THE EPIDEMIOLOGY AND COST OF TREATING DIARRHOEA IN SOUTH AFRICA

Volume 1: Prevalence of diarrheagenic pathogens in water sources in the Vhembe District of the Limpopo Province

N Potgieter, TG Barnard, LS Mudau and AN Traore

WATER RESEARCH COMMISSION

TT 760/18

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Volume 1

Prevalence of diarrheagenic pathogens in water sources in the Vhembe District of the Limpopo Province

Report
to the Water Research Commission

by

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This report emanates from the Water Research Commission project, titled: *Epidemiological and economic implications of diarrhoea in water sources from rural and peri-urban communities in the Limpopo Province, South Africa* (K5/7150). The outputs of this research project are presented in three separate publications:

- **Volume I: Prevalence of diarrheagenic pathogens in water sources in the Vhembe District of the Limpopo Province (this report).**
- **Volume II: Prevalence and antibiotic profiles of diarrheagenic pathogens in children under the age of 5 years – A case of Vhembe District of the Limpopo Province. (TT 761/18)**
- **Volume III: The cost of treating diarrhoea in children under the age of 5 years in rural and peri-urban communities – A case study of Vhembe District of the Limpopo Province. (**TT **762/18)**

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EXECUTIVE SUMMARY

BACKGROUND

Contaminated water can lead to waterborne disease outbreaks (Levy, 2015). Water is a vehicle for the transmission of pathogenic bacteria, viruses and parasites to humans, which cause waterborne illnesses such as diarrhoea (Lewin et al., 2007; Samie et al., 2009; Batabyal et al., 2013; Mobasa et al., 2017). Multiple routes of transmission on exposure as well as some multiple infectious and non-infectious diarrhoea pathogens make it difficult to impossible to accurately identify the specific pathogen(s) responsible for a specific diarrhoea episode (Levy, 2015). Several studies have shown that it is not always possible to make an association between water quality and diarrhoea (Gundry et al., 2004). Even if a water sample is collected from a household storage container on a specific day and moment, this does not guarantee that this water caused the diarrhoea seen in the child or any other member of the household. Many factors within a household and environment could be responsible for a diarrhoea episode (Gundry et al., 2004).

Prevention of water pollution requires effective and continuous monitoring of physical, chemical and microbiological parameters to ascertain the possible risks associated with water from a particular source (Chandra et al., 2006). This is however not done regularly in developing countries due to limited or non-existing resources, which results in some diarrheal outbreaks going unnoticed (Zamxaka et al., 2004). In South Africa, almost 80% of the population still depend on surface water and groundwater as their main source of water for domestic purposes. This water is stored in various types of container without any treatment (Venter, 2001). As a result, many vulnerable people in rural and peri-urban communities are exposed to waterborne pathogens (Smith et al., 2006).

There are very few studies and reports on waterborne diarrhoea in the rural communities in the Vhembe region and it is highly possible that some diarrhoea cases go unreported. A possible link between diarrhoea and water quality in the Vhembe region has been established (Bessong et al., 2009). Therefore, continuous monitoring of water quality is necessary to assess the possible risks associated with water sources used in the rural communities in the Vhembe region. The continuous epidemiological and prevalence assessment of diarrhoeagenic pathogens in different water sources will provide information to health statisticians on the prevalent strains circulating in these communities. This data will add to the knowledge of treatment effectiveness and intervention strategies. Thus, the aim of this study is to determine the prevalence of diarrhoea-causing pathogens in water used in the rural and peri-urban communities in the Venda District for drinking and domestic purposes.

OBJECTIVES OF THE STUDY

The objectives of the study are:

- Assess the prevalence of total coliform bacteria, *Escherichia coli* and pathogenic bacteria (*Vibrio cholerae*, *Salmonella* spp. and *Shigella* spp.) using the Colilert Quanti-Tray®/2000 technique and membrane filtration method respectively.
- Characterise the isolated pathogenic bacteria using established multiplex polymerase chain reaction (PCR) protocols (Water and Health Research Centre, University of Johannesburg).
- Determine the presence of enteric viruses [adenovirus, reovirus, norovirus (GI, GII), enterovirus, astrovirus, sapovirus, rotavirus and hepatitis A virus] using cell culture and molecular protocols (University of Pretoria).
- Determine the presence of protozoan parasites (*Cryptosporidium* and *Giardia*) using the United States Environmental Protection Agency (USEPA) Method 1623.1 protocol (Rand Water Board).
- Make recommendations to the relevant policymakers to improve water quality in rural and peri-urban communities in South Africa.

METHODOLOGY USED

Water was sampled over a 12-month period during the winter months (June–August 2016) and during the summer months (October–December 2016) from ten river water sources used by rural and peri-urban communities for drinking purposes. In three rural villages, water was collected from household water storage containers and boreholes. The Colilert Quanti-Tray®/2000 technique (IDEXX) was used to isolate total coliforms and *E. coli*. Target bacteria were isolated from river water using membrane *Clostridium perfringens* agar, *Salmonella–Shigella* agar and thiosulfate–citrate bile salts–sucrose agar. Presumptive isolates were confirmed by an oxidase test, API-20E test, Gram-staining and standardised published multiplex PCR protocols. Enteric viruses were analysed according to the South African National Standards for drinking water. The USEPA Method 1623.1 was employed for parasites.

RESULTS AND DISCUSSION

The results showed high *E. coli* counts in the Madadzhe River used for irrigation; *C. perfringens* was more prominent in summer. There was high contamination of household storage containers. Only 11% of boreholes were contaminated with *E. coli*. All six diarrhoeagenic strains of *E. coli* were isolated in the river and household storage container water samples. The enterohemorrhagic *E. coli* (EHEC) strain was found to be the most recorded strain in summer in river water samples. In borehole waters, only enteropathogenic *E. coli*, enteroaggregative *E. coli* and EHEC were detected. The presence of pathogenic *E. coli* strains in water could be a concern for potential health risks since strains such as EHEC – associated with bloody diarrhoea –have the potential for causing outbreaks (Isaäcson, 1993; Anon, 2000).

V. cholera spp. could only be detected with PCR in the Luvuvhu river. There was no *Shigella* spp. detected. *Salmonella* spp. were identified by PCR. Although this study did not determine the pathogenic nature of *Salmonella* and *Vibrio* spp., the results of this study pose a concern since evidence of diarrhoea outbreaks have previously been reported in the Vhembe region (Bessong et al., 2009). Noroviruses, rotaviruses, adenoviruses, several other endemic viruses as well as *Cryptosporidium* and *Giardia* parasites were detected from the river water samples – especially in winter.

LIMITATIONS OF THE STUDY

- Due to the cost to test for parasites and viruses in environmental samples, the project had to limit the number of samples assessed for viruses and parasites.
- Sediment samples from rivers, which are mostly associated with parasites and spores, were not included.

CONCLUSION

The microbial quality of raw water used by the rural communities in the Vhembe District was poor and is therefore a potential vehicle for the transmission of diarrhoea-causing pathogens to humans. Various pathogenic strains of *E. coli* are circulating in the rural households in the Vhembe District. This is a cause of concern since *E. coli* is implicated in serious diarrhoea outbreaks across the world. Although *C. perfringens* is a reliable indicator for parasites, the low prevalence of parasites could have been due to cysts or oocysts being stuck in sediments.

Government has an obligation to ensure safe drinking water to consumers – they must reduce the number of people living without adequately treated water supply. Risk analysis of potential pollution hazards in communities must be done regularly and proactive management must be employed to limit outbreaks and medical costs due to the treatment of diarrhoea. Contingency plans must be in place and all role players from government to the consumer must be accountable for the protection of water from catchment to the point of use and back to the environment.

RECOMMENDATIONS FOR FUTURE RESEARCH

- Assess environmental samples continuously for the prevalence of diarrhoeal-causing bacteria, viruses and parasites.
- Identify and document hazardous events/situations, their causes and scenarios in rural and peri-urban communities.
- Estimate the level of risk for each of the identified hazards by assessing the likelihood and severity of each.

