J.M, (1992). Indicators of food microbial in quality and safety. In: *Modern Food Microbiology*. 4th Edition. p701-703. New York: Chapman and Hall.

Jay, J.M. (1997). Do background microorganisms play a role in the safety of fresh foods? *Trends in Food Science & Technology*, 8, 421- 424.

Jean, J., D'Souza, D.H. & Jaykus, L. (2004). Multiplex nucleic acid sequence-based amplification for simultaneous detection of several enteric viruses in model ready-to-eat foods. *Applied and Environmental Microbiology*, 70:6603-6610.

Jin, S., Fallgreen, P.H., Morris, J.M. & Chen, Q. (2007). Removal of bacteria and viruses from water using layered double hydroxide nanocomposites. *Science and Technology of Advanced Materials*, 8, 67-70.

Johnston, L.M., Jaykus, L., Moll, D., Martinez, M.C., Anciso, J., Mora, B. & Moe, C.L. (2005). A field study of the microbiological quality of fresh produce. *Journal of Food Protection,* 68(9), 1840-1847.

Johnston, L.M., Moe, C.L., Moll, D. & Jaykus, L. (2006). The epidemiology of produce-associated outbreaks of foodborne disease. In: *Microbial Hazard Identification in Fresh Fruits and Vegetables* (edited by J. James). p38-52. New Jersey: John Wiley & Sons.

Jokela, P., Joki-Korpela, P., Maaronen, M., Glumoff, V. & Hyypiä, T. (2005). Detection of human picornaviruses by multiplex reverse transcriptase-PCR and liquid hybridization. *Journal of Clinical Microbiology* 43, 1239-1245.

Jones, T.F., McMillian, M.B., Scallan, E., Frenzen, P.D., Cronquist, A.B., Thomas, S. & Angulo, F.J. (2006). A population-based estimate of the substantial burden of diarrhoeal disease in the United States; Foodnet, 1996-2003. *Epidemiology and Infection*, 135, 293-301.

Jothikumar, N., Cromeans, T.L., Sobsey, M.D. & Robertson, B.H. (2005). Development and evaluation of a broadly reactive TaqMan assay for rapid detection of hepatitis A virus. *Applied and Environmental Microbiology*, 71, 3359-3363.

Kautter, D.A., Solomon, H.M., Lake, D.E., Bernard, D.T. & Mills, D.C. (1992). *Clostridium botulinum* and its toxins. In: *Compendium of Methods for the Microbiological Examination of Foods* (Edited by Vanderzant, C. & Splittstoesser, D.F.). p605-621. Washington DC: American Public Health Association.

Kaysner, C.A., Tamplin, M.L. & Twedt, R.M. (1992). *Vibrio*. In: *Compendium of Methods for the Microbiological Examination of Foods* (Edited by Vanderzant, C. & Splittstoesser, D.F.). p451-473. Washington DC: American Public Health Association.

Keddy, K. (1998). The global toll of gastroenteritis. *South African Journal of Epidemiology and Infection*, 13, 2-3.

Kehl, O., Wichern, M., Lübken, M. & Horn, H. (2009). Analysis of design approaches for stabilization ponds under different boundary conditions – A comparison. *Ecological Engineering*, 35, 1117-1128.

Kfir, R., Hilner, C., Du Preez, M. & Bateman, B. (1995). Studies on the prevalence of *Giardia* cysts in South African water. *Water Science & Technology,* 31, 435-438.

King, A.W.Q., Brown, F., Hovi, T., Knowles, N., Lemon, S.M. & Minor, P.D. (2000). Picornaviridae. In: *Virus Taxonomy: Classification and nomenclature of viruses (*Edited by Van Regenmortel, M.H.V., Fauquet, C.M., Bishop, D.H.L., Carstens, E.B., Estes, M.K. & Lemon, S.M.). p671-673. San Diego: Academic Press.

Kirsten, J.F. & Van Zyl, J. (1990). Die ekonomiese impak van bepsproeiingslandbou in die Suidwes-Vrystaat – 'n toepassing van streeksinset-uitset tabelle. *Agrekon*, 29, 162-170.

Kleiboeker, S.B. (2004). Development of real-time, multiplex PCR/RT-PCR assays for improved PRDC pathogen detection. Research Report Swine Health. National Institute for Animal Agriculture, Colorado, USA.

Kniel, K.E., Lindsay, D.S., Summer, S.S., Hackney, C.R., Pierson, M.D. & Dubey, J.P. (2002). Examination of attachment and survival of *Toxoplasmagondii* oocysts on raspberries and blueberries. *Journal of Parasitology,* 88,790-793.

Konnerup, D., Koottatep, T. & Brix, H. (2009). Treatment of domestic wastewater in tropical, subsurface flow constructed wetlands planted with *Canna* and *Heliconia*. *Ecological Engineering*, 35, 248-257.

Koopmans, M. & Duizer, E. (2004). Foodborne viruses: an emerging problem. *International Journal of Food Microbiology*, 90, 23-41.

Koopmans, M., Vennema, H., Heersma, H., van Strien, E., van Duynhoven, Y., Brown, D., Reacher, M. & Lopman, B. (2003). Foodborne viruses – for the European Consortium on Foodborne Viruses. *Emerging Infectious Diseases*, 9, 1136-1142.

Koopmans, M., von Bonsdorff, C-H., Vinjé, J., de Medici, D. & Monroe, S. (2002). Foodborne viruses. *FEMS Microbiolology Reviews*, 26, 187-205.

Korsten, L. & Zagory, D. (2006). Pathogen survival on fresh fruit in ocean cargo and warehouse storage. In: *Microbial Hazard Identification in Fresh Fruits and Vegetables* (edited by J. James). p95-109. New Jersey: John Wiley & Sons.

Köster, W., Egli, T., Ashbolt, N., Botzenhart, K., Burlion, N., Endo, T., Grimont, P., Guillot, E., Mabilat, C., Newport, L., Niemi, M., Payment, P., Prescott, A., Renaud, P. & Rust, A. (2003). Analytical methods for microbiological water quality testing. OECD/WHO guidance document. In: *Assessing Microbial Safety of Drinking Water: Improving Approaches and Methods.* (Edited by E. Ronch, J. Bartram). p237-292. Paris: OECD/WHO. ISBN 9264099468.

Kou, X., Wu, Q., Wang, D. & Zhang, J. (2008). Simultaneous detection of norovirus and rotavirus in oysters by multiplex RT-PCR. *Food Control* 19, 722-726.

Kroupitski, Y., Golberg, D., Belausov, E., Pinto, R., Swartzberg, D., Granot, D. & Sela, S. (2009). Internalization of *Salmonella enterica* in leaves is induced by light and involves chemotaxis and penetration through open stomata. *Applied and Environmental Microbiology*, 75, 6076-6086.

Krtinić, G., Đurić, P. & Ilić, S. (2010). Salmonellae in food stuffs of plant origin and their implications on human health. *European Journal of Clinical Microbiology and Infectious Disease*, 29, 1321-1325.

Kumar, C.G. & Anand, S.K. (1998). Significance of microbial biofilms in food industry: a review. *International Journal of Food Microbiology*, 42, 9-27.

L'Homme Y, Sansregret R, Plante-Fortier É, Lamontagne A-M, Ouardani M, Lacroix G, Simard C. (2009). Genomic characterization of swine caliciviruses representing a new genus of Caliciviridae. *Virus Gene*, 39, 66-75.

Lambertini, E., Spencer, S.K., Bertz, P.D., Lodge, F.J., Kieke, B.A & Borchardt, M.A. (2008). Concentration of enteroviruses, adenoviruses, and noroviruses from drinking water by use of glass wool filters. *Applied and Environmental Microbiology* 74, 2990-2996.

Lane, S. & Lloyd, D. (2002). Current trends in research into the waterborne parasite *Giardia*. *Critical Reviews in Microbiology,* 28, 123-147.

Lappalainen, M., Chen, R.W., Maunula, L., von Bonsdorff, C-H., Plyusnin, A. & Vaheri, A. (2001). Molecular epidemiology of viral pathogens and tracing of transmission routes: hepatitis-, calici- and hantaviruses. *Journal of Clinical Virology,* 21,177-185.

Lauri, A. & Mariani, P.O. (2009). Potentials and limitations of molecular diagnostic methods in food safety. *Gene and Nutrition,* 4,1-12.

Lawrence, P., Adham, S. & Barrott, L. (2002). Ensuring water re-use projects succeed – institutional and technical issues for treated wastewater re-use. *Desalination*, 152, 291-298.

Lazarova, V., Janex, M.L., Fiksdal, L., Oberg, C., Barcina, I. & Pommepuy, M. (1998). Advanced wastewater disinfection technologies: short and long term efficiency. *Water Science & Technology*, 38(12), 109-117.

Lee, S-H. & Kim, S.J. (2002). Detection of infectious enteroviruses and adenoviruses in tap water in urban areas in Korea. *Water Research*, 36, 248-256.

Lefay, D., Naciri, M., Poirier, P. & Chermette, R. (1999). Prevalence of *Cryptosporidium* infection in calves in France. *Veterinary Parasitology*, 89, 1-9.

Leggitt, P. & Jaykus, L. (2000). Detection methods for human enteric viruses in representative foods. *Journal of Food Protection*, 63, 1738-1744.

Legnani, P.P. & Leoni, E. (2004). Effect of processing and storage conditions on the microbiological quality of minimally processed vegetables. *International Journal of Food Science and Technology*, 39, 1061-1068.

Liberti, L. & Notarnicola, M. (1999). Advanced treatment and disinfection for municipal wastewater reuse in agriculture. *Water Science & Technology*, 40(4-5), 235-245.

Lopez, A., Pollice, A., Lonigro, A., Masi, S., Palese, A.M., Cirelli, G.L., Toscano, A. & Passino, R. (2006). Agricultural wastewater reuse in southern Italy. *Desalination*, 187, 323-334.

López-Gálvez, F., Posada-Izquierdo, G.D., Selma, M.V., Pérez-Rodríguez, F., Gobet, J., Gil, M.I. & Allende, A. (2012). Electro-chemical disinfection: An efficient treatment to inactivate Escherichia coli O157:H7 in process wash water containing organic matter. *Food Microbiology*, 30, 146-156.

Lund, B.M. & O'Brien, S.J. (2011). The occurrence and prevention of foodborne disease in vulnerable people. *Foodborne Pathogens and Disease*, 8, 961-973.

Lund, B.M. (1983). Bacterial spoilage. In: *Post-harvest Pathology of Fruits and Vegetables* (Edited by Dennis, C.). p219- 223. London: Academic Press.

Lynch, M.F., Tauxe, R.V. & Hedberg, C.W. (2009). The growing burden of foodborne outbreaks due to contaminated fresh produce: risks and opportunities. *Epidemiology and Infection*, 137, 307-315.

Macarisin, D., Bauchan, G. & Fayer, R. (2010). *Spinacia oleracea* L. leaf stomata harboring *Cryptosporidium parvum* oocysts: a potential threat to food safety. *Applied and Environmental Microbiology,* 76, 555-559.

MacGowan, A.P., Bowker, K., McLauchlin, J., Bennet, P.M. & Reeves, D.S. (1994). The occurrence and seasonal changes in the isolation of *Listeria* sp in shop bought food stuffs, human feces, sewage and soil from urban sources. *International Journal of Food Microbiology, 21*, 325- 334.

Maciorowski, K.G., Herrera, P., Jones, F.T., Pillai, S.D. & Ricke, S.C. (2007). Effects on poultry and livestock of feed contamination with bacteria and fungi. *Animal Feed Science and Technology*, 133, 109-136.

Mahbub, I., Michael, P.D., Sharad, C.P., Patricia, M. & Xiuping, J. (2004). Persistence of enterohemorrhagic Escherichia coli 0157:H7 in soil and on leaf lettuce and parsley grown in fields treated with contaminated manure composts or irrigation water. *Journal of Food Protection,* 67(7), 1365-1370.

Mancini, G., Barone, C., Roccaro, P. & Vagliasindi, F.G.A. (2007). The beneficial effects of storage on the quality of wastewater for irrigation: a case study in Sicily. *Water Science & Technology*, 55(1-2), 417-424.

Manojkumar, R. & Mrudula, V. (2006). Applications of real-time reverse transcription polymerase chain reaction in clinical virology laboratories for the diagnosis of human diseases. *American Journal of Infectious Diseases,* 2, 204-209.

Mara, D. & Cairncross, S. (1989). *Guidelines for the Safe Use of Wastewater and Excreta in Agriculture and Aquaculture*. p187-190. Geneva: World Health Organization, Switzerland.

Mara, D.D. & Pearson, H.W. (1998). System for wastewater reuse for both restricted and unrestricted crop irrigation. *Water Research*, 33(2), 591-594.

Mara, D.D., Sleigh, P.A., Blumenthal, U.J. & Carr, R.M. (2007). Health risks in wastewater irrigation: Comparing estimates from quantitative microbial risk analyses and epidemiological studies. *Journal of Water and Health*, 5, 39-50.

Martínez, M.A., Alcalá, A.C., Carruyo, G., Botero, L., Liprandi, F. & Ludert, J.E. (2006). Molecular detection of porcine enteric caliciviruses in Venezuelan farms. *Veterinary Microbiology*, 116, 77-84.

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

Marx, F.E. (1997). *Detection of human astroviruses in South Africa*. PhD dissertation. Pretoria: University of Pretoria.

Matthews, K.R. (2006). Microorganisms associated with fruits and vegetables. In: *Microbiology of Fresh Produce* (edited by K.R. Matthews). p1-21. Washington DC: ASM Press.

Mattison, K. & Bidawid, S. (2009). Analytical methods for food and environmental viruses. *Food and Environmental Virology,* 1, 107-122.

Mattison, K., Brassard, J., Gagne, M.J., Ward, P., Lessard, L., Simard, C., Shukla, A., Pagotto, F., Jones, T.H. & Trottier, Y.L. (2009). The feline calicivirus as a sample process control for the detection of food and waterborne RNA viruses. *International Journal of Food Microbiology*, 132:73-77.

Mazollier, J. (1988). IVe`me gamme. Lavage-de´sinfection des salades. *Infos-Ctifl,* 41, 20-23.

McMahon, A.S. & Wilson, I.G. (2001). The occurrence of enteric pathogens and *Aeromonas* species in organic vegetables*. International Journal of Food Microbiology,* 70, 155-162.

Mead, P.S., Slutsker, L., Dietz, V., McCraig, L.F., Bresee, J.S., Shapiro, C., Griffin, P.M. & Tauxe, R.V. (1999). Food-related illness and death in the United States. *Emerging Infectious Diseases*, 5, 607-625.

Mederios, L., Hillers, V., Kendall, P. & Mason, A. (2001). Evaluation of food safety education for consumers. *Journal of Nutrition Education and Behavior,* 33, S27-S34.

Meinhardt, P.L. (2006). Recognizing waterborne disease and the health effects of water contamination: a review of the challenges facing the medical community in the United States. *Journal of Water and Health,* 4[Suppl], 27-34

Midgley, G.F., Chapman, R.A., Hewitson, B. & Johnston, P. (2005). A status quo, vulnerability assessment of the physical and socio-economic effects of climate change in the Western Cape. Report to the Western Cape Government, Cape Town, South Africa. CSIR Report No. ENV-S-C 2005-703.

Mintz, E.D., Hudson-Wragg, M., Mshar, P., Cartter, M.L. & Hadler, J.L. (1993). Foodborne giardiasis in a corporate office setting. *The Journal of Infectious Diseases,* 167, 250-253.

MMWR (Mortality and Morbidity Weekly Reports). (2010). CDC Surveillance summaries June 11, 59, SS-6.

Moe, C.L. & Rheingans, R.D. (2006). Global challenges in water, sanitation and health. *Journal of Water and Health*, 4 [Suppl], 41-57.

Mohle-Boetani, J.C., Farrar, J., Bradley, P., Barak, J.D., Miller, M., Mandrell, R., Mead, P., Keene, W.E., Cummings, K., Abbott, S. & Werner, S.B. (2009). *Salmonella* infections associated with mung bean sprouts: epidemiological and environmental investigations. *Epidemiology and Infection*, 137, 357-366.

Monge, R. & Chinchilla, M. (1996). Presence of *Cryptosporidium* oocysts in fresh vegetables. *Journal of Food Protection,* 59, 202-203.

Moore, J.E., Millar, B.C., Kenny, F., Lowery, C.J., Xiao, L., Rao, J.R., Nicholson, V., Watabe, M., Heaney, N., SUNNOTEL, O., McCORRY, K., ROONEY, P.J., SNELLING, W.J. & DOOLEY, J.S.G. (2007). Detection of *Cryptosporidium parvum* in lettuce. *International Journal of Food Science and Technology,* 42, 385-393.

Moreno-Espinosa, S., Farkas, T. & Jiang. (2004). Human calicivirus and pediatric gastroenteritis. *Seminars in Pediatric Infectious Diseases*, 15, 237-245.

Moriarty, E.M., Duffy, G., McEvoy, J.M., Caccio, S., Sheridan, J.J., McDowell, D. & Blair, I.S. (2005). The effect of thermal treatments on the viability and infectivity of *Cryptosporidiumparvum* on beef surfaces. *Journal of Applied Microbiology,* 98, 618-623.

Morse, L.J., Bryan, J.A., Hurley, J.P., Murphy, J.F., O'Brien, T.F. & Wacker, W.E. (1972). The Holy Cross college football team hepatitis outbreak. *Journal of American Medical Association* 219, 702-708.

Mukherjee, A., Speh, D., Jones, A.T., Buesing, K.M. & Diez-Gonzales, F. (2006). Longitudinal microbiological survey of fresh produce grown by farmers in the upper midwest. *Journal of Food Protection*, 69(8), 1928-1936.

Mullendore, J.L., Sobsey, M.D. & Shieh, Y.C. (2001). Improved method for the recovery of hepatitis A virus from oysters. *Journal of Virological Methods*, 94, 25-35.

Murphy, K.O.H. (2006). A Scoping Study to Evaluate the Fitness-for-Use of Greywater in Urban and Peri-Urban Agriculture. WRC Report 1479/1/06. Water Research Commission, Pretoria.

NACMCF (National Advisory Committee on Microbiological Criteria for Foods) (1999). Microbiological Safety Evaluations and Recommendations on fresh produce. *Food Control, 10*, 117-143.

Nadan, S., Walter, J.E., Grabow, W.O.K., Mitchell, D.K. & Taylor, M.B. (2003). Molecular characterization of astroviruses by reverse transcriptase PCR and sequence analysis: Comparison of clinical and environmental isolates from South Africa. *Applied and Environmental Microbiology*, 69, 747-753.

Nainan, O.V., Xia, N., Vaughan, G. & Margolis, H.S. (2006). Diagnosis of hepatitis A virus infection: a molecular approach. *Clinical Microbiology Reviews*, 19, 63-67.

NDA (National Department of Agriculture) (2007). Agricultural statistics. National Department of Agriculture, South Africa.

NDH (National Department of Health) (1998). South African Standard Treatment Guidelines and Essential Drugs List for Hospital level, 1998 Edition. National Department of Health, South Africa.

Ndiame, D. & Jaffe, S.M. (2005). Fruits and vegetables: Global trade and competition in fresh and processed product markets. In: *Global Agricultural Trade and Developing Countries* (Edited by Aksoy, M.A & Beghin, J.C.). World Bank. Geneva. p237-257.

Newell, D.G., Koopmans, M., Verhoef, L., Duizer, E., Aidara-Kane, A., Sprong, H., Opsteegh, M., Langelaar, M., Threfall, J., Scheutz, F., van der Giessen, J. & Kruse, H. (2010). Food-borne diseases – The challenges of 20 years ago still persist while new ones continue to emerge. *International Journal of Food Microbiology,* 139, S3-S15.

NFCS (National Food Consumption Survey) (2000). The National Food Consumptions Survey: Children aged 1-9 years, South Africa, 1999. Department of Health, South Africa.

Nguyen-the, C. & Carlin, F. (1994). The microbiology of minimally processed fresh fruits and vegetables. *Critical Review of Food Science and Nutrition, 34(4)*, 371-401.

NICD (National Institute of Communicable Diseases) (2011). *Communicable Diseases Surveillance Bulletin*, 9(2), 1-32.

Nieuwoudt, L. & Groenewald, J. (2003). *The Challenge of Change – Agriculture, Land and the South African Economy*. p31-33. University of Natal Press, Pietermaritzburg.

NMMP (2002). National Microbial Monitoring Programme for Surface Water. Department of Water Affairs and Forestry. Implementation Manual. Second Edition. Pretoria. South Africa.

NWRS (National Water Resource Strategy) (2004). First Edition, September 2004. Department of Water Affairs & Forestry, South Africa.

Nygård, K., Lassen, J., Vold, L., Andersson, Y., Fisher, I., Löfdahl, S., Threlfall, J., Luzzi, I., Peters, T., Hampton, M., Torpdahl, M., Kapperud, G. & Aavitsland, P. (2008). Outbreak of *Salmonella* Thompson infections linked to imported rucola lettuce. *Foodborne Pathogens and Disease*, 5, 165-173.

Okhuysen, P.C., Chappell, C.L., Crabb, J.H., Sterling, C.R. & DuPont, H.L. (1999). Virulence of three distinct *Cryptosporidium parvum* isolates for healthy adults. *Journal of Infectious Diseases,* 180, 1275-1281.

Olaniran, A.O., Naicker, K., & Pillay, B. (2009). Antibiotic resistance profiles of *Escherichia coli* isolates from river sources in Durban, South Africa. *Water SA*, *25*, 1743.

Ölmez, H. & Kretzschamr, U. (2009). Potential alternative disinfection methods for organic fresh-cut industry for minimizing water consumption and environmental impact. *Food Science and Technology*, 42, 686-693.

Ong, C., Moorhead, W., Ross, A. & Issac-Renton, J. (1996). Studies of *Giardia* spp. and *Cryptosporidium* spp. in two adjacent watersheds. *Applied and Environmental Microbiology,* 62, 2798-2805.

Oron, G., Gillerman, L., Bick, A., Buriakovsky, N., Manor, Y., Ben-Yitshak, E., Katz, L. & Hagin, J. (2006). A two stage membrane treatment of secondary effluent for unrestricted reuse and sustainable agricultural production. *Desalination*, 187, 335-345.

Ortega, Y.R., Roxas, C.R., Gilman, R.H., Miller, N.J., Cabrera, L., Taquiri, C. & Sterling, C.R. (1997). Isolation of *Cryptosporidium parvum* and *Cyclospora cayetanensis* from vegetables collected in markets of an endemic region in Peru. *American Journal of Tropical Medicine and Hygiene,* 57, 683-686.

Ouattara, N.K., Passerat, J. & Servais, P. (2011). Faecal contamination of water and sediment in the rivers of the Scheldt drainage network. *Environmental Monitoring and Assessment*, DOI 10.1007/s10661-011-1918-9.

Pachepsky, Y., Shelton, D.R., McLain, J.E.T., Patel, J., & Mandrell, D.E. (2011). Irrigation waters as a source of pathogenic microorganisms in produce: A review. *Advances in Agronomy*, *113*, 73-138.

Pakalniskiene, J., Falkenhorst, G., Lisby, M., Madsen, S.B., Olsen, K.E.P., Nielsen, E.M., Mygh, A., Boel, J. & Mølbak, K. (2009). A foodborne outbreak of enterotoxigenic *E. coli* and *Salmonella* Anatum infection after a high-school dinner in Denmark, November 2006. *Epidemiology & Infection*, 137, 396-401.

Parashar, U.D. & Monroe, S.S. (2001). ‘Norwalk-like viruses’ as a cause of foodborne disease outbreaks. *Reviews in Medical Virology*, 11, 243-252.

Parish, M.E. (1997). Public health and nonpasteurized fruit juices. *Critical Review of Microbiology, 23*, 109-119.

Patel, M.M, Hall, A.J., Vinjé, J & Parashar, U.D. (2009). Noroviruses: A comprehensive review. *Journal of Clinical Virology,* 44, 1-8.

Paulse, A.N., Jackson, V.A., & Khan, W. (2009). Comparison of microbial contamination at various sites along the Plankenburg and Diep Rivers, Western Cape, South Africa. *Water SA*, *35 (4)*, 469-478.