CAPACITY BUILDING

Student name	Degree (University)	Title of study	Supervisors	Degree awarded
Lee Heine	DTech (University of Johannesburg)	Incidence and antimicrobial resistance of diarrhoeagenic bacteria in Vhembe region of South Africa	Prof. TG Barnard Prof. N Potgieter	In final stage
Tshikani Basil Minyuku	MTech (Tshwane University of Technology)	Cost implications of diarrhoea treatment related to water, sanitation and hygiene of children under 5 years in public health clinics of Vhembe District, Limpopo Province, South Africa	Dr LS Mudau Prof. MI Mokgobu	In final stage
Jean Pierre Kabue Ngandu	PhD (University of Venda)	Molecular characterization of norovirus strains circulating in rural communities of the Limpopo Province of South Africa	Prof. N Potgieter Dr E Meader Prof. PR Hunter	Graduated 2018
Ellen Ledwaba	MSc (University of Venda)	Prevalence of selected bacterial and viral entero-pathogens in children less than 5 years of age in Limpopo Province of South Africa	Prof. N Potgieter Prof. AN Traore	Graduated 2016
Simbarashe Karambwe	MSc (University of Venda)	Prevalence of diarrhoea-causing bacteria, viruses and parasites in water sources in the rural communities in the Vhembe District	Prof. N Potgieter Prof. AN Traore	Graduated 2017

LIST OF RESEARCH OUTPUTS

Published journal articles:

- Jean Pierre Kabue, Emma Meader, Paul R. Hunter and Natasha Potgieter. (2016). Human norovirus prevalence in Africa: A review of studies from 1990 to 2013. *Tropical Medicine and International Health* 21(1): 2–17.
- Jean Pierre Kabue, Emma Meader, Paul R. Hunter and Natasha Potgieter. (2016). Norovirus prevalence and estimated viral load in symptomatic and asymptomatic children from rural communities of Vhembe District, South Africa. *Journal of Clinical Virology* 84:12–18.
- Jean Pierre Kabue, Emma Meader, Paul R. Hunter and Natasha Potgieter. (2017). Genetic characterisation of norovirus strains in outpatient children from rural communities of Vhembe District, South Africa: 2014–2015. *Journal of Clinical Virology* 94:100–106.

Conference attendance:

- Jean Pierre Kabue, Emma Meader, Paul R. Hunter and Natasha Potgieter. (2016). Molecular characterization of norovirus strains circulating in rural communities of the Limpopo Province of South Africa. Oral presentation. 8th International Water and Health seminar. June 27–29. Cannes, France.
- Jean Pierre Kabue, Emma Meader, Paul R. Hunter and Natasha Potgieter. (2018). Genetic diversity of norovirus strains in outpatient children from rural communities of Vhembe District, South Africa, 2014-2015. Oral presentation. 20th International conference on Infectious Diseases. January 29–30. Sydney, Australia. *Presentation won best presentation award.*

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Name	Organisation	Role
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M Makhado	Management Sciences for Health	Data analysis
N Zitha	University of Venda	Sample collection
T Banda	University of Venda	Intern – Administrative assistant
P Mukhuvha	University of Venda	Administrative assistant

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ACRONYMS AND ABBREVIATIONS

API-20E	–	Analytical Profile Indexing for *Enterobacteriaceae*
cfu	–	Colony Forming Units
CDC	–	Centers for Disease Control and Prevention (USA)
CPE	–	Cytopathogenic Effects
DAPI	–	4′,6-diamidino-2-phenylindole (differential interference contrast)
DIC	–	Differential Interference Contrast
DNA	–	Deoxyribonucleic Acid
DO	–	Dissolved Oxygen
DWAF	–	Department of Water and Forestry
EAEC	–	Enteroaggregative *Escherichia coli*
EHEC	–	Enterohaemorrhagic *E. coli*
EIEC	–	Enteroinvasive *E. coli*
EPEC	–	Enteropathogenic *E. coli*
ETEC	–	Enterotoxigenic *E. coli*
HAV	–	Hepatitis A Virus
m-CP	–	Membrane *Clostridium perfringens*
MPN	–	Most Probable Number
m-PCR	–	Multiplex Polymerase Chain Reaction
NaCl	–	Sodium/Natrium Chloride
Nov GI	–	Norovirus Genotype I
Nov GII	–	Norovirus Genotype II
PCR	–	Polymerase Chain Reaction
PAST	–	PAleontological STatistics
PLC/PRF/5	–	Primary Liver Carcinoma Hepatoma Cell Line
RT-PCR	–	Reverse Transcriptase Polymerase Chain Reaction
S-S	–	*Salmonella–Shigella*
SANS	–	South African National Standards
spp.	–	Species
TC	–	Total Coliforms
TCBS	–	Thiosulfate-Citrate Bile Salts Sucrose Agar
TCVA	–	Total Culturable Virus Assay
TDS	–	Total Dissolved Salts
TID	–	Total Infectious Dose
UNICEF	–	United Nations Children Educational Fund
USEPA	–	United States Environmental Protection Agency
WHO	–	World Health Organization
WSA	–	Water Services Authority

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1 INTRODUCTION

1.1 Problem Statement

South Africa is classified as a water-stressed country. Of South Africa's water, 9% is supplied from groundwater sources, 14% is sourced from sewage and effluent purification, and 77% is obtained from surface water such as dams and rivers (Van Vuuren, 2009). In addition, nearly 80% of the population in South Africa relies on surface water as the main source of water; this figure alone implies that people still use raw surface water (untreated water) for domestic purposes (Venter, 2001). The urban population in South Africa enjoys well-developed water infrastructure and thus uses potable water. However, in rural and most peri-urban communities, a well-established water infrastructure is unlikely (Obi et al., 2007). Settlements in rural and peri-urban areas are sparsely distributed due to the rough terrain, which hinders the capacity to provide a centralised drinking water system (Sibanda & Okoh, 2013). Therefore, rural populations obtain water on an individual or household basis from the closest surface water and groundwater sources – the microbial quality of which is often unknown (Peter-Varbanets et al., 2009). In the Vhembe District (Limpopo Province, South Africa), most communities rely on river water sources, which are not treated, and water collected from boreholes for their domestic water needs. Most river water is of poor microbiological quality and unsafe for consumption (Obi et al., 2002; Palamuleni & Akoth, 2015). In addition, due to its infrequent availability in developing countries, households store water in containers (Brick et al., 2004).

Because of the poor water quality in rural areas of South Africa, a considerable number of residents are exposed to waterborne pathogens that can cause enteric diseases such as diarrhoea (Smith et al., 2006). Water contaminated by pathogenic microorganisms increases the risk of acquiring waterborne infections such as diarrhoea and gastroenteritis following consumption (Karanis et al., 2010). According to the World Health Organization, WHO (2017), the percentage of deaths in developing countries due to consuming contaminated water has considerably decreased but still caused 1.4 million deaths. In South Africa, most deaths due to diarrhoea have been linked to unsafe water, sanitation and hygiene (Lewin et al., 2007). Diarrheal incidence may be used as a call for the need to identify potential environmental hazards such as surface water sources (Obi et al., 2007; Wenhold & Faber, 2009). Drinking water sources are contaminated by the introduction of faeces from humans, wild and/ or farm animals that are flushed into surface waters (Hunter & Thompson, 2005). In addition, sewage disposal and poorly managed waste treatment plants are also sources of water pollution (Dungeni & Momba, 2010). Human and animal activities such as laundry, diaper-dumping, grazing around the water source, and farming have been shown to affect the quality of surface water sources in the Vhembe District (Traore et al., 2016).

The lack of demographic statistics in rural communities poses difficulties in evaluating the impact of the quality of the drinking water supply on the health of human beings. It is thus imperative to know the prevalence of pathogenic organisms and the incidences of diseases occurring in rural areas due to polluted water (Zamxaka et al., 2004). The effective monitoring of physical, chemical and microbiological parameters can assist in mitigating the pollution of surface waters (Chandra et al., 2006). Very few studies and reports on waterborne diarrhoea in the rural communities in the Vhembe region are available. It is highly possible that some diarrhoea cases are not reported. A possible link between diarrhoea and water quality in the Vhembe region has been established (Bessong et al., 2009); therefore, continuous monitoring of water quality is necessary to assess the possible risks associated with water sources used in the rural communities in the Vhembe region. The continuous epidemiological and prevalence assessment of diarrhoeagenic pathogens in different water sources will provide information to health statisticians regarding the prevalent strains circulating in these communities. This data will add to the knowledge of treatment effectiveness and intervention strategies. Thus, the aim of this study was to determine the prevalence of diarrhoea-causing pathogens in water used in the rural and peri-urban communities in the Venda District for drinking and domestic purposes.

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2 WATER AS A VECTOR FOR DIARRHEAL PATHOGENS – A REVIEW

2.1 Provision of Water and Sanitation Services In South Africa

South Africa is still behind in providing a continuous water supply and adequate sanitation in most of its municipalities, which has negative impacts on the occurrence of diarrhoea. Consequently, escalating the health care budget in both public and primary health care facilities. Chola et al. (2015) reported that if water and sanitation are provided promptly as part of the 13 interventions identified and a full coverage of services is provided, South Africa could reduce the mortality of children under the age of 5 by 5 million in 2030. According to a study conducted by Leatt & Berry (2006), Limpopo Province is one of the poorest regions of South Africa – only 32% of children have access to basic sanitation. These environmental impacts could contribute to the burden of diarrhoea.