Payment, P. & Franco, E. (1993). *Clostridium perfringens* and somatic coliphages as indicators of the efficiency of drinking water treatment for viruses and protozoan cysts. *Applied and Environmental Microbiology,* 59, 2418-2424.

Pelly, H., Cormican, M., O'Donovan, D., Chalmers, R.M., Hanahoe, B., Cloughley, R., McKeown, P. & Corbett-Feeney, G. (2007). A large outbreak of cryptosporidiosis in western Ireland linked to public water supply: a preliminary report. *Eurosurveillance,* 12, 1-2.

Pepper, I., Gerba, C.P. & Brusseau, M. (1996). *Environmental Microbiology: A Laboratory Manual.* San Diego: Academic Press.

Petala, M., Tsiridis, V., Samaras, P., Zouboulis, A. & Sakellaropoulos, G.P. (2006). Wastewater reclamation by advanced treatment of secondary effluents. *Desalination*, 195, 109-118.

Petersen, L.R., Cartter, M.L. & Hadler, J.L. (1988). A food-borne outbreak of Giardia lamblia. *The Journal of Infectious Diseases,* 157, 846-848.

Pintó, R.M,, Costafreda, M.I,, Pérez-Rodriguez, F.J., D'Andrea, L. & Bosch, A. (2010). Hepatitis A virus: State of art. *Food and Environmental Virology* 2, 127-135.

Pintó, R.M. & Bosch, A. (2008). Rethinking viruses detection in food. In: *Food-borne viruses: Progress and Challenges* (Edited by M.P.G. Koopmans, D.O. Cliver, A. Bosch). p171-188. Washington DC.: ASM Press.

Pintó, R.M. & Saiz, J-C. (2007). Enteric hepatitis viruses. In: *Human Viruses in Water*. (Edited by A. Bosch). p9-67. Amsterdam: Elsevier B.V.

Pintó, R.M., Costafreda, M.I. & Bosch, A. (2009). Risk assessment in shellfish-borne outbreaks of hepatitis A. *Applied and Environmental Microbiology,* 75, 7350-7355.

Pollice, A., Lopez, A., Laera, G., Rubino, P. & Lonigro, A. (2004). Tertary filtered municipal wastewater as alternative water source in agriculture: a field investigation in Southern Italy. *Science of the Total Environment*, 324, 201-210.

Ponka, A., Kotilainen, H., Rimhanen-Finne, R., Hokkanen, P., Hanninen, M.L., Kaarna, A., Meri, T. & Kuusi, M. (2009). A Foodborne Outbreak due to *Cryptosporidium parvum* in Helsinki, November 2008. *Eurosurveillance,* 14, 1-3.

Porter, J.D.H., Gaffney, C., Heymann, D. & Parkin, W. (1990). Food-borne outbreak of *Giardia lamblia*. *American Journal of Public Health,* 80, 1259-1260.

Prazak, A.M., Murano, E.A., Mercado, I. & Acuff, G.R. (2002). Prevalence of *Listeria monocytogene* during production and post-harvest processing of cabbage. *Journal of Food Protection, 65(11),* 1728-1734.

Quick, R., Paugh, K., Addiss, D., Kobayashi, J. & Baron, R. (1992). Restaurant-associated outbreak of giardiasis. *The Journal of Infectious Diseases,* 166, 673-676.

Redder, A., Dürr, M., Daeschlein, G., Baeder-Bederski, O., Koch, C., Müller, R., Exner, M. & Borneff-Lipp, M. (2010). Constructed wetlands – Are they safe in reducing protozoan parasites? *International Journal of Hygiene and Environmental Health*, 213, 72-77.

Reinders, F. (2000). Water Usage in South Africa. ARC – Institute for Agricultural Engineering, Pretoria, South Africa. p5-15.

Rhodes, M.W. & Kator, H. (1988). Survival of *Escherichia coli* and *Salmonella* spp. in estuarine environments. *Applied and Environmental Microbiology,* 54, 2902-2907.

Richards, G.P. (1999). Limitations of molecular biological techniques for assessing the virological safety of foods. *Journal of Food Protection*, 62, 691-697.

Richards, G.P. (2005). Food and waterborne enteric viruses. In: *Foodborne Pathogens Microbiology and Molecular Biology* (Edited by Fratamico, P.M., Bhunia, A.K., Smith, J.L.). p121-143. Norfolk: Caister Academic Press.

Robertson, B,H., Jansen, R.W., Khanna, B., Totsuka, A., Nainan, O.V., Siegl, G., Widell, A., Margolis, H.S., Isomura, S., Ito, K., Ishizu, T., Moritsugu, Y. & Lemon, S.M. (1992). Genetic relatedness of hepatitis A virus strains recovered from different geographical regions. *Journal of General Virology,* 73, 1365-1377.

Robertson, L.J. & Gjerde, B. (2001a). Occurrence of parasites on fruits and vegetables in Norway. *Journal of Food Protection,* 64, 1793-1798.

Robertson, L.J. & Gjerde, B. (2001b). Occurrence of *Cryptosporidium* oocysts and *Giardia* cysts in raw waters in Norway. *Scandinavian Journal of Public Health,* 29, 200-207.

Robertson, L.J. & Smith, H.V. (1992). *Cryptosporidium* and Cryptosporidiosis. Part 1: current perspective and present technologies. *European Microbiology,* 1, 20-29.

Robertson, L.J., Campbell, A.T. & Smith, H.V. (1992). Survival of *Cryptosporidium parvum* oocysts under various environmental pressures. *Applied and Environmental Microbiology,* 58, 3494-3500.

Robertson, L.J., Forberg, T., Hermansen, L., Gjerde, B.K., Alvsvag, J.O. & Langeland, N. (2006). *Cryptosporidium parvum* Infections in Bergen, Norway, during an expensive outbreak ok waterborne Giardiasis in autumn and winter 2004. *Applied and Environmental Microbiology,* 72, 2218-2220.

Robinson, I. & Adams, R.P. (1978). Ultra-violet treatment of contaminated irrigation water and its effect on the bacteriological quality of celery at harvest. *Journal of Applied Bacteriology, 45, 83-90.*

Rose, J.B., Dickson, L.J., Farrah, S.R. & Carnahan, R.P. (1996). Removal of pathogenic and indicator microorganisms by a full-scale water reclamation facility. *Water Research*, 30(11), 2785-2797.

Rosenblum, L.S., Mirkin, I.R., Allen, D.T., Safford, S. & Hadler, S.C. (1990). A multifocal outbreak of hepatitis A traced to commercially distributed lettuce. *American Journal of Public Health,* 80, 1075-1079.

Rutjes, S.A., Italiaander, R., van der Berg, H.H.J.L., Lodder, W.J. & de Roda Husman, A.M. (2005). Isolation and detection of enterovirus RNA from large-volume water samples by using the Nuclisens miniMAG system and real-time nucleic acid sequence-based amplification. *Applied and Environmental Microbiology,* 71, 3734-3740.

Rzezutka, A., Nicholas, R.A.B., Connelly, L., Kaupke, A., Kozyra, I., Cook, N., Birrell, S. & Smith, H.V. (2010). Cryptosporidium oocysts on fresh produce from areas of high livestock production in Poland. *International Journal of Food Microbiology,* 139, 96-101.

Sadovski, A., Fattal, Y.B. & Goldberg, D. (1978). Microbial contamination of vegetables irrigated with sewage effluent by the drip method. *Journal of Food Protection, 41,* 336-340.

Sadovski, A.Y. & Ayala, F. (1980). *Streptococcus faecalis* and *Streptococcus faecium* in frozen vegetables. Incidence and Survival after treatments commonly used at the vegetable freezing plants. *Journal of Food Safety, 2*, 59-73.

Samie, A., Bessong, P.O., Obi, C.L., Sevilleja, J.E.A.D., Stroup, S., Houpt, E. & Guerrant, R.L. (2006). *Cryptosporidium* species: Preliminary descriptions of the prevalence and genotype distribution among school children and hospital patients in the Venda regions, Limpopo Province, South Africa. *Experimental Parasitology,* 114, 314-322.

Sánchez, G., Bosch, A. & Pintó, R.M. (2007). Hepatitis A virus detection in food: current and future prospects. *Letters in Applied Microbiology* 45, 1-5.

Sapers, G.M. (2001). Efficacy of washing and sanitizing methods for disinfectants of fresh fruit and vegetable products. *Food Technology Biotechnology, 39,* 305-311.

Satcher, D. (2000). Food safety: A growing global health problem. *Journal of the American Medical Association*, 283, 1817-1823.

Savichtcheva, O. & Okabe, S. (2006). Alternative indicators of fecal pollution: Relations with pathogens and conventional indicators, current methodologies for direct pathogen monitoring and future application perspectives. *Water Research*, 40, 2463-2476.

Schiemann, D.A. & Wauters, G. (1992). *Yersinia*. In: *Compendium of Methods for the Microbiological Examination of Foods* (Edited by Vanderzant, C. & Splittstoesser, D.F.). p433-450. Washington DC: American Public Health Association.

Schlundt, J., Toyofuku, H., Jansen, J. & Herbst, S.A. (2004). Emerging food-borne zoonoses. *Revue Scientifique et Technique (International Office of Epizootics),* 23, 513-533.

Schultz, B., (2001). Irrigation, drainage and flood protection in a rapidly changing world. *Irrigation and Drainage, 50*, 261- 277.

Schutes, R.B.E. (2001). Artificial wetlands and water quality improvement. *Environment International*, 26, 441-447.

Schwaiger, K., Helmke, K., Hölzel, C.S. & Bauer, J. (2011). Comparative analysis of the bacterial flora of vegetables collected directly from farms and from supermarkets in Germany. *International Journal of Environmental Health Research*, 21, 161-172.

Scipioni, A., Mauroy, A., Vinje, J & Thiry, E. (2008). Animal noroviruses. *Veterinary Journal,* 178, 32-45.

Scott, T.M., Rose, J.B., Jenkins, T.M., Farrah, S.R. & Lukasik, J. (2002). Microbial source tracking: current methodology and future directions. *Applied and Environmental Microbiology,* 68, 5796-5803.

Seymour, I.J. & Appleton, H. (2002). Foodborne viruses and fresh produce. *Journal of Applied Microbiology*, 91, 759-773.

Shan, X.C., Wolffs, P. & Griffiths, M.W. (2005). Rapid and quantitative detection of hepatitis A virus from green onion and strawberry rinses by use of real-time reverse transcription-PCR. *Applied and Environmental Microbiology,* 71, 5624-5626.

Shan, X.C., Wolffs, P. & Griffiths, M.W. (2005). Rapid and quantitative detection of hepatitis A virus from green onion and strawberry rinses by use of real-time reverse-transcription-PCR. *Applied and Environmental Microbiology*, 71, 5624-5626.

Sharrer, M.J. & Summerfelt, S.T. (2007). Ozonation followed by ultraviolet irradiation provides effective bacteria inactivation in a freshwater recirculating system. *Aquacultural Engineering*, 37, 180-191.

Shirasaki, N., Matsushita, T., Matsui, Y., Oshiba, A. & Ohno, K. (2010). Estimation of norovirus removal performance in a coagulation-rapid sand filtration process by using recombinant norovirus VLPs. *Water Research*, 44, 1307-1316.

Shuval, H.I., Adin, A., Fattal, B., Rawitz, E. & Yekutiel, P. (1986). Wastewater irrigation in developing countries: health effects and technical solutions. *Technical Paper Number 51*. World Bank, Washington DC.

Simpson, G., Miller, R.F., Laxton, G.D. & Clement, W.R. (2000). A focus on chlorine dioxide: the 'ideal' biocide. Houston, Texas Unichem International Incorporated.

Smith, H.V., Grimason, A.M., Thitai, W.N., Smith, P.G., Jackson, M.H & Girdwood, R.W.A. (1993). Occurrence and removal of cryptosporidium spp oocysts and Gadia spp cysts in Kenyan waste stabilization pond. *Water Science & Technology*, 27(3-4), 97-104.

Smith, J.L. & Buchanan, R.L. (1992). *Shigella*. In: *Compendium of Methods for the Microbiological Examination of Foods* (Edited by Vanderzant, C. & Splittstoesser, D.F.). p423-431. Washington DC: American Public Health Association.

Smith, P.G. & J.S. Scott. (2002). *Dictionary of Water and Waste Management*. IWA Publishing. Butterworth: Oxford.

Solomon, E.B., Yaron, S. & Matthews, H.R. (2002). Transmission of *Escherichia coli* O157:H7 from contaminated manure and irrigation water to lettuce plant tissue and its subsequent internalization. *Applied and Environmental Microbiology*, 68(1), 397-400.

Souza, D.H.D., Sair, A., William, K., Papafragkou, E., Jean, J., Moore, C. & Jaykus, L. (2006). Persistence of calicivirus on the environmental surfaces and their transfer to food. *International Journal of Food Microbiology*, 180, 84-91.

Spradling, P.R., Martin, A. & Feinstone, S.M. (2009). Hepatitis A virus. In: *Clinical Virology*. 3rd ed. (Edited by D.D. Richards, R.J. Whitley, F.G. Hayden). p1083-1108. Washington DC: ASM Press.

Statistics South Africa (2005). Census of commercial agriculture 2002: Financial and production statistics. Report No. 11-02-02(2002). p54-60. Statistics South Africa: Pretoria.

Steel, M., Mahdi, A. & Odumeru, J. (2005). Microbial assessment of irrigation water used for production of fruit and vegetables in Ontario, Canada. *Journal of Food Protection, 68(7),* 1388-1392.

Steele, M. & Odumeru, J. (2004). Irrigation water as source of foodborne pathogens on fruit and vegetables. *Journal of Food Protection*, 67(12), 2839-4901.

Stevens, D.P. (1982). Giardiasis: host-pathogen biology. *Reviews of Infectious Diseases,* 4, 851-859

Stine, S. (2004). Survival of enteric pathogens on the surface of fresh produce. PhD Dissertation, University of Arizona, USA.

Stine, S.W., Song, I., Choi, C.Y. & Gerba, C.P. (2005). Application of microbial risk assessment to the development of standards for enteric pathogens in water used to irrigate fresh produce. *Journal of Food Protection,* 68(5), 913- 918.

Stopforth, J.D., Kottapalli, B., & Sofos, J.N. (2010). Interventions for hazard control in foods preharvest. In: *Pathogens and toxins in foods: challenges and interventions* (Edited by Juneja, V.K. & Sofos, J.N.) p357-395. Washington DC: ASM Press.

Strauss, M. (1996). Health (pathogen) considerations regarding the use of human waste in aquaculture. *Environmental Research Forum*, 5-6, 83-98.

Swaminathan, A., Torresi, J., Schlagenhauf, P., Thursky, K., Wilder-Smith, A., Connor, B.A., Schwartz, E., Von Sonnenberg, F., Keystone, J. & O'Brien, D.P. (2009). A global study of pathogens and host risk factors associated with infectious gastrointestinal disease in returned international travellers. *Journal of Infection,* 59, 19-27.

Szostakowska, B., Kruminis-Lozowska, W., Racewicz, M., Knight, R., Tamang, L., Myjak, P. & Graczyk, T.K. (2004). *Cryptosporidium parvum* and *Giardia lamblia* recovered from flies on a cattle farm and in a landfill. *Applied and Environmental Microbiology,* 70, 3742-3744.

Taban, B.M. & Halkman, A.K. (2011). Do leafy green vegetables and their ready-to-eat (RTE) salads carry a risk of foodborne pathogens? *Anaerobe*, 17, 286-287.

Tallon, P., Magajna, B., Lofranco, C. & Leung, K.T. (2005). Microbial indicators of faecal contamination in water: a current perspective. *Water, Air, and Soil Pollution,* 166, 139-166.

Taylor, M.B. (2011). Water- and foodborne viruses: Current developments. *Continuing Medical Education,* 29, 207-209

Taylor, M.B., Cox, N., Very, M.A. & Grabow, W.O.K. (2001). The occurrence of hepatitis A and astroviruses in selected river and dam waters in South Africa. *Water Research*, 35, 2653-2660.

Taylor, M.B., Schildhauer, C.I., Parker, S., Grabow, W.O.K., Jiang, X., Estes, M.K. & Cubitt, W.D. (1993). Two successive outbreaks of SRSV-associated gastroenteritis in South Africa. *Journal of Medical Virology*, 41, 18-23.

Teltsch, B. & Katzenelson, E. (1978). Airborne enteric bacteria and viruses from spray irrigation with wastewater. *Applied and Environmental Microbiology, 35(2),* 290-296.

Templeton, T.J., Lancto, C.A., Vigdorovich, V., Liu, C., London, N.R., Hadsall, K.Z. & Abrahamsen, M.S. (2004). The *Cryptosporidium* oocyst wall protein is a member of a multigene family and has a homolog in *Toxoplasma*. *Infection and Immunity,* 72, 980-987.

Teunis, P.F.M., Moe, C.L., Liu, P., Miller, S.E., Lindesmith, L., Baric, R.S., Le Pendu, J & Calderon, R.L. (2008). Norwalk virus: How infectious it is. *Journal of Medical Virology* 80, 1468-1476.

Thompson, M.W. (1999). South African natural land cover database project. CSIR. Internet Document – www.sac.co.za.

Thompson, R.C.A. (2000). Giardiasis as a re-emerging infectious disease and its zoonotic potential. *International Journal for Parasitology,* 30, 1259-1267.

Thornton, A.C., Jennings-Conklin, K.S. & McCormick, M.I. (2004). Noroviruses: agents in outbreaks of acute gastroenteritis. *Disaster Management and Response*, 2, 4-9.

Tiefenthaler, L., Stein, E.D. & Schiff, K.C. (2011). Levels and patterns of fecal indicator bacteria in stormwater runoff from homogenous land use sites and urban watersheds. *Journal of Water and Health*, 9, 279-290.

Toze, S. (2006). Reuse of effluent water – benefits and risks. *Agricultural Water Management*, 80, 147-159.

Tuan Zainazor, C., Hidayah, M.S.N., Chai, L.C., Tunung, R., Ghazali, F.M. & Son, R. (2010). The scenario of norovirus contamination in food and food handlers. *Journal of Microbiology and Biotechnology,* 20, 229-237.

Turantas, F. (2002). Incidence of faecal streptococci as an indicator of sanitation in ice cream and frozen vegetables. *International Journal of Food Science and Technology,* 37, 239- 243.

Turon, C. Comas, J. & Poch, M. (2009). Constructed wetland clogging: A proposal for the integration and reuse of existing knowledge. *Ecological Engineering*, 35, 1710-1718.

Tzipori, S. & Ward, H. (2002). Cryptosporidiosis: biology, pathogenesis and disease. *Microbes and Infection,* 4, 1047-1058.

United Nations (2000). We the Peoples: The Role of the United Nations in the 21st Century. United Nations Millennium Summit Report of the Secretary-General of the United Nations, New York, USA.

United Nations (2003). Human Development Report. Millennium Development Goals: A compact among nations to end human poverty. New York: United Nations Development Programme. NY.

USDA (United States Department of Agriculture) (1998). The 1998 farm and ranch irrigation survey: census of agriculture. Internet Document – www.nass.usda.gov.innopac.up.ac.za:80/census97.

Van Den Ende, G.M. (1986). *Cryptosporidium* among black children in hospital in South Africa. *Journal of Infection,* 13, 25-30.

Van Elfen, J. (2001). Cholera. In: *Dokter in die Huis* (Edited by Du Toit, D.). p108-109. Cape Town: Tafelberg Publishers.

Van Der Merwe, M. & Britz, T.J. (1994). Characterisation and numerical analysis of the microbial community in raw Baker's yeast factory effluent. *Water SA*, *20*(2), 161-171.

van Heerden, J., Ehlers, M.M., Heim, A. & Grabow, W.O.K. (2005). Prevalence, quantification and typing of adenoviruses detected in river and treated drinking water in South Africa. *Journal of Applied Microbiology,* 99, 234-242

Van Zyl, W.B., Page, N.A., Grabow, W.O.K., Steele, A.D. & Taylor, M.B. (2006). Molecular epidemiology of group A rotaviruses in water sources and selected raw vegetables in southern Africa. *Applied and Environmental Microbiology,* 72, 4554-4560.

Van Zyl, W.B., Williams, P.J., Grabow, W.O.K. & Taylor, M.B. (2004). Application of a molecular method for the detection of group a rotaviruses in raw and treated water. *Water Science & Technology*, 50, 223-228

Vaz da, S.M., Mara, D.D. & Vargas-Lopez, C.E., (1991). Residual faecal contamination on effluent-irrigated lettuces. *Water Science & Technology, 24(9),* 89-94.

Velaudapillai, T, Niles, G.R. & Nagaratnam, W. (1969). *Salmonella*, *Shigella*, and enteropathogenic *Escherichia coli* in uncooked food. *Journal of Hygiene,* 67, 187-91.

Velusamy, V., Arshak, K., Korostynska, O., Oliwa, K. & Adley, C. (2010). An overview of foodborne pathogen detection: In the perspective of biosensors. *Biotechnology Advances*, *28*(2), 232-254.

Venter, J.M.E. (2004). The incidence of hepatitis A virus in selected water sources and associated risk of infection in South Africa. MSc thesis. Pretoria: University of Pretoria

Venter, J.M.E., van Heerden, J., Vivier, J.C., Grabow, W.O.K. & Taylor, M.B. (2007). Hepatitis A virus in surface water in South Africa: What are the risks? *Journal of Water and Health*, 5, 229-240.

Verheyen, J., Timmen-Wego, M., Laudien, R., Boussaad, I., Sen, S., Koc, A,, Uesbeck, A., Mazou, F. & Pfister, H. (2009). Detection of adenoviruses and rotaviruses in drinking water sources used in rural areas of Benin, West Africa. *Applied and Environmental Microbiology,* 75, 2798-2801.

Vetterling, J.M. & D.J. Doran. (1969). Storage polysaccharide in coccidial sporozoites after excystation and penetration of cells. *Journal of Protozoology,* 16, 772-775.

Walmsley, R.D., Walmsley, J.J. & Silberbauer, M. (1999). Freshwater Systems and Resources. In: *National State of the Environment Report*. Department of Environmental Affairs and Tourism. p. i. South Africa.

Walter, J.E. & Mitchell, D.K. (2000). Role of astrovirus in childhood diarrhea. *Current Opinions in Pediatrics*, 31, 275-279.

Wand, H., Vacca, G., Kuschk, P., Krüger, M. & Kästner, M. (2007). Removal of bacteria by filtration in planted and non-planted sand columns. *Water Research*, 41, 159-167.

Wang, G., Zhao, T. & Doyle, M.P. (1996). Fate of enterohemorrhagic *Escherichia coli* O157:H7 in bovine feces. *Applied Environmental Microbiology,* 62(7), 2567-2570.

Wang, Q-H., Souza, M., Funk, J.A., Zhang, W. & Saif, L. (2006). Prevalence of noroviruses and sapoviruses in swine of various ages determined by reverse transcription-PCR and microwell hybridization assays. *Journal of Clinical Microbiology*, 44, 2057-2062.

Water Research Commission. (1993). *Water and Waste Management in the Wine Industry*. *WRC Project No. 145 TT 51/90.* Water Research Commission, Pretoria, South Africa.

WRC (Water Research Commission) (2003). *The Evaluation of Alternative Disinfection Processes for the Removal of Protozoan Oocysts and Cysts and other Micro-organisms, in the Treatment of Final Wastewater*. *WRC Project No. 1030/1/03*, Water Research Commission, Pretoria, South Africa.

WCASA (Water Conservation Alliance of Southern Arizona) (2004). Residential graywater reuse study. Tucson: WCASA.

Weiss, J. & Seeliger, H.P. (1975). Incidence of *Listeria monocytogenes* in nature*. Applied Microbiology, 29*, 29-32.

Weissinger, W.R., Chantarapanont, W. & Beuchat, L.R. (2000). Survival and growth of *Salmonella baildon* in shredded lettuce and diced tomatoes and effectiveness of chlorinated water as a sanitizer*. International Journal of Food Microbiology,* 62, 123-131.

Wenhold, F. & Faber, M. (2006). *Nutritional Status of South Africans with Specific Reference to Malnutrition: Links to Agriculture and Water*. WRC Report K5/1579/4. October 2006. Water Research Commission, Pretoria.

WESGRO (2003). Fruit sector fact sheet. Western Cape Trade and Investment Promotion Agency. p2-3.

WESGRO (2006). Fruit processing sector brief. Western Cape Trade and Investment Promotion Agency. p36-40.