Globally, an estimated 748 million people still use water from unsafe sources; 2.5 billion people have no basic sanitation; and 1.1 billion people still practise open defecation (WHO/UNICEF, 2015). On 21 March 2012, the South African Human Rights Commission highlighted that 16 million South Africans still do not have adequate access to safe water and sanitation, compounding the problem of waterborne disease. Nationally in 2015, 93% households had improved drinking water whereas 7% households still used unimproved water sources, which pose a risk of infectious diseases. Statistics further indicate that in 2015, 66% of South Africans had improved sanitation and 34% had unimproved sanitation. The reports furthermore indicate that 30% of households in urban areas and 34% of households in rural areas had unimproved sanitation (WHO/UNICEF, 2015). In households where a toilet is available, there is usually no water close to wash hands after defecation, which may contribute to the transmission of pathogenic agents on hands.

A review of the literature on maternal education suggests that mothers who have a basic, secondary or higher education tend to practise good hygiene and better child-feeding, which help to protect their children against infectious diseases. Such mothers are also more aware of disease-causing factors and prevention measures that can be taken (Boadi & Kuitunen, 2005). Several studies have reviewed the behavioural determinants of faecal indicator bacterial levels on hands of caregivers to classify the most promising household-level interventions for preventing diarrhoea in children (Mattioli et al., 2014). A study done in the Vhembe region of South Africa found that mothers and caretakers of infants and young children used their bare hands to mash *vhuswa* into a weaning pap, which is prepared under unhygienic conditions (Potgieter et al., 2005). A study conducted by Ghuliani & Kaul (1995) showed that 79% of the mothers' fingers were contaminated. This contributed to the transfer of causative organisms from the mother's hands to the child's hands, as well as utensils, her own teats and eventually to the child during feeding. The incidence of diarrhoea among children was significantly higher in families where mothers did not wash their hands before feeding their children (Ghuliani & Kaul, 1995).

2.2 Environmental Transmission of Diarrheal Pathogens

2.2.1 Pathways to diarrhoeal diseases

The Global Disease Report from the Centres for Disease Control and Prevention (CDC) in the United States of America (CDC, 2014) have compiled different scenarios, which highlight the major pathways to diarrhoeal diseases (Figure 1).

- In Scenario 1, sick people without proper sanitation facilities defecate in or near a water source; the water source is contaminated with faeces; farmers use contaminated water to irrigate their crops; crops irrigated with contaminated water are used to prepare meals; and families eat and drink contaminated food and water.
- In Scenario 2, animals defecate in or near a water source; the water source is contaminated with faeces; farmers use contaminated water to irrigate their crops; crops irrigated with contaminated water are used to prepare meals; and families eat and drink contaminated food and water.

- In Scenario 3, sick people without proper sanitation facilities defecate in or near a water source; the water source is contaminated with faeces; people use contaminated water for drinking and food preparation; and families eat and drink contaminated food and water.
- In Scenario 4, animals defecate in or near a water source; the water source is contaminated with faeces; people use contaminated water for drinking and food preparation; and families eat and drink contaminated food and water.
- In Scenario 5, caregivers change a sick baby's diaper; contaminate their hands or they touch objects and other people, contaminating surfaces they touch; caregivers prepare foods with unwashed hands, contaminating the food; and families eat and drink contaminated food and water.

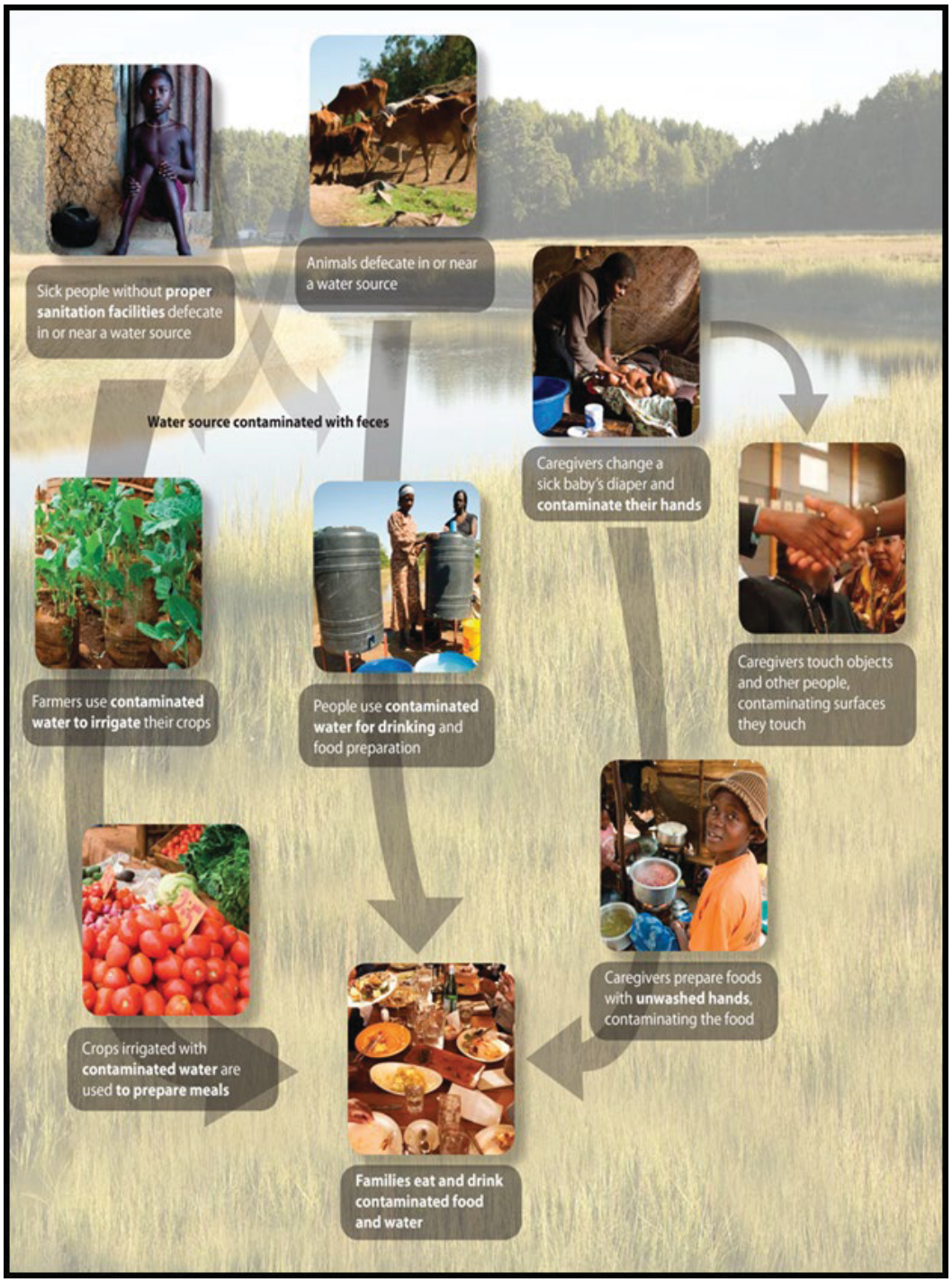


Figure 1: Major pathways to diarrhoeal diseases (CDC, 2014)

2.2.2 The faecal–oral contamination pathway

The faecal–oral contamination pathway is an important pathway in the spread of diseases, which is as a result of transmission of pathogens through flies, fingers, food, water, soil and faeces (Brown et al., 2013). Figure 2 shows the faecal–oral contamination pathway. In rural households where poor sanitation practices are a major influence on disease transmission, the absence of a good quality water supply and no or poorly constructed sanitation infrastructure and also without a place to wash hands could be largely responsible for disease transmission caused by bacteria, viruses, fungi and parasites (Nataro et al., 2006). These excreta-related infections are acquired through ingestion or inhalation and are likely to cause infections in susceptible individuals, especially children under the age of 5 years.

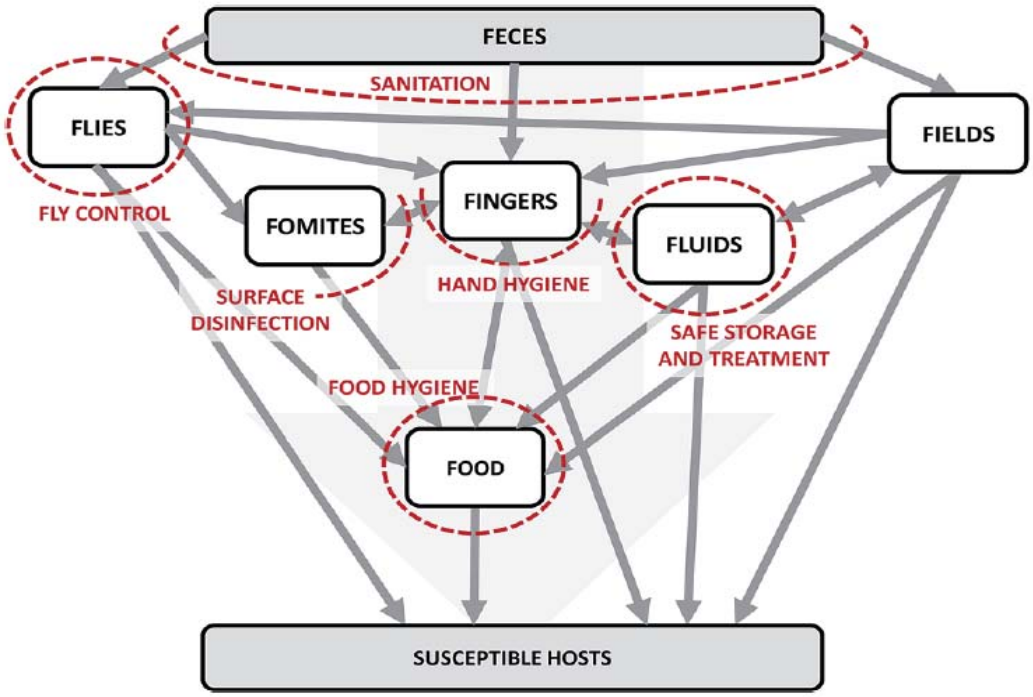


Figure 2: The faecal–oral contamination pathway (Julian, 2016)

Previous epidemiologic studies have showed that factors determining the occurrence of diarrhoea in children are multifaceted and the relative influence of each factor varies as a function of the interaction between socio-economic, environmental and behavioural variables (Yassin, 2000; Boadi & Kuitunen, 2005). Table 1 summarises the pathogenic carriers and transmitters.

Table 1: Summary of frequently reported sources considered hazardous (Keshav et al., 2015)

Source of contamination	Contact	Risk involved	References
Dishcloths	Indirect	Moist conditions are ideal for survival and growth of bacterial cells	Kagan et al., 2002, Mattick et al., 2003 a, 2003b, Bloomfield et al., 2007, Rossi et al., 2013
Food	Direct/ indirect	Serves as nutritional sources for bacteria	De Jong et al., 2008, Pickering et al., 2011, Beltran et al., 2013

Source of contamination	Contact	Risk involved	References
Floors, furniture and wall surfaces	Indirect	Bacterial cells survive in dust	Bloomfield et al., 2007, Sinclair & Gerba 2011, Chang et al., 2013
Fomites	Indirect	Carrier for bacterial cells	Sinclair & Gerba 2011
Personal hygiene	Direct	Lack of knowledge	Curtis et al., 2003
Toilets, bath, sink	Direct	Bacterial cells adhere to surface and can be viable for several hours	Kagan et al., 2002, Ojima et al., 2002, Sinclair & Gerba 2011
Utensils, equipment	Indirect	Carrier for bacterial cells	Soares et al., 2012, Jensen et al., 2013
Water	Direct/ indirect	Temperature of water used for various household chores is generally below 50°C	De Jong et al., 2008, Mattick et al., 2003a, 2003b
Water disposal	Indirect	Serves as a nutritional source for bacteria	Scott, 1999

For a child to be infected with a diarrhoeal disease, they must first be infected or exposed to a range of pathogens. The more the child is exposed, the more likely it is that they will become infected. However, very little data is currently available on the interaction between the pathogen and human environment. Contaminated water used for washing baby utensils may be a basis of faecal contamination of food. Infant feeding utensils become contaminated with bacteria if they are poorly cleaned or rinsed with contaminated water. Using improperly cleaned cups and feeding bottles leads to contamination of baby formula, and also provides a chance of allowing bacterial growth (Redmond & Griffith, 2009). Studies in developing countries have found that home-prepared infant formula feedings were contaminated with 10^2–10^6 coliform/ml due to preparing milk with contaminated water or washing utensils with contaminated water (Andresen et al., 2007). It is also believed that parents who change diapers and prepares bottles without washing their hands could also contribute to the transmission of pathogenic microorganisms during preparation of food and feeding the baby (Motarjemi et al., 1993). Improperly cleaned and dried dishcloths and sponges provide a good breeding environment for bacteria and can serve as a transmission route of faecal contamination (Enriquez et al., 1997; Rusin et al., 1998). This is because dishcloths are used all over the kitchen to wipe dirty surfaces as well as hands and remain wet after washing, which creates an ideal environment where pathogens may survive in the moist environment for a long time.

In addition, dishcloths could also be used to wipe baby feeding utensils, which further contaminates them. Thus, contaminating organisms are introduced to children during feeding with contaminated utensils (Keshav et al., 2015). Majuru et al. (2011) reported that an intermittent water supply increases the number of diarrhoea illnesses in households. Most studies also indicated high contamination of water kept inside containers, which if influenced by an intermittent water supply is a challenge as it could lead to the occurrence of diarrhoea; especially for children under the age of 5 (Clasen, 2006; Potgieter et al, 2009; Mudau et al., 2016). It is therefore critically important to have effective barriers in place to prevent this major transmission route. Improved sanitation alone could reduce diarrhoea-related morbidity by more than a third; improved sanitation combined with hygiene awareness and behaviours could reduce it by two-thirds. Such behaviours include consistent use of a toilet or latrine by each person in the household, safe disposal of young children's faeces, and hand-washing with soap or ash after defecation and before eating.

The number of outbreaks of infectious diarrhoea caused by faecal–oral pathogens provides clear evidence of the efficiency of faecal–oral contamination pathways. It is therefore important to monitor the concentration of these organisms to evaluate the health risk associated with different pathways (Keshav et al., 2015). Infection is more common when there is a shortage of sufficient sanitation and hygiene and safe water for drinking, cooking and cleaning (WHO, 2000). Often the unhygienic conditions in rural areas are attributed to inadequate water and sanitation facilities, lack of access to health care and hygiene education, and poor disposal of water and domestic wastes (DWAF, 1998).

2.3 Microbial Contamination of Water Sources

2.3.1 Overview

Contamination of water is inevitable and yet water is essential to sustain life. Microbial hazards continue to be a threat in both developing and developed countries. According to Cabral (2010), the main risk to public health due to microbes in water is associated with the ingestion of water contaminated with human and animal excreta. Hurst et al. (2002) linked animal faeces with the possible presence of various pathogens such as enteric bacteria including *Vibrio*, protozoa and enteric viruses. Various human and animal activities have been documented in literature as contributing to surface water pollution. A study done by Traore et al. (2016) in the Vhembe District showed that human activities such as doing laundry, baby diaper-dumping, bathing, agriculture, car washing and animal grazing were identified in the various river environments (Figure 3 to Figure 6). The same study also observed that the presence of faecal matter from human, and wild and domestic animals was the most probable source affecting water quality since they were among the top activities, while farming was the second-highest activity. *E. coli*, *Salmonella* spp. and *Shigella* spp., being allochthonous in nature, are introduced through agriculture, urban surface run-off, waste water discharges and domestic waste (Fujioka, 2002; Robin et al., 2012).

Figure 3: Laundry-washing at the water source

Figure 4: Domestic animals grazing around the water source

Figure 5: Surface run-off from agricultural lands

Figure 6: Dumping of diapers into water sources

2.3.2 Surface water contamination by diarrhoea pathogens

River water is an important reservoir for *V. cholerae,* which is the aetiological agent of cholera (Madoraba & Momba, 2010; Wang et al., 2010). *V. cholerae* has been isolated from surface water (Percival et al., 2004; Fraga et al., 2007). The occurrence of *V. cholerae* in water sources can be linked to faecal pollution (Cox et al., 2005). In addition, *Salmonella* has been detected repeatedly in various types of natural water such as rivers, lakes, coastal waters, estuaries as well as contaminated ground-water (Moganedi et al., 2007; Haley et al., 2009; Wilkes et al., 2009; Levantesi et al., 2010). The presence of *Salmonella* spp. in natural water resources has also been attributed to run-off from fields with animal husbandry and the disposal of untreated sewage (Moganedi et al., 2007; Jenkins et al., 2008). A study by Potgieter et al. (2005) on surface water used as a drinking water source in the Vhembe District has shown that the water was positive for *Salmonella.* The findings from previous studies that detected *Shigella* in surface waters indicate that surface waters could potentially transport *Shigella* strains (Wose Kinge & Mbewe, 2010).