Wheeler, C., Vogt, T.M., Armstrong, G.I., Vaughan, G., Weltman, A., Nainan, O.V., Dato, V., Xia, G., Waller, K., Amon, J., Lee, T.M., Hembree, C., Evenson, S., Ruta, M.A., Williams, I.T., Flore, A.E. & Bell, B.P. (2005). An outbreak of hepatitis A associated with green onions. *New England Journal of Medicine*, 353, 890-897.

WHO (World Health Organisation) (1989). Health guidelines for the use of wastewater in agriculture and aquaculture. Technical Report Series 778. World Health Organisation: Geneva, Switzerland.

WHO (World Health Organisation) (2000). Human Health and dams, the World Health Organisation. Submission to the World Commission on Dams (WCD); Geneva, World Health Organisation (Document WHO/SDE/WSH/00.01.

WHO (World Health Organisation) (2001a). Health impacts assessment (HIA), report of an inter regional meeting on the harmonization and mainstreaming of HIA in the World Health Organisation of a partnership meeting on the institutionalization of HIA capacity building in Africa, Arusha, 31 October to 3 November 2000. Geneva. World Health Organisation.

WHO (World Health Organisation) (2002). Food safety and foodborne illnesses, Fact Sheet No 236. Geneva: World Health Organization, Switzerland.

WHO (World Health Organisation) (2003). The present state of foodborne disease in QECD countries. Geneva, Switzerland: World Health Organisation. Report.

WHO (World Health Organisation) (2004). *Guidelines for Drinking Water Quality*, 3rd ed. Geneva: World Health Organization, Switzerland.

WHO (World Health Organisation) (2006). *WHO Guidelines for the Safe Use of Wastewater, Excreta and Greywater: Volume II, Wastewater Use in Agriculture*. p1-176. Geneva: World Health Organization, Switzerland.

WHO (World Health Organization) (2001b). *Foreword.* Water Quality – Guidelines, Standards and Health: Assessment of risk and risk management for water-related infectious disease. Eds. Fewtrell L, Bartram J. IWA Publishing: London (on behalf of WHO). pvii-viii

WHO (World Health Organization) (2008). Protozoan parasites (*Cryptosporidium*, *Giardia*, *Cyclospora*). www.who.int/entity/water_sanitation/admicrob5. Accessed 18 August 2008.

Wisniewski, C. (2007). Membrane bioreactor for water reuse. *Desalination*, 203, 15-19.

Wolfe, S.M. (1992). Giardiasis. *Clinical Microbiology Reviews,* 5, 93-100.

Wood, J.D., Bezanson, G.S., Gordon, R.J. & Jamieson, R. (2010). Population dynamics of *Escherichia coli* inoculated by irrigation into the phyllosphere of spinach grown under commercial production conditions. *International Journal of Food Microbiology*, 143, 198-204.

Wood, R.C., Hedberg, C. & White, K. (1991). A multistate outbreak of Salmonella Javiana infections associated with raw tomatoes. In: *CDC Epidemic Intelligence Service*, 40th Annual Conference, Atlanta. US Department of Health and Human Services, Public Health Service. p69-200.

Wu, J., Rodriguez, R.A., Stewart, J.R. & Sobsey, M.D. (2011). A simple and novel method for recovering adenovirus 41 in small volumes of source water. *Journal of Applied Microbiology,* 110, 1332-1340.

Wyn-Jones, A.P. & Sellwood, J. (2001). Enteric viruses in the aquatic environment. *Journal of Applied Microbiology,* 91, 945-962.

Wyn-Jones, A.P. (2007). The detection of waterborne viruses. In: Human Viruses in Water (Edited by A. Bosch). p177-203. Amsterdam: Elsevier B.V.

Xu, P., Janex, M.L., Savoye, P., Cockx, A. & Lazarova, V. (2002). Wastewater disinfection by ozone: main parameters for process design. *Water Research*, 36, 1043-1055.

Yuk, H-Y., Yoo, M-Y., Yoon, J.W., Moon, K.D., Marshall, D.L. & Oh, D.H. (2006). Effect of combined ozone and organic acid treatment for control of *Escherichia coli* O157:H7 and *Listeria monocytogenes* on lettuce. *Journal of Food Science*, 71 (3), 83-87.

Zachoval, R. & Deinhardt, F. (1993). Natural history and experimental models. In: *Viral hepatitis: Scientific basis and clinical management* (Edited by Zuckerman, A.J. & Thomas, H.C.). p35-53. New York: Churchill Livingstone Inc.

Zamxaka, M., Pironcheva, G., & Muyima, N.Y.O. (2004). Bacterial community patterns of domestic water sources in the Gogogo and Nkonkobe areas of the Eastern Cape Province, South Africa. *Water SA*, *30*(3), 341-346.

Zheng, D-P, Ando, T., Fankhauser, R.L., Beard, R.S., Glass, R.I. & Monroe, S.S. (2006). Norovirus classification and proposed strain nomenclature. *Virology,* 346, 312-323.

Zheng, H., Zhu, G., Jiang, S., Tshukudu, T., Xiang, X., Zhang, P. & He, Q. (2011). Investigations of coagulation-flocculation process by performance optimization, model prediction and fractal structure of flocs. *Desalination*, 269, 148-156.

Zhou, T., Mckellar, R., Jones, S., Fisher, P., Zhou, H., Knight, K. & Li, X. Z. (2005). Irrigation treatments and the presence of *Escherichia coli* on Ontario field straw berries. *Food Protection Trends,* 25(8)*,* 622-625.

Zimmerman, F.J. (2000). Barriers to participation of the poor in South Africa's land redistribution. *World Development,* 28 (8)*,* 1439-1460.

CHAPTER 2

RESEARCH OUTPUTS

2.1 PUBLICATIONS

2.1.2 Publications

Bidawid, S., Bosch, A., Cook, N., Greening, G., Taylor, M. & Vinjé, J. (2009). Editorial. *Food and Environmental Virology*. 1, 1-2.

Bosch, A., Sanchez, G., Abbaszadegan, M., Carducci, A., Guix, S., Le Guyader, F.S., Netshikweta, R., Pinto, R.M., Van Der Poel, W.H.M., Rutjes, S., Sando, D., Taylor, M.B., Van Zyl, W.B., Rodriguez-Lazaro, D. & K. Sellwood, J. (2011). Analytical methods for virus detection in water and food. *Food Analytical Methods,* 4, 4-12.

Gemmell, M.E. & Schmid, S. (2012). Microbiological assessment of river water used for the irrigation of fresh produce in a sub-urban community in Sobantu, South Africa. *Food Research International,* **47**, 300-305.

Ijabadeniyi, A.O., Debusho, L.K., Vanderlinde, M. & Buys, E.M. (2011). Irrigation water as a potential preharvest source of bacterial contamination of vegetables. *Journal of Food Safety,* **31**, 452-461.

Ijabadeniyi, O.A., Minnaar, A. & Buys, E.M. (2011). Effect of attachment time followed by chlorine washing on the survival of inoculated Listeria monocytogenes on tomatoes and spinach. *Journal of Food Quality* 34, 133-141.

Kiulia, N.M., Netshikweta, R., Page, N.A., van Zyl, W.B., Kiraithe, M.M., Nyachieo, A., Mwenda, J.M. & Taylor, M.B. (2010). The detection of enteric viruses in selected urban and rural river water and sewage in Kenya, with special reference to rotaviruses. *Journal of Applied Microbiology*, 109, 818-828.

Mans, J., de Villiers, C. & Taylor, M.B. (2009). Norovirus infection as a cause of acute gastroenteritis in paediatric patients in South Africa: underestimated or overlooked? [Abstract]. *Southern African Journal of Epidemiology and Infection*; 24, 29.

Mans, J., de Villiers, J.C., du Plessis, N., Avenant, T. & Taylor, M.B. (2010). Emerging norovirus GII.4 2008 variant detected in hospitalised paediatric patients in South Africa. *Journal of Clinical Virology*, 49, 258-264.

Potgieter, N., de Beer, M.C., Taylor, M.B. & Steele, D.A. (2012). The prevalence and diversity of rotavirus strains in children with acute diarrhoea from rural communities in the Limpopo Province, South Africa. *Journal of Infectious Diseases,* 202 [Suppl]:S148-S155.

Taylor, M.B. 2011. Water and food-borne viruses: current developments. *Continuing Medical Education*, 29, 207-209.

2.1.2 Book chapters

Gemmell, M.E., & S. Schmidt. (2010). Potential links between irrigation water quality and microbiological quality of food in subsistence farming in KwaZulu-Natal, South Africa. In: *Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology*. Ed. A. Méndez-Vilas. Volume. 2:1190-1195. Formatex, Spain. ISBN: 978-84-614-6195-0.

2.2 CONFERENCES

2.2.1 National conferences

Brand, A., G.O. Sigge & T.J. Britz. (2009). Assessment of microbiological quality of the Berg River as a source of irrigation water for raw and minimally processed crops. EnviroWater Congress. Stellenbosch. 2-4 March 2009.

Brand, A.S., Sigge, G.O., Niemann, N., Kikine, T. & Britz, T.J. (2010). Comparison of Colilert-18® with traditional multiple tube fermentation for the enumeration of faecal coliforms and E.coli from irrigation water used on minimally processed produce. 15th IUFoST World Congress of Food Science and Technology, Cape Town, South Africa, 22-26 August 2010.

Britz, T.J. (2009). Is polluted irrigation water a threat – give your profits the trots! *Keynote Speaker*. *Bien Donné Agricultural Expo 2009.* Bien Donné. 5 November 2009.

Britz, T.J., A. Ackerman & G.O. Sigge. (2009). Microbially polluted irrigation water: Potential impact on food safety. *Keynote Speaker*. EnviroWater Congress, Stellenbosch. 2-4 March 2009.

de Ridder, G.A., Taylor, M.B. & Cook, N. (2011). Amplification controls for the real-time PCR analysis of noroviruses in water and food: which is best [Poster]. 2nd Regional Conference of the Southern African Young Water Professionals, 3-5 July 2011. CSIR International Convention Centre, Pretoria, South Africa.

de Ridder, G.A., Taylor, M.B. & Cook, N. (2011). Amplification controls for the real-time PCR detection of noroviruses in water and food samples [Presentation]. Faculty Day, Faculty of Health Sciences, University of Pretoria 30-31 August 2011. HW Snyman Building North, Pretoria.

Gemmell, M.E. & S. Schmidt. (2011). Microbiological quality of the Msunduzi River, KwaZulu-Natal, commonly used for the irrigation of minimally processed crops. Young Water Professionals Conference, Pretoria, South Africa, 3-5 July 2011.

Gemmell, M.E., & S. Schmidt. (2010). Investigation into potential links between irrigation water quality and microbiological quality of food. [oral presentation] Faculty Research Day, Faculty of Science and Agriculture, University of KwaZulu-Natal, Pietermaritzburg, September 23, 2010.

Huisamen, N., G.O. Sigge & T.J. Britz. (2009). Investigation of the water quality of the Eerste and Plankenburg Rivers as irrigation sources and the impact on the safety of fresh produce. EnviroWater Congress. Stellenbosch. 2-4 March 2009.

Kikine, T, G.O. Sigge, & T.J. Britz. (2010). Assessment of the Plankenburg River as an acceptable irrigation source for produce that will be consumed raw or undergo minimal processing. 15th World Congress of Food Science and Technology. Cape Town, South Africa, 22-26 August 2010.

Mans, J., Page, N.A. & Taylor, M.B. (2010). Diverse norovirus genotypes identified in individuals affected by a multifactorial waterborne gastroenteritis outbreak in Mpumalanga, 2007. [Poster]. Faculty Day, Faculty of Health Sciences, University of Pretoria 17-18 August 2010: HW Snyman Building, Pretoria.

Mans, J., Page, N.A. & Taylor, M.B. (2010). Diverse norovirus genotypes identified in individuals affected by a multifactorial waterborne gastroenteritis outbreak in Mpumalanga, 2007 [Poster]. Pathvine 2010: 50th Annual Conference of the Federation of South African Societies of Pathology (FSASP) 2-5 September 2010: Lord Charles Hotel, Somerset West, South Africa.