A study done by Traore et al. (2016) on some of the rivers in the Venda region detected several pathogenic strains of *E. coli* such as enteroinvasive *E. coli* (EIEC), enterotoxigenic *E. coli* (ETEC) and enteropathogenic *E. coli* (EPEC), which are strains known for causing diarrhoea in children (Omar & Barnard, 2014). *E. coli* is a member of faecal coliform bacteria that are inherent to the gastro-enteric tracts of humans. It is mostly associated with faeces and has been chosen as a prime indicator of faecal pollution. Its presence in water is predictive of other pathogenic microbes such as enteric bacteria, viruses and parasites. Environmental monitoring of surface waters has employed *E. coli* on several occasions due to its easy detection by simple laboratory methods. However, *E. coli* is not reliable when predicting the presence of parasites and viruses. Other indicators include *Enterococci, Clostridium perfringens* and bacteriophages (Savichtcheva & Okabe, 2006). *C. perfringens* is an alternative indicator of faecal pollution: its detection in water signifies the possible presence of protozoan parasites and enteric viruses. *C. perfringens* is also regarded as a conservative indicator of faecal pollution (Brookes et al., 2005). Bacteriophages indicate the possible presence of viruses in water (McMinn et al., 2017).

River water has been shown to be subject to contamination by protozoan parasites such as *Cryptosporidium* and *Giardia* due to point or non-point pollution sources (Dreelin et al., 2014). According to Robertson et al. (2006), both *Cryptosporidium* and *Giardia* are associated with sewage. Thus, contamination of water sources by sewage threatens human health due to their low infectivity dose, which is as low as 10 oocysts/cyst (Dallingham et al., 2002; Wright, 2005). Outbreaks of cryptosporidiosis and giardiasis have been linked to sewage contaminating drinking water (Gaffga et al., 2007). Reports on parasitic organisms in waste water are rare in South Africa and yet *Cryptosporidium* and *Giardia* are reported to be the most prevalent parasites in waste water samples (Samie & Ntekele, 2014).

Gastro-enteric viruses such as rotaviruses and noroviruses together with human adenoviruses are the most frequently detected human viruses in water samples (Calgua et al., 2013). Studies focused on river water have detected noroviruses and human adenoviruses in the Llobregat River in Barcelona (Spain), and the Macacos and Fairas Timbo rivers in Rio de Janeiro (Brazil) (Calgua et al., 2013). In Africa, rotaviruses and adenoviruses were detected in surface water in the rural areas of Benin, West Africa (Verheyen et al., 2009). In South Africa, a study conducted in the Tyme river in the Eastern Cape Province detected both rotaviruses and noroviruses; the detection of the noroviruses was sporadic (Sibanda & Okoh, 2013). Mans et al. (2013) also highlighted that noroviruses are still detectable in sewage-polluted rivers in South Africa. However, there is no data regarding the detection of enteric viruses in surface waters in the Vhembe District except for a study done by Obi et al. (2002) that noted the detection of somatic phages, which is a likely indicator of viral water contamination (Grabow, 2001; Hot et al., 2003; Yousefi & Zazouli, 2008). Hence, little is known about the frequency and pattern of viral contamination of drinking water sources in resource-poor settings such as rural areas in the Vhembe District (Verheyen et al., 2009).

2.3.3 Physico-chemical parameters affecting microbial water quality

Physical parameters together with microbiological parameters are a direct measure to assess pollution levels. Therefore, effective monitoring of these parameters may help mitigate pollution of surface waters (Chandra et al., 2006). The natural processes of pathogen reduction restrict the microbial density in water within limits. However, following pollution, the concentration of parameters change and in most cases, this expands the microbial density (Gerardi & Zimmerman, 2005).

2.3.3.1 Temperature

Evaluation of the quality of potable water depends mainly on temperature, which affects many phenomena such as the rate of chemical reactions in the water body, reduction in solubility of gases and amplifications of tastes and colours of water (Olajire & Imeokparia, 2001). A normal temperature range supportive of good surface water quality is between 0°C and 30°C (Chapman, 1996). Research has suggested that warmer temperatures during summer coincide with *Salmonella, Campylobacter* or *E. coli* infections (Kovats et al., 2004; Fleury et al., 2006). Low winter temperatures favour viruses such as rotaviruses and noroviruses (Levy et al., 2009; Keller et al., 2010). According to Bishankha et al. (2013), an increase in temperature or high temperatures has a negative impact on water quality by significantly enhancing microbial growth. Conversely, a negative correlation of temperature and enteric bacterial counts was established in India (Borade et al., 2015).

2.3.3.2 pH

The pH of most raw surface water sources ranges from 6.0 to 8.5 (WHO, 2002; Jordaan & Bezuidenhout, 2012). During the wet season, the pH of surface water is generally low at about 7.0 or below, but the pH is above 7.0 during the dry season (Oyhakilome et al., 2012). This observation is also positively correlated to temperature (Frimpong et al., 2015). The relationship between pH and both *E. coli* and TC has established that the bacterial counts are high in the pH range from 6.0 to 7.0, and bacterial counts are low at a pH above 7.0 (Oyhakilome et al., 2012; Frimpong et al., 2015). This observation is also

supported by a negative correlation of pH and bacterial counts observed by Borade et al. (2015). However, Bishankha et al. (2013) observed no significant effect of pH on microbial growth.

2.3.3.3 Total dissolved solids

Total dissolved solids (TDS), which is a quantitative measure of dissolved salts (Olawale, 2016), mainly assess the general water quality (DeZuane, 1977). Water sources gain TDS concentration levels following sewage disposal, run-off from agricultural lands and industrial waste water discharge (DWAF, 1996; WHO, 2003). However, previous studies have associated wet seasons with low TDS concentrations due to dilution effects while dry seasons had high levels of TDS (Oyhakilome et al., 2012; Olawale, 2016). An acceptable limit for water quality standards based on TDS is 1000 mg/L. However, there is no data regarding the health effects associated with consuming such water, but high TDS levels affect the aesthetic value of water and taste negatively (DWAF, 1996; WHO, 2003).

2.3.3.4 Conductivity

The freshness of a water source relies on its conductivity (Aiyesanmi et al., 2006). There is an established direct relationship between conductivity and TDS as conductivity depends on the dissolved salts in water (Arimieari et al., 2014). According to McKelvie (2004), the freshness and potability of a water source based on conductivity are only credible within the 0–325 µS/cm range. However, the South African Water Quality Guidelines on water quality for domestic use do not specify a guideline (DWAF, 1996).

2.3.3.5 Dissolved oxygen

The levels of dissolved oxygen (DO) in water affect the freshness of water as well as aquatic life (Arimieari et al., 2014). According to Olawale (2016), a community water supply with high levels of DO provides water of good taste. The factors that affect DO levels in surface water sources, such as rivers, include domestic, agricultural, industrial effluent and waste discharges (Olawale, 2016). Aiyesanmi et al. (2006) highlighted that DO levels above 6 mg/L in surface water have acceptable quality while DO levels below 4 mg/L signify pollution and thus poor water quality.

3 METHODS

3.1 Description of the Study Area

3.1.1 Overview

The Vhembe District (25 597.42 km^2) is largely a rural area faced with infrastructure backlogs for water, sanitation and electricity. Its communities (1 294 722 inhabitants) use water sources outside their households. The Vhembe District is bounded in the north by the Limpopo River, in the west by the Sand River, and in the south and east by the Luvuvhu River. The district is served by four local municipalities, namely, Makhado, Musina, Mutale and Thulamela (Stats SA, 2011). The Venda tribe is the dominant ethnic group followed by the Dembeles or Tsonga tribe.

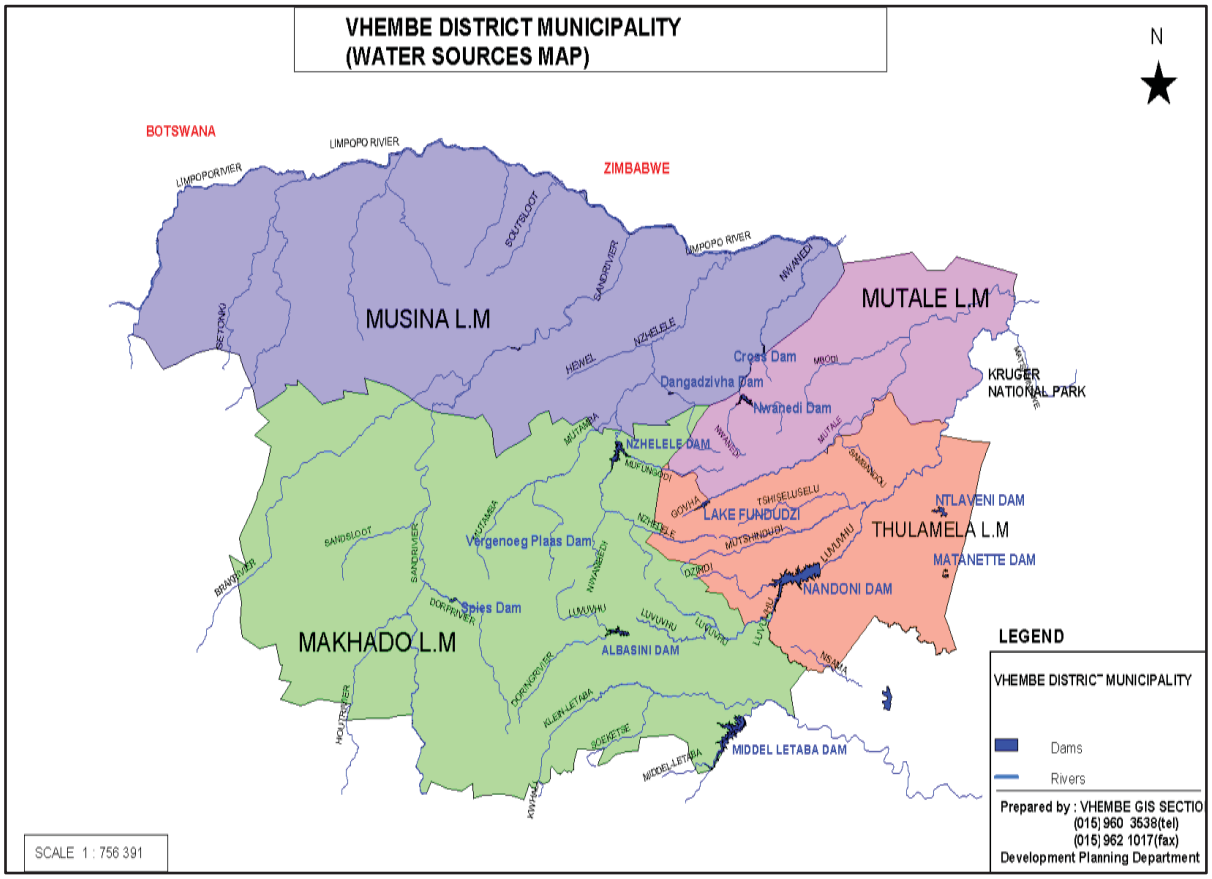


Figure 7: Geographical location of the study area (adapted from Google Maps)

3.1.2 Water sources

3.1.2.1 Surface waters

Rivers were identified as the prime water source used by people in rural and peri-urban areas as an alternative means of meeting their water supply demands (Obi et al., 2002). This study focused on ten rivers, which were identified prior to commencing with sampling. The rivers were chosen based on their proximity to rural and peri-urban communities and public health care clinics. The water abstraction points were chosen as sampling points. In the Thulamela catchment, the Tshinane, Mutshundudi, Sambandou, Luvuvhu (Mutoti site), Luvuvhu (Mhinga site), Dzindi and Madadzhe rivers were tested. In the Mutale catchment, the Mutale River was tested. In the Makhado catchment, the Nzhelele and Luvuvhu (Tshino site) rivers were tested. Sampling was done to cover two seasons. The collection of samples covered winter months (June–August 2016) and summer months (October–December 2016). The various activities taking place around river sources were also noted.

3.1.2.2 Groundwater sources

Water was collected from nine functional boreholes in three different rural villages (Muraga, Basani and Tshikonelo). These boreholes are small (less than 300 mm in diameter) machine-drilled wells, which are typically 30–90 m deep (Figure 8). These sources are all cased, screened and equipped with a sanitary seal and drainage aprons.

Figure 8: Typical borehole found in the Vhembe District

3.1.3 Household water storage containers

Water storage containers (Figure 9) include plastic drums and buckets with lids. A total of 89 households in three different villages (Muraga, Basani and Tshikonelo) were visited randomly to sample water from water storage containers used the day of collection for drinking and cooking.

Figure 9: Examples of household water storage containers in the Vhembe District

3.2 Study Design

3.2.1 Collection of water samples

River water samples were collected in sterile plastic 500 ml Nalgene containers for bacterial analysis. Sterile 10 L containers were used to collect river water for analysis of parasites and viruses. The samples were kept on ice while being transported to the University of Venda and the Rand Water Board for parasite analysis, and the Enteric Virus and Environmental Research Group at the University of Pretoria (for virus analysis). Water samples from household water storage containers and boreholes were collected in 500 ml sterile bottles between August 2015 and September 2016. The samples were kept on ice while being transported to the University of Venda.

3.3 Analyses of Water Samples

3.3.1 Physical parameters

Physical parameters included pH, temperature, salinity, electrical conductivity, TDS and DO, which were measured on-site using a portable multi-meter reader (Bante 900P model; Shanghai, China).

3.3.2 Detection of indicator organisms

3.3.2.1 TC and E. coli

E. coli and TC were assessed using the Colilert Quanti-Tray/2000 technique (IDEXX). A volume of 100 ml water sample was poured into a graduated 100 ml IDEXX vessel whereafter the content of one packet of Colilert-18 reagent was added. The vessel was capped and the mixture shaken until all reagent had dissolved. This reagent mixture was poured into a Quanti-Tray and sealed in an IDEXX Quanti-Tray® Sealer. The trays were incubated at 35.5°C and the results recorded after 24 hours. After incubation, the Quanti-Trays/2000 were examined under long wave (366 nm) ultraviolet light. Wells that turned yellow and had fluorescence were counted as *E. coli*, while all wells that turned yellow without fluorescence were counted as TC (Omar et al., 2010).

3.3.2.2 C. perfringens

Water samples were analysed by the membrane filtration method followed by culturing of the filters. Membrane *C. perfringens* (m-CP) agar plates as described by Bisson & Cabelli (1979) were used. According to the published method (EPA, 1996) for clean surface water, 10 ml, 30 ml and 50 ml may be filtered. In the case of turbid surface water, 3 ml, 10 ml and 30 ml may be filtered.

In this study, 3 ml, 10 ml and 30 ml were considered and 0.45 µm, 47 mm cellulose acetate membrane filters (Sartorius Biolab Products) were used. The filters with the trapped microorganisms were overlaid on m-CP agar (Oxoid CM0992, pH 7.6) using sterile forceps. The plates were incubated anaerobically at 42°C (Bonde, 1963) for 24 hours in a jar containing AnaeroGen sachets (Oxoid). Yellow colonies that turned pink on exposure to ammonium hydroxide fumes were considered presumptive and were Gram-stained following the protocol described by Prescott (2002) and Willey et al. (2014).

3.3.3 Detection of pathogenic bacteria

3.3.3.1 V. cholerae

The isolation of *V. cholerae* from water samples was performed according to the methodology published by Choopun et al. (2002). A volume of 100 ml of each sample was filtered through a 0.45 µm pore size 47 mm cellulose acetate filter (Sartorius Biolab Products, Lasec). The filters were enriched in 100 ml alkaline peptone water (Sigma-Aldrich, 2% NaCl) at 30°C for 24 hours. A volume of 10 µL of the enriched samples was streaked onto thiosulfate–citrate–bile salts–sucrose (TCBS) agar plates (pH 8.6, Davies Diagnostics Pty, Limited). The plates were incubated at 37°C for 24 hours.

All yellow colonies together with green-yellow colonies were considered presumptive positive. The presumptive isolates were sub-cultured onto TCBS agar prior to nutrient agar and subjected to the

oxidase test (Oxidase strips, Sigma-Aldrich) and API-[1]20E test (bioMérieux Product, supplied by Quantum Biotech) following the manufacturer's instructions. Gram-staining was done according to the protocol described by Prescott (2002) and Wiley et al. (2014). The isolates that showed consistency with the expected results of oxidase test, Gram-staining and API-20E were preserved in nutrient broth and stored at −20°C until further analysis.