Mans, J., Netshikweta, R., Magwalivha, M., van Zyl, W.B. & Taylor, M.B. (2009). Molecular detection and characterisation of noroviruses occurring in selected surface waters in South Africa [Presentation]. Faculty Day, Faculty of Health Sciences, University of Pretoria 18-19 August 2009: HW Snyman Building, Pretoria.

Mans, J., Page, N.A. & Taylor, M.B. (2010). Diverse norovirus genotypes identified in individuals affected by a multifactorial waterborne gastroenteritis outbreak in Mpumalanga, 2007 [Poster]. Pathvine 2010: 50th Annual Conference of the Federation of South African Societies of Pathology (FSASP) 2-5 September 2010: Lord Charles Hotel, Somerset West, South Africa.

Netshikweta, R., van Zyl, W.B., Mans, J. & Taylor, M.B. (2009). Optimisation and application of real-time RT-PCR techniques for the detection of selected food and waterborne viruses [Poster]. Faculty Day, Faculty of Health Sciences, University of Pretoria 18-19 August 2009: HW Snyman Building, Pretoria.

Netshikweta, R., van Zyl, W.B., Mans, J. & Taylor, M.B. (2010). The application of real-time RT-PCR for the detection of selected enteric viruses in irrigation water in South Africa [Presentation].1st Regional Conference of the Southern African Young Water Professionals 19-20 January 2010: CSIR International Convention Centre, Pretoria, South Africa.

Ngiba, F. & S. Schmidt. (2010). Assessment of river water used in KwaZulu-Natal for irrigation for the presence of *Staphylococcus aureus*. [oral presentation] 23rd SASM (South African Society for Microbiology) KZN symposium, Durban, October 29, 2010.

Niemann, N., Brand, A.S., Sigge, G.O. & Britz, T.J. (2010). Investigating the microbial loads of water from the upper Berg River used for the irrigation of fresh produce. 15th IUFoST World Congress of Food Science and Technology, Cape Town, South Africa, 22-26 August 2010.

Niemann, N., G.O. Sigge & T.J. Britz. (2010). Monitoring microbial loads in the irrigation water of the Upper Berg River. 1st Regional Conference of Southern Africa. Young Water Professionals (YWP). CSIR International Convention Centre, Pretoria, South Africa. 19-20 January 2010.

Shelembe, M.R. & S. Schmidt. (2010). Evaluation of the MPN (most probable number) method to detect faecal coliforms in river water used for domestic consumption and irrigation in KwaZulu-Natal. [oral presentation] 23rd SASM (South African Society for Microbiology) KZN symposium, Durban, October 29, 2010.

Taylor, M.B. (2010). Emerging and re-emerging viral infections: the whys and the wherefores [Invited lecture]" South African Water Fitness Association (SAWFA) Central workshop 20 February 2010 Mondeor, Johannesburg

van Zyl, W.B. & Taylor M.B. (2011). Detection of human picobirnaviruses in South African surface and drinking water [Presentation]. Faculty Day, Faculty of Health Sciences, University of Pretoria 30-31 August 2011. HW Snyman Building North, Pretoria.

van Zyl, W.B. & Taylor, M.B. (2009). Detection and characterisation of human polyomaviruses in South African untreated and treated water samples [Presentation]. Faculty Day, Faculty of Health Sciences, University of Pretoria 18-19 August 2009: HW Snyman Building, Pretoria

Yasvoin, T., Mans, J. & Taylor, M.B. (2011). First detection of human sapoviruses in river water in South Africa [Presentation]. 2nd Regional Conference of the Southern African Young Water Professionals 3-5 July 2011: CSIR International Convention Centre, Pretoria, South Africa.

Yasvoin, T., Mans, J. & Taylor, M.B. (2011). First detection of human sapoviruses in river water in South Africa [Presentation]. Faculty Day, Faculty of Health Sciences, University of Pretoria 30-31 August 2011. HW Snyman Building North, Pretoria.

Yasvoin, T., Mans, J., Page, N., & Taylor, M.B. (2011). Genetic diversity of human sapoviruses in South Africa: First report [Presentation]. Virology Africa 2011 organised by the Institute of Infectious Disease and Molecular Medicine, University of Cape Town and the Poliomyelitis Research Foundation, 29 November-2 December 2011, UCT Graduate School of Business, Portswood Rd, V&A Waterfront, Cape Town.

2.2.2 International conferences

Brand, A.S., G.O. Sigge & T.J. Britz. (2009). Assessment of the microbiological quality of the upper Berg River (South Africa) as an irrigation source for raw and minimally processed produce. 15th International Symposium on Health Related Water Microbiology, Naxos, Greece. May 31-June 5 2009

Brand, A.S., G.O. Sigge., C. Lamprecht, & T.J. Britz. (2010). Ubiquity and specificity of MTF for enumeration of *E. coli* from water used to irrigate fresh produce. 22nd International FoodMicro Congress, Copenhagen, Denmark. 30 August-3 September.

Brand, A.S., Sigge, G.O., Lamprecht, C. & Britz, T.J. (2010). Ubiquity and specificity of MTF for enumeration of *E.coli* from water used to irrigate fresh produce. 22nd International ICFMH Symposium – Food Micro 2010, Copenhagen, Denmark, 30 August-3 September 2010.

Brand, A.S., Sigge, G.O., Niemann, N., Kikine, T. & Britz, T.J. (2010). Comparison of Colilert-18® with traditional multiple tube fermentation for the enumeration of faecal coliforms and E.coli from irrigation water used on minimally processed produce. 15th IUFoST World Congress of Food Science and Technology, Cape Town, South Africa, 22-26 August 2010.

Britz, T.J. & Sigge, G.O. (2010). Food Safety and irrigation water. Water Research Commission, 40 Year Celebration Conference. Emperor's Palace, Kempton Park, South Africa. 31 August-1 September 2011.

Britz, T.J., A. Ackerman & G.O. Sigge. (2009). *Escherichia coli* survival profiles on beans, peas and tomatoes irrigated with polluted river water. 15th International Symposium on Health Related Water Microbiology, Naxos, Greece. May 31-June 5 2009

Crous, M., Sigge, G.O. & Buys, E. (2010). Survival of *Listeria monocytogenes* on spray irrigated broccoli. 15th IUFoST World Congress of Food Science and Technology, Cape Town, South Africa, 22-26 August 2010.

Duhain, G., Minnaar, A. & Buys, E.M. (2011). Cryptosporidium viability on vegetables. Poster presented at Copenhagen, Denmark.

Gemmell, M.E. & S. Schmidt. (2011). Microbiological assessment of river water and irrigated produce in subsistence farming in KwaZulu-Natal (South Africa). 111th American Society of Microbiology General Meeting, New Orleans, United States of America, 21-24 May 2011.

Gemmell, M.E. & S. Schmidt. (2010). Investigation into potential links between irrigation water quality and microbiological quality of food in KwaZulu-Natal. [poster presentation] IUFoST 15th World congress of food science and technology, Cape Town, August 22-26, 2010.

Huisamen, N., G.O. Sigge & T.J. Britz. (2009). Investigating the water quality of the Plankenburg and Eerste Rivers as irrigation sources and the subsequent impact on the quality of crops produced with the intention to be consumed in a minimally processed state. 15th International Symposium on Health Related Water Microbiology, Naxos, Greece. May 31-June 5 2009

Kikine, T., Sigge, G.O. & Britz, T.J. (2010). Assessment of the Plankenburg River as an acceptable irrigation source for fresh produce consumed raw or undergo minimal processing. 15th IUFoST World Congress of Food Science and Technology, Cape Town, South Africa, 22-26 August 2010.

Kiulia, N.M., Netshikweta, R., Page, N.A., van Zyl, W., Kiraithe, M.M., Mwenda, J.M. & Taylor, M.B. (2010). Detection of rotavirus in surface water in Kenya: A pilot study [Poster]. (2010). "Realizing the Impact of Rotavirus Vaccines" 9th International Rotavirus Symposium 2-3 August 2010 Sandton Sun Convention Centre, Johannesburg, South Africa

Kiulia, N.M., Netshikweta, R., Page, N.A., van Zyl, W., Kiraithe, M.M, Mwenda, J.M. & Taylor, M.B. (2010). Detection of rotavirus in surface water in Kenya: A pilot study [Poster]. 6th African Rotavirus Symposium 4 August 2010 National Institute for Communicable Diseases, Johannesburg, South Africa

Mans, J., Netshikweta, R., Magwalivha, M., van Zyl, W.B. & Taylor, M.B. (2010). Human caliciviruses detected in river water in a densely populated area of Gauteng, South Africa [Poster presentation]. 4th International Conference on Caliciviruses 16-19 October 2010 Hotel Santa Cruz Plaza, Santa Cruz, Chile.)

Mans, J., de Villiers, J.C., du Plessis, N.M., Avenant, T. & Taylor, M.B. (2010). Characterisation of noroviruses identified in hospitalised paediatric patients in Pretoria, South Africa during 2008 [Presentation]. 4th International Conference on Caliciviruses 16-19 October 2010 Hotel Santa Cruz Plaza, Santa Cruz, Chile.

Mans, J., Netshikweta, R., Magwalivha, M., van Zyl, W.B. & Taylor, M.B. (2010). Human caliciviruses detected in river water in a densely populated area of Gauteng, South Africa [Poster presentation]. 4th International Conference on Caliciviruses 16-19 October 2010 Hotel Santa Cruz Plaza, Santa Cruz, Chile.

Mans. J., Netshikweta,. R., Magwalivha, M., van Zyl, W.B. & Taylor, M.B. (2009). Molecular detection and characterisation of noroviruses occurring in selected surface waters in South Africa [Presentation]. WaterMicro 2009 15th International Symposium on Health Related Water Microbiology: 31 May-06 June 2009: Naxos, Greece.

Mans, J., Nadan, S., Netshikweta, R., Page, N. & Taylor, M.B. (2011). Norovirus genogroup II diversity in children with diarrhoea in South Africa in 2009 [Poster]. Vaccines for Enteric Diseases, VED 2011, 14-16 September 2011. The Novotel Cannes Montfleury, Cannes, France.

Netshikweta, R., van Zyl, W.B., Mans, J., Wolfaardt, M. & Taylor, M.B. (2010).The application of real-time RT-PCR for the detection of selected enteric viruses on fresh produce in South Africa [Poster] IUFoST 2010 15th World Congress of Food Science & Technology 22-26 August 2010: Cape Town International Convention Centre, South Africa.

Niemann, N., Brand, A.S., Sigge, G.O. & Britz, T.J. (2010). Investigating the microbial loads of water from the upper Berg river used for the irrigation of fresh produce. 15th IUFoST World Congress of Food Science and Technology, Cape Town, South Africa, 22-26 August 2010.

Potgieter, N., Thoma, I. & Barnard, T.G. (2009). The microbiological contamination of minimal processed food products from rural subsistence farmers in the Limpopo Province of South Africa. Water and Health Conference, University of North Carolina, North Carolina, USA 01 -05 October 2011.

van Zyl, W.B. (2011). “Principles of the real-time (RT)-PCR assay” and “Application of real-time PCR in rotavirus detection and data interpretation” WHO funded 11th African Rotavirus Network Workshop and hosted by RRL-South Africa/MRC Diarrhoeal Pathogens Research Unit. 07 June 2011 Department of Virology, University of Limpopo (Medunsa Campus), Pretoria, South Africa

van Zyl, W.B. & Taylor, M.B. (2009). Detection and characterisation of human polyomaviruses in South African untreated and treated water samples [Poster]. WaterMicro 2009 15th International Symposium on Health Related Water Microbiology: 31 May-06 June 2009: Naxos, Greece.

2.3 SEMINARS

De Ridder, G. (2010). The development and application of multiplex real-time RT-PCR assays for the detection of enteric viruses in food and water samples with special reference to berry fruits [Progress report] Research meeting, Department of Medical Virology, University of Pretoria, Pretoria 17 September 2010.

Yasvoin, T. (2011). Genetic diversity of human sapoviruses in South Africa [Progress report]. Research meeting, Department of Medical Virology, University of Pretoria, Pretoria 11 November 2011.

2.4 WORKSHOPS AND TECHNOLOGY TRANSFER

Workshop on the standardized research methodology for the assessment of the impact of irrigation water on food safety. 25 May 2007, held at the WRC Offices, Pretoria. The following researchers attended: G. Backeberg, T.J. Britz, G.O. Sigge, E.M. Buys, M.B. Taylor and N. Potgieter. Seven post-graduate students from the different research teams also attended.