3.3.3.2 Salmonella and Shigella

Water samples (100 ml) were concentrated using the membrane filtration technique to isolate *Salmonella* spp. and *Shigella* spp. Membrane filters (0.45 µm, 47 mm cellulose acetate, Sartorius Biolab Products, Lasec) were used. The membrane filters were submerged into 100 ml buffered peptone water (Oxoid CM0509, pH 7.2). The flasks were shaken by hand for 5 minutes to mix the trapped bacteria on the filter pads with the pre-enrichment broth. Thereafter, they were incubated at 30°C and 37°C for 24 hours respectively. A successive selective enrichment step in Rappaport-Vassiliadis soya peptone broth (Oxoid CM0866, pH 5.2), nutrient broth (Sigma-Aldrich pH 7.5) and Selenite cysteine broth (Difco, BD Product pH 7.0) accompanied by incubation at 42°C for 48 hours was observed. Next, a loopful of the enriched samples was streaked on selective media. *Salmonella–Shigella* (S-S) agar (Difco, BD Product pH 7.0), a selective media for *Salmonella* and *Shigella* spp., and the plates were incubated at 37°C for 24 hours. Presumptive isolates were sub-cultured onto S-S agar prior to sub-culturing onto nutrient agar and then subjected to biochemical tests; oxidase test following the manufacturer's instructions (Oxidase strips, Sigma-Aldrich) and API-20E test following the manufacturer's instructions (bioMérieux Product, supplied by Quantum Biotech). The isolates that showed consistency with the expected results of the oxidase test, Gram-staining and API-20E were preserved in nutrient broth and stored at −20°C until further analysis.

3.3.4 Multiplex PCR (M-PCR) for pathogenic bacteria

3.3.4.1 Vibrio spp., Salmonella spp. and Shigella spp. confirmation

The m-PCR protocol published by Mieta et al (2010) was used. The m-PCR targeted:

- The *ial* gene for EIEC and *Shigella* spp.
- The *ipa*B gene for *Salmonella* spp.
- The *ipa*H gene for *Shigella* spp.
- The *sod*B gene for *Vibrio* spp.

The primers used for the m-PCR are shown in Table 2. The Bio-Rad MyCycler Thermal cycler was used for all PCR reactions that were in a total volume of 20 µL. A positive control was a 2 µL mixture of *V. cholera* O1, EIEC, *S. dysenteriae* and *S. typhimurium* DNA. The negative control excluded template deoxyribonucleic acid (DNA).

Table 2: Primers used for the multiplex PCR (Mieta et al., 2010)

Strain	Target gene	Primer (5'–3')	Size (bp)	Reference
EIEC and *Shigella*	*ial*	(F): GGTATGATGATGATGAGTGGC	630	Paton & Paton (1998)
		(R): GGAGGCCAACAATTATTTCC		
Shigella	*ipa*H	(F): CCTTGACCGCCTTTCCGATA	606	Kong et al. (2002)
		R): CAGCCACCCTCTGAGGTACT		
Salmonella	*ipa*B	(F): GGACTTTTTAAAAGCGGCGG	314	Kong et al. (2002)
		(R): GCCTCTCCCAGAGCCGTCTGG		

[1] Analytical profile indexing for *Enterobacteriaceae*

Strain	Target gene	Primer (5'–3')	Size (bp)	Reference
Vibrio	*sod*B	(F): AAGACCTCAACTGGCGGTA	248	Tarr et al. (2007)
		(R): GAAGTGTTAGTGATCGCCAGAGT		

F – forward primer; R – reverse primer

3.3.4.2 E. coli confirmation

The samples were analysed by the Water and Health Research Centre at the University of Johannesburg. Briefly, this method was performed as described by Omar et al. (2010) and Traore et al. (2016). A volume of 2 ml was extracted from randomly selected ten positive wells of the Colilert Quanti-Tray using a 1 ml disposable syringe and aliquoted into 2 ml Eppendorf tubes. The tubes were stored at −20°C until further analysis. The extraction of DNA and m-PCR reactions was performed according to the methodology described by Omar & Barnard (2014). The extracted DNA was used as a template in all PCR reactions. All PCR reactions proceeded in a Bio-Rad MyCycler™ Thermal cycler in a total volume of 20 µL. The targeted genes for the pathogenic *E. coli* strains and their primers are listed in Table 3.

Table 3: Primers used in molecular detection of pathogenic *E. coli* strains (Omar & Barnard, 2014)

***E. coli* strain**	**Target gene**	**Primer (5'–3')**	**Primer concen-tration (µM)**	**Size (bp)**	**Reference**
Commensal *E. coli*	*Mdh*	(F): GGT ATG GAT CGT TCC GAC CT (R): GGC AGA ATG GTA ACA CCA GAG T	0.1	304	Tarr et al. (2002)
EIEC	*Ial*	(F): GGT ATG ATG ATG ATG AGT CCA (R): GGA GGC CAA CAA TTA TTT CC	0.2	650	López-Saucedo et al (2003)
EHEC/atyp EPEC	*eaeA*	(F): CTG AAC GGC GAT TAC GCG AA (R): CCA GAC GAT ACG ATC CAG	0.3	917	Aranda et al. (2004)
Typ EPEC	*Bfp*	(F): AAT GGT GCT TGC GCT TGC TGC (R): TAT TAA CAC CGT AGC CTT TCG CTG AAG TAC CT	0.2	410	Aranda et al. (2004)
EAEC	*Eagg*	(F): AGA CTC TGG CGA AAG ACT GTA TC (R): ATG GCT GTC TGT AAT AGA TGA GAA C	0.2	194	Pass et al. (2000)
EHEC	*stx1*	(F): ACA CTG GAT GAT CTC AGT GG (R): CTG AAT CCC CCT CCA TTA TG	0.5	614	Moses et al. (2006)
	stx2	(F): CCA TGA CAA CGG ACA GCA GTT (R): CCT GTC AAC TGA GCA CTT TG	0.3	779	Moses et al. (2006)
ETEC	*Lt*	(F): GGC GAC AGA TTA TAC CGT GC (R): CGG TCT CTA TAT TCC CTG TT	0.1	360	Pass et al. (2000)
	St	(F): TTT CCC CTC TTT TAG TCA GTC AAC TG (R): GGC AGG ATT ACA ACA AAG TTC ACA	0.5	160	Pass et al. (2000)
***E. coli* toxin**	*astA*	(F): GCC ATC AAC ACA GTA TAT CC (R): GAG TGA CGG CTT TGT AGT C	0.2	106	Kimata et al. (2005)
External control	*Gapdh*	(F): GAG TCA ACG GAT TTG GTC GT (R): TTG ATT TTG GAG GGA TCT CG	0.2	238	Mbene et al. (2009)

F – forward primer; R – reverse primer

3.3.5 Detection of protozoan parasites

Due to the cost, only river water samples were sent to Rand Water to detect the prevalence of parasites. The Rand Water laboratory is a SANS-accredited laboratory that complies with ISO/IEC 17025. The method 1623.1 as described by USEPA was employed. Water samples (10 L) were filtered using the PALL system employing Envirochek capsules. The trapped oocysts and cysts together with extraneous materials were eluted using an elution buffer solution. The eluate was centrifuged at 1500 × g to pellet the oocysts and cysts. The supernatant was aspirated. Thereafter, the oocysts and cysts were separated from the extraneous materials using an immunomagnetic separation technique employing paramagnetic beads (Dynabeads® GC-Combo) conjugated to anti-*Cryptosporidium* and anti-*Giardia* antibodies. In the presence of oocysts or cysts, a paramagnetic complex is formed, which is attracted by a magnet, thus separating the oocysts/cysts from the extraneous materials. Next, the beads were detached from the oocysts/cysts. The oocysts and cysts were stained on well slides with fluorescently labelled monoclonal antibodies and 4′,6-diamidino-2-phenylindole (DAPI), a fluorescent stain that binds strongly to adenosine–thymine rich regions in DNA. The stained samples were examined using fluorescence differential interference contrast (DIC) microscopy. Qualitative analysis was first performed by scanning each slide well for objects that met the size, shape and fluorescent characteristics of *Cryptosporidium* oocysts or *Giardia* cysts. Quantitative analysis was achieved by counting the total number of objects on the slides that met functional assay, DAPI and DIC criteria for oocysts or cysts.

3.3.6 Detection of enteric viruses

Only river water samples were sent for analyses by the Enteric Virus and Environmental Virology Research Lab at the University of Pretoria, according to SANS 241-1 (2015) Ed. 2 for drinking water. This was due to the cost factor.