From 11-16 January 2009 Prof Albert Bosch and Dr Rosa Pintó from the Enteric Virus Group, Dept. Microbiology, University of Barcelona, Spain visited the laboratory of Prof Maureen Taylor to review their collaborative work on the development and optimisation of methods for the recovery and detection of food- and waterborne enteric viruses.

From 25-30 January 2009 Mr Rembuluwani Netshikweta visited the laboratory of Prof Trevor Britz, Department of Food Science, University of Stellenbosch, where he trained students in the techniques used for the recovery of viruses from water and fresh produce samples.

From 22 April-12 May 2010. Dr Nigel Cook from the Food and Environmental Research Agency, Sand Hutton, United Kingdom visited the laboratory of Prof Maureen Taylor to develop an Internal Amplification Control for the NoV GII real-time RT-PCR assay for the detection of NoV in food and water samples. On 23 April 2010 he presented a lecture “Importance of harmonisation of nucleic acid testing for foodborne pathogens” to staff and post-graduate students of the Depts. of Medical Virology, Medical Microbiology and Microbiology and Plant Pathology, University of Pretoria. The visit was sponsored by the University of Pretoria, Cost Action 929 of the European Cooperation in

Science and Technology and the European Commission Research Framework 5 Network of Excellence "Monitoring and Quality Assurance in the Food Supply Chain (MoniQA)"**.**

From 8 May-12 May 2010. Dr Nigel Cook from the Food and Environmental Research Agency, Sand Hutton, United Kingdom visited the laboratory of Prof Trevor Britz and presented a lecture "Importance of harmonisation of nucleic acid testing for foodborne pathogens" to staff and post-graduate students of the Department of Food Science. The costs of the visit was sponsored by the University of Pretoria, Cost Action 929 of the European Cooperation in Science and Technology and the European Commission Research Framework 5 Network of Excellence "Monitoring and Quality Assurance in the Food Supply Chain (MoniQA)"**.**

From 15-17 November 2010 Prof MB Taylor visited the laboratory of Prof A Bosch, Department of Microbiology, University of Barcelona, Barcelona, Spain where the framework for joint publication on the recovery and detection of viruses in irrigation water and fresh produce samples was discussed.

As part of an on-going National Research Foundation South African Co-operation for Scientific Research and Technological Development-funded collaborative project between the Department of Medical Virology, University of Pretoria, South Africa and the Enteric Viruses Group, Institute of Primate Research (IPR) & Kenya Medical Research Institute (KEMRI) (Kenya), Dr Jason Mwenda and Mr Nicholas Kiulia were trained in the techniques used for the recovery and detection of viruses from water and fresh produce samples.

2.5 GENERAL PRESENTATIONS (Keynote and Invited)

Britz, T.J. (2009). Is polluted irrigation water a threat – give your profits the trots! *Keynote Speaker*. *Bien Donné Agricultural Expo 2009.* Bien Donné. 5 November 2009.

Britz, T.J. (2009). Microbially polluted irrigation water: Potential impact on food safety. *Keynote Speaker*. EnviroWater Congress, Stellenbosch. 2-4 March 2009.

Britz,. T.J. (2010). Carry-over links of potential pathogens from irrigation water to fresh produce. *Invited speaker*. ILSI Management Committee. Cape Town. 12 November 2010.

Britz,. T.J. (2010). Is polluted irrigation water a threat? *Invited speaker*. AgriSA Managers Meeting. Pretoria. 9 March 2010

Britz,. T.J. (2010). Linking pathogens from irrigation water to pathogens on fresh produce. *Invited speaker*. CEO Forum of the Minister of Agriculture. Pretoria. 26 July 2010.

Britz. T.J. (2010). Carry-over links of potential pathogens from irrigation water to fresh produce. *Invited speaker*. ILSI South Africa Management Committee. Cape Town. 12 November 2010.

Taylor, M.B. (2010). Hepatitis A virus in surface water in South Africa: what are the risks? *Invited lecture*. 2^{nd} COST 929 Symposium: Future Challenges in Food and Environmental Virology 7-9 October 2010. Point Hotel Barbaros, Istanbul, Turkey.

Taylor, M.B. (2010). Hepatitis A virus in surface water in South Africa: what are the risks? *Invited lecture*. 2^{nd} COST 929 Symposium: Future Challenges in Food and Environmental Virology 7-9 October 2010 Point Hotel Barbaros, Istanbul, Turkey.

Taylor, M.B. (2009).Viruses in a South African river: The impact of improved technology [Invited lecture]. COST929 ENVIRONET Workshop and Management Committee meeting, 6-7 October 2009. Instituto Superor Tecnico, Av. Rovisco Pais, Lisbon, Portugal

Britz, T.J. (2011). Is ons voedsel veilig. *Invited speaker*. Stellenbosch Studie- en Besprekingsgroep. 8 November 2011.

Britz, T.J. & Sigge, G.O. (2011). Food Safety and irrigation water. *Invited speaker*. Water Research Commission, 40 Year Celebration Conference. Kempton Park, South Africa. 31 Aug-1 Sept 2011.

Taylor, M.B. (2011). Occurrence of viruses in irrigation water and fresh produce. *Invited lecture*. South African Association for Food Science and Technology (SAAFoST), Water Safety and Quality Workshop. 08 July 2011. South Africa, Kelvin Drive, Woodmead, Johannesburg.

2.6 AWARDS AND HIGHLIGHTS

T. Yasvoin was awarded the prize for the Best Platform Presentation for the presentation entitled "*First detection of human sapoviruses in river water in South Africa"* at the 2nd Regional Conference of the Southern African Young Water Professionals 03-05 July 2011: CSIR International Convention Centre, Pretoria, South Africa.

T. Yasvoin was awarded a bursary from the Poliomyelitis Research Foundation to attend the Virology Africa 2011 Congress. 29 November-2 December 2011, UCT Graduate School of Business, Portswood Rd, V&A Waterfront, Cape Town.

J. Mans was awarded the prize for the best poster entitled *"Diverse norovirus genotypes identified in individuals affected by a multifactorial waterborne gastroenteritis outbreak in Mpumalanga, 2007*" in the Medical Microbiology section, at the Pathvine 2010: 50th Annual Conference of the Federation of South African Societies of Pathology (FSASP) 2-5 September 2010: Lord Charles Hotel, Somerset West, South Africa.

M. Crous was awarded a travel bursary by the University of Pretoria to spend 3 months in Germany to further her studies in Food Science.

G. Duhain was awarded a travel bursary to attend a quality assurance course at the University of Wageningen in the Netherlands.

R. Netshikweta was awarded the third prize in the category Junior Researcher: Basic Sciences [Posters] at the Faculty of Health Sciences Faculty Day 18-19 August 2009 for his poster entitled *"Optimisation and application of real-time RT-PCR techniques for the detection of selected food and waterborne viruses".*

R. Netshikweta was awarded the prize for the best abstract at the 1st Regional Conference of the Southern African Young Water Professionals 19-20 January 2010 for his presentation entitled: "*The application of real-time RT-PCR for the detection of selected enteric viruses in irrigation water in South Africa".*

J. Mans was awarded the prize in the Medical Microbiology section at Pathvine 2010: 50th Annual Conference of the Federation of South African Societies of Pathology (FSASP) 2-5 September 2010 for the best poster entitled *"Diverse norovirus genotypes identified in individuals affected by a multifactorial waterborne gastroenteritis outbreak in Mpumalanga, 2007.*

R. Netshikweta was awarded the third prize in the category Junior Researcher: Basic Sciences [Posters] at the Faculty of Health Sciences Faculty Day 18-19 August 2009 for his poster entitled *"Optimisation and application of real-time RT-PCR techniques for the detection of selected food and waterborne viruses".*

M.B. Taylor was invited to be an editor for the "Journal of Applied Microbiology" and "Letters in Applied Microbiology".

2.7 CAPACITY BUILDING

2.7.1 Students in training as part of the project K51773

Institution	Student	Gender	Race	Degree
University of Stellenbosch	Marlize Jordaan	F	W	MSc Food Sc
University of Stellenbosch	Nika Schoeman	F	W	MSc Food Sc
University of Stellenbosch	Marko Romanis	M	W	MSc Food Sc
University of Pretoria	Matthew Aijuka	M	B	MSc Food Sc
University of Pretoria	Gabriël de Ridder	M	W	MSc (Med Virology)
University of Pretoria	Vurayai Ruhanya	M	B	MSc (Med Virology)
University of Pretoria	Tanya Yasvoin	F	W	PhD (Med Virology)
University of Venda	Given Maruvhani	M	B	BSc Hons (Micro)
University of Venda	Sammy Mathebula	M	B	BSc Hons (Micro)
University of Venda	P Shilawhila	F	B	BSc Hons (Micro)
University of Venda	Lucky Baloyi	M	B	MSc (Micro)
University of KZN	Dumsani Ngcamu	M	B	MSc (Micro)
University of KZN	Megan Gemmell	F	W	MSc (Micro)
University of Venda	Mapula Razwinani	F	B	MSc (Micro)
University of Venda	Phyllis Sidzhangi	F	B	BSc Hons
University of Venda	Ivy Thomas	F	Indian	BScHons (Micro)

2.7.2 Students who have completed their post-graduate training as part of the project K51773

Institution	Student	Gender	Race	Degrees awarded
University of Stellenbosch	Alison Ackermann	F	W	MSc Food Sc
University of Stellenbosch	Marijké Lötter	F	W	MSc Food Sc
University of Stellenbosch	Nicola Huisamen	F	W	MSc Food Sc
University of Stellenbosch	Tshepo Kikine	M	B	MSc Food Sc
University of Stellenbosch	Anneri van Blommestein	F	W	MSc Food Sc
University of Stellenbosch	Amanda Brand	F	W	PhD
University of Pretoria	Lauren Smith	F	W	Hon Food Science
University of Pretoria	Oluwatosin A. Ijabadeniyi	M	B	PhD Food Sc
University of Pretoria	Géraldine Duhain	F	W	MSc Food Sc
University of Pretoria	Mignon Crous	F	W	MSc Food Sc
University of Pretoria	Siphiwe Dube	F	B	Hon Food Sc
University of Pretoria	Rembuluwani Netshikweta	M	B	MSc (Medical Virology)
University of KZN	Mlungisi Shelembe	M	B	BScHons (Micro)
University of KZN	Fezile Ngiba	F	B	BScHons (Micro)

2.7.3 Post-doctoral Fellows as part of the project K51773

Institution	Student	Gender	Race	Qualification level
University of Pretoria	Dr Janet Mans	F	W	Post-doctoral Fellow
University of Stellenbosch	Dr Corne Lamprecht	F	W	Post-doctoral Fellow

2.8 THESES AND DISSERTATIONS

2.8.1 Honours studies completed

F. Ngiba, F. (2011). Assessment of river water used in KwaZulu-Natal for irrigation for the presence of *Staphylococcus aureus*. University of KwaZulu- Natal, Pietermaritzburg.

Shelembe, M.R. (2011). Evaluation of the MPN (most probable number) method to detect faecal coliforms in river water used for domestic consumption and irrigation in KwaZulu-Natal. Department of Microbiology, KwaZulu-Natal University, Pietermaritzburg.

Dube, S. (2011). The microbiological quality of unpackaged and modified atmosphere packaged lettuce, Swiss chard and tomatoes. Department of Food Science, University of Pretoria, Pretoria.

Smith, L. (2012). The attachment rate and build-up of *E. coli* on butter head lettuce. Department of Food Science, University of Pretoria, Pretoria.

2.8.2 Masters studies completed

2.8.2.1 Ackermann, A. (2010). Assessment of microbial loads present in irrigation water and their survival kinetics. University of Stellenbosch, Stellenbosch, South Africa. Supervisors: Prof TJ Britz & Dr GO Sigge.

Abstract – In an exploratory study over a five month period, the microbiological and water chemistry of three selected sites from the upper Berg and two from the Plankenburg Rivers were assessed. From the exploratory study it was concluded that the water from all the sites were not suitable for use in irrigation practices as they regularly exceeded the guidelines for faecal coliforms and *E. coli* as set out by SA authorities.

In a second study the carry-over and survival of *E. coli* on green beans, sugar-snap peas and cocktail tomatoes was assessed under controlled laboratory conditions. The produce was exposed to *E. coli* under different exposure and drying times and different inoculum concentrations. In all cases a reduction of at least one log value was found. None of these parameter changes affected the variation in

numbers for the same inoculum or the *E. coli* survivors. With the exception of the 10^2 and 10^3 inoculum ranges, *E. coli* survivors detected always exceeded the guidelines for fresh produce. If similar survival patterns are to be found in the environment then these results should serve as a warning that the Plankenburg river water is unsafe for use in the irrigation of fresh produce.

2.8.2.2 Lötter, M. (2010). Assessment of microbial loads present in two Western Cape rivers used for irrigation of vegetables. University of Stellenbosch, Stellenbosch, South Africa. Supervisors: Dr G.O. Sigge & Prof T.J. Britz.

Abstract – The purpose of this study was to determine the microbial types and loads in river water, irrigation water and on irrigated produce. A baseline study was done on four sites in two Western Cape rivers. These sites were chosen to allow for the sampling of river water, irrigation water and irrigated produce to determine whether a link between the use of contaminated irrigation water and the microbial population found on irrigated produce exists. From the data it was evident that the two rivers contained faecal indicators at levels much higher than those in national and international guidelines for safe irrigation.

Based on the results a more intensive study on the microbial loads of the river and irrigation water as well as irrigated produce from the Mosselbank site was done. Lettuce and cabbages from commercial farmers were chosen as the irrigated produce. While the counts of indicator bacteria in the irrigation water was often lower, faecal coliform counts as high as 1 600 000 cfu.100 mL^{-1} and several other potential pathogens were found on the irrigated produce. This indicated a possible “build-up” of contamination on the produce with the repeated application of the tainted irrigation water.

2.8.2.3 Crous, M. (2011). Effect of irrigation intervals and processing on the survival of *Listeria monocytogenes* on spray irrigated broccoli. University of Pretoria, Pretoria, South Africa. Supervisors: Prof E. Buys & Dr G.O. Sigge.

Abstract – The first aim was to determine the effect of irrigation intervals on the survival of *L. monocytogenes* on spray irrigated broccoli under field trial conditions, and subsequent survival of the pathogen on broccoli during postharvest processing procedures. The non-pathogenic *L. innocua* was used as surrogate organism to *L. monocytogenes*. It was found that the presence of high levels of *Listeria* contamination in irrigation water used for vegetable crops, can be associated with increased microbial concentrations on the crop surface.

The effect of processing on organism survival post-harvest was also assessed. The results showed that even though chlorine is effective in reducing *L. innocua* numbers during minimal processing, it does not suffice alone to eliminate pathogens from vegetables, just as MAP storage is only effective as part of a hurdle procedure. Cooking is essential in destroying *L. innocua* present and to ensure vegetables are safe for consumption in terms of pathogenic exposure. Thus the behaviour of *L. monocytogenes* and the risk associated with the application of contaminated irrigation water to fresh produce can be better understood and the hazard managed.

2.8.2.4 Huisamen, N. (2011). Assessment of microbial levels in the Plankenburg and Eerste Rivers and subsequent carry-over to fresh produce using source tracking as indicator. University of Stellenbosch, Stellenbosch, South Africa. Supervisors: Dr G.O. Sigge & Prof T.J. Britz.

Abstract – A base-line of the microbial loads in the Plankenburg and Eerste Rivers was established using standard microbial methods for the detection of indicator organisms such as total and faecal coliforms, *Escherichia coli* and Enterococci as well as potential pathogens that included *Salmonella*, *Listeria*, *Staphylococcus*, endospore formers and aerobic colony counts. High faecal coliform and *E. coli* concentrations were detected. The recommended irrigation water guidelines were frequently exceeded, indicating faecal pollution. A potential health risk was confirmed when potential pathogens such as *Aerococcus viridans*, *Klebsiella*, *L. monocytogenes* and *Salmonella* Typhimurium were detected.

The carryover of organisms from rivers to produce was also investigated by comparing the microbial population of the Plankenburg and Eerste Rivers to the population recovered from irrigation water and the surface of fresh produce. A species similarity between the microbial populations in the river, the irrigation water and produce was found. The build-up of organisms on the surface of produce because of multiple irrigations was also confirmed. Microbial source-tracking techniques including multi-antibiotic resistance profiling and the phenotypic classifications were used to determine similarities. A high degree of similarity indicating a high probability of carry-over was confirmed. It was concluded that a high risk is associated with the use of contaminated irrigation water and the transfer of potentially harmful organisms to the surface of fresh produce.

2.8.2.5 Kikine, T. (2011). Profiling of potential pathogens from Plankenburg river water used for the irrigation of fresh produce. University of Stellenbosch, Stellenbosch, South Africa. Supervisors: Prof T.J. Britz & Dr G.O. Sigge.

Abstract – A previous baseline study showed large variations in microbial loads over a 4 year period. Thus to determine whether the count variations represented an over- or underestimation of the contamination

levels, an microbial assessment of the weekly, daily and hourly variations was conducted on the Plankenburg river. This river was specifically used as it is an irrigation source point for upstream and downstream fresh produce farmers and is downstream from an informal settlement. It was found that temperature and pH had no major impact on either the total coliform or *E. coli* counts. The increase in total coliform and *E. coli* counts found during the weekly, daily and hourly variation trend studies clearly proves that sampling time is an important factor to consider when evaluating considering microbial levels of a river. Both the Plankenburg and Eerste Rivers were found to be unsuitable for the irrigation of fresh produce intended to be consumed raw due to the high levels of faecal contamination that exceeded DWAF and WHO guidelines.

2.8.2.6 Netshikweta, R. (2011). Optimisation and assessment of real-time PCR techniques for the detection of selected food- and waterborne viruses. University of Pretoria, Pretoria, South Africa. Supervisors: Prof M.B. Taylor & Dr W.B. van Zyl.

<u>*Abstract*</u> – The aim of this investigation was to develop and optimise methods for the concentration and detection of NoV GII and HAV in irrigation water and fresh produce. These methods were then applied to field samples of irrigation water and fresh produce to try and establish a link between viral contamination detected in irrigation water and that on associated irrigated fresh produce.

86 irrigation water and 72 fresh produce samples were collected from commercial and subsistence farms, street vendors and commercial outlets and analysed for HAV, NoV GI and NoV GII. Overall, 16.3% and 12.5% of irrigation water and fresh produce samples tested positive for one or more human pathogenic viruses, namely NoV GII and HAV, respectively. A direct link between contaminated irrigation water and contamination of fresh produce could not be established, but irrigation water was identified as a possible source of contamination of the fresh produce. The results also suggested that food handlers contributed significantly to the viral contamination of the fresh produce.

2.8.2.7 Duhain, G. (2012). Occurrence of *Cryptosporidium* spp. in South African irrigation waters and the survival of *C. parvum* during vegetable processing. University of Pretoria, Pretoria, South Africa. Supervisor: Prof E. Buys.

<u>*Abstract*</u> – The first phase was a field survey to determine the incidence of *Cryptosporidium, Giardia* and *Salmonella* spp. in rivers from 3 provinces of South Africa used for irrigation as well as on irrigated vegetables. Out of the 30 water samples analysed, 43% were positive for *Cryptosporidium* oocysts, 23% positive for *Giardia* cysts and 27% were positive for *Salmonella* spp. No *Cryptosporidium* oocysts or *Giardia* cysts were found on the vegetables analysed.

In a second study the individual and combined effects of chlorine, blanching, blast freezing and microwave heating on *C. parvum* oocysts inoculated on green peppers were investigated. The results of the challenge tests indicate that *C. parvum* oocysts on vegetables are inactivated by blanching and microwave heating but survive blast freezing and exposure to chlorine. Boiling and microwave heating of vegetables should be sufficient to kill *C. parvum*. On the other hand, ready-to-eat vegetables could be at risk of carrying live *C. parvum* oocysts as the use of chlorine in the washing bath is not expected to inactivate *C. parvum* oocysts present on vegetables. The data indicate that *C. parvum* oocysts are sensitive to heat and, to some extent, to freezing temperature but are resistant to chlorine. The results show the presence of *Cryptosporidium* and *Giardia* in irrigation waters and thus a possible health risk associated with the consumption of raw vegetables as those can become contaminated via the irrigation water.

2.8.2.8 Van Blommestein, A. (2012). Impact of selected environmental factors on *E. coli* growth in river water and an investigation of carry-over to fresh produce during irrigation. University of Stellenbosch, Stellenbosch, South Africa. Supervisors: Prof T.J. Britz & Dr G.O. Sigge.

<u>*Abstract*</u> – The purpose of the first study was to determine the impact of different environmental factors: carbon levels; temperature; incubation time; and initial microbial load on the growth of *E.coli* and other "indigenous" microbes present in irrigation river water. It was found that in non-sterile river water the *E.coli* levels increased with increase in incubation temperature but that *E.coli* die-off was more rapid when the nutrient levels were low. It was concluded that the carbon level is a major growth limiting factor in river water.

The purpose of the second study was to determine the effect of daily irrigation on carry-over, the effect of "once-off" irrigation on the survival of *E.coli* on the produce, identifying types of *E.coli* in the irrigation water and those on irrigated fresh produce, and then linking the *E.coli* types. The results showed that *E.coli* is carried over from irrigation water to the irrigated green beans, especially when the *E.coli* levels in the river water were high. The results also showed that these isolates were related and originated from the same pollution source. For further linking confirmation, DNA sequencing was done and *E.coli* strains and some *E.cloacae* and *K.pneumoniae* strains from the water and from produce could successfully be linked. This confirmed the carry-over of faecal coliforms from irrigation water to fresh produce.

2.8.3 PhD studies completed

2.8.3.1 Ijabadeniyi O.A. (2010). Effect of irrigation water quality on the microbiological safety of fresh vegetables. University of Pretoria, Pretoria, South Africa. Supervisor: Prof EM Buys.

Abstract – The effect of source water from the Olifants and Wilge Rivers on the bacterial quality of water in the Loskop Canal and subsequent contribution to the bacterial contamination of fresh vegetables was determined for 12 months. Also the effect of attachment time on the survival of *Listeria monocytogenes* and the effect of chlorine on *L. monocytogenes* attached to vegetables were determined. Finally, a step-wise logistic regression analysis was made to determine whether various predictor variables could be used to predict the occurrence of *L. monocytogenes*, *Salmonella* spp and intestinal *Enterococcus* in irrigation water and vegetables (i.e., cauliflower and broccoli). This research also indicated that *L. monocytogenes* could attach to both surface and subsurface structures of both tomatoes and spinach within 30 min, and that even after 72 h, it still remained viable. It also indicated that chlorine treatment is more effective against surface *L. monocytogenes* compared with subsurface inoculated *L. monocytogenes.*

2.8.3.2 Brand, A.S. (2012). Critical evaluation of the accuracy of the enumeration methodology of coliform and *E. coli* in water from rivers used for the irrigation of fresh produce. University of Stellenbosch, Stellenbosch, South Africa. Supervisors: Prof T.J. Britz & Dr G.O. Sigge.

Abstract – The accuracy of methods for the enumeration of coliforms and *Escherichia coli* present in river water intended for the irrigation of fresh produce has been critically evaluated to determine whether the results of the traditional method were reliable in indicating faecal pollution. The potential of rapid alternative methods were also explored. This research confirmed that MTF is accurate in the enumeration of coliforms and *E. coli*. Inaccuracies are primarily attributable to atypical organisms which are considered to make up a small proportion of the total bacterial population. Colilert-18 was shown to be an acceptably accurate alternative, and its rapid production of results can be highly advantageous in the monitoring of irrigation water used for MPFs.

CHAPTER 3

ARCHIVING OF DATA GENERATED DURING THE PROJECT

Large volumes of data were generated by the various research teams collaborating on this WRC research project. The raw data generated during this project is thus being archived by the respective research organisations. These include:

Department of Food Science, University of Stellenbosch, Stellenbosch, Western Cape

Department of Food Science, University of Pretoria, Pretoria, Gauteng

Discipline of Microbiology, University of KwaZulu-Natal, Pietermaritzburg, KwaZulu-Natal

Department of Microbiology, University of Venda, Thohoyandou, Limpopo

Department of Medical Virology, University of Pretoria / National Health Laboratory Service