3.3.6.1 Detection of viruses by cell culture

Viruses were recovered from 10 L surface water samples by standard glass wool adsorption-elution protocols followed by a secondary concentration step by organic flocculation with beef extract as recommended by Calgua et al. (2013). A modification of the glass wool adsorption-elution method developed by Vilagines et al. (1993) as described by Mans et al. (2013) and Kiulia et al. (2014) was used. Briefly, the modified method described by Mans et al. (2013) used 15 g of glass wool per column and a steel gauze grid (pore size = 1 mm^2, 30 mm diameter) inserted between each of the three 5 g portions of glass wool. Positively charged glass wool columns were used to capture the viruses, which are generally negatively charged (Kiula et al., 2014). The negatively charged viruses, which had adsorbed to the glass wool, were eluted with 100 ml of sterile glycine-beef-extract buffer of pH 9.5 (0.3754% w/v glycine, 0.5 % beef extract), which reverses the ionic charges of the viruses and releases them from glass wool. The pH of the eluate was adjusted to neutral with 1 M HCl. In the secondary concentration step, the 100 ml eluate was concentrated to a final volume of 20 ml in sterile phosphate buffered saline (pH 7.4, Sigma-Aldrich Co., USA) by polyethylene glycol/sodium chloride precipitation as described by Minor (1985) and Vilagines et al (1997). The recovered concentrate was stored at −20°C until further processing.

Generally, epidemiological important viruses [hepatitis A virus (HAV), human rotaviruses, human astrovirus] are difficult to culture in vitro while some cannot be propagated (noroviruses) in cell cultures (Duizer et al., 2004; Formiga-Cruz et al., 2005; Greening, 2006). In this study, the cell culture was tested for adenoviruses and enteroviruses only. Direct PCR was used to test for adenoviruses, enteroviruses, HAV, norovirus genotype I (NoV GI) and norovirus genotype II (NoV GII), rotaviruses and sapoviruses. Based on the findings by Rodriguez et al. (2008) and supported by Van Heerden et al (2003), the human hepatoma cell line *PLC/PRF/5* has been shown to enable the enumeration of culturable viruses from sewage-contaminated water. In this study, three cell lines were used, namely, the *PLC/PRF/5 cell line* [European Collection of Cell Cultures (ECACC 85061113), Salisbury, UK] (Alexander et al., 1976), the

BGM African Green Monkey Kidney cell line (ECACC 90092601), (Dahling et al., 1974) and the *Vero African Green Monkey Kidney cell line* (ECACC 84113001).

All cell lines were propagated, maintained and infected using standard cell culture techniques as described by Grabow et al. (1999), Vivier et al. (2004) and Mans et al. (2013). Briefly, the confluent monolayers of the *PLC/PRF/5 cell line* and *BGM African Green Monkey cell line* prepared in 25 cm^2 plastic flasks were infected with 1 ml of the antibiotic and anti-mycotic treated viral concentrate (Vivier et al., 2004). The infected cells were incubated for 14 days inclusive of a blind passage at day 7 after infection. The infected cells were harvested for molecular analysis as well as for blind passage onto monolayers of Vero cells in cell culture tubes with flying coverslips. After a further incubation of 7 days at 37°C, the infected cell cultures were examined for cytopathogenic effects (CPE), stained with haematoxylin and eosin, and examined for virus-specific inclusion bodies (Malherbe & Strickland-Cholmley, 1980).

3.3.6.2 Virus detection by molecular based assays

Recovered viral concentrate (1 ml) was seeded with 10 µL of mengovirus (5×10^5 copies) as an extraction control. The extracted nucleic acid was eluted in 100 µL, aliquoted into small volumes and stored at −70°C. The harvested cell culture suspensions (500 µL) were subjected to three cycles of freezing and thawing prior to nucleic acid extraction. Nucleic acids were extracted from 200 µL of the harvested cell culture extracts using the NucliSENS® easyMAG® instrument (bioMérieux, Marcy l 'Etoile, France) according to the manufacturer's instructions. The extracted nucleic acids were eluted in 50 µL, aliquoted in smaller volumes and stored at −70°C.

Commercial real-time reverse transcriptase PCR (RT-PCR) assays using TaqMan® technology were applied to the direct detection of selected human pathogenic enteric viruses in the recovered virus concentrate, namely, adenoviruses, astroviruses, enteroviruses, HAV, mengovirus, norovirus GI, norovirus GII, rotaviruses and sapoviruses. The TaqMan® Environmental Master Mix 2.0 kit (Applied Biosystems, Foster City, CA) was used for adenoviruses. The Transcriptor First Strand cDNA synthesis kit (Roche) in conjunction with the LightCycler® TaqMan® master kit (Roche) was used for molecular amplification of and real-time detection of sapoviruses. For astroviruses, enteroviruses, HAV, mengovirus, norovirus GI, norovirus GII and rotaviruses, the ceeramTools® using the TaqMan® technology was used to analyse all samples. Artificially seeded samples were used to analyse mengoviruses.

The primers and probes used for the direct analysis of viruses are shown in Table 4. For the integrated cell culture-molecular based assay, enteroviruses were detected with a one-step real-time RT-PCR assay using the Quantitect Probe RT-PCR Kit (Qiagen, Hilden, Germany) and primers and a hydrolysis probe as previously described by Fuhrman et al. (2005) and the adenoviruses using primers and probes as described by Heim et al. (2003) in the TaqMan® Environmental Master Mix 2.0 kit.

3.4 Data Analysis

Means and standard deviations were calculated for the numbers of *E. coli* detected from water samples after extraction using each method. Differences between the amounts of DNA extracted using each method were tested for significance using a student's t-test. Statistical significance was defined at a p value of less than 0.05. All statistical analyses were performed using PAleontological STatistics (PAST) Version 3.0.

Table 4: Primers and Probes used in real-time RT-PCR

Virus	Primer (5'->3')	Reference
Adenovirus	50-GCC-ACG-GTG-GGG-TTT-CTA-AAC-TT-30, Adenoquant 1 (AQ1) 50-GCC-CCA-GTG-GTC-TTA-CATGCA-CAT-C-30, Adenoquant 2 (AQ2) 50-TGC-ACC-AGA-CCC-GGG-CTC-AGGTAC-TCC-GA-30 (Adenoprobe, AP) FAM labelled	Heim et al., 2003
HAV	R: HAV240 (5'-GGAGAGCCCTGGAAGAAA G-3') F: HAV68 (5'-TCACCGCCGTTTGCCTAG-3') Probe: HAV150 (5'-TTAATTCCTGCAGGTTCAGG-3')	Costafreda et al., 2006
Mengovirus	R: Mengo209 (5'-GAAGTAACATATAGACAGACGCACAC-3') F: Mengo110 (5'-GCGGGTCCTGCCGAAAGT-3') FAM-MGB probe Mengo147 (5'-ATCACATTACTGGCCGAAGC-3')	Pinto & Saiz, 2007
Norovirus GI	F: QNIF4 (CGCTGGATGCGNT TCCAT) R: NV1LCR (CCTTAGACGCCATCATCATTTAC) (33) Probe: NV1LCpr(6-carboxyfluorescein–TGGACAGGAGAYCGCRA TCT–6-carboxytetramethylrhodamine)	Da Silva et al., 2007 Svraka et al., 2007
Norovirus GII	COG2F: CARGARBCNATGTTYAGRTGGATGAG (+)5003c COG2R: TCGACGCCATCTTCATTCACA (-)5100c Probe: RING2-TPFAM-TGGGAGGGCGATCGCAATCT-TAMRA (+)5048c	Kageyama et al., 2003 Loisy et al., 2005
Sapovirus	CU-SV-F1 GAC CAG GCT CTC GCY ACC TAC 5074–5094a CU-SV-F2 TTG GCC CTC GCC ACC TAC 786–803b CU-SV-R CCC TCC ATY TCA AAC ACT AWT TTG 5177–5154a CU-SV-Probec TGG TTY ATA GGY GGT AC 5101–5117a	Chan et al., 2006
Enterovirus	R:Ev1[5-GATTGTCACCATAAGCAGC-3 F:Ev2 5-CCCCTGAATGCGGCTAATC-3 Ev-probe (5-FAM-CGGAACCGACTACTTTGGGTGTCCGT-BHQ-Phosphor-3)	Fuhrman et al., 2005

a GenBank accession number NC 006269
b GenBank accession number U95644
c Corresponding nucleotide position of Camberwell virus (accession no. AF145896) of the 5-end

4 RESULTS AND DISCUSSION

4.1 Human and Animal Activities at Surface Water Sources

The various activities taking place around the rivers were recorded and are listed in Table 5. Many of these activities, such as doing laundry, washing cars and littering, can contaminate river water. The results agree with the findings by Nevondo & Cloete (1999) and Traore et al. (2016) that most rivers in rural areas of South Africa are subjected to pollution through activities such as washing clothes, bathing and animal activities. These are all risk factors that compromise drinking water quality in communities. The problem in rural and peri-urban communities is that operational monitoring to assess these potential pollution hazards is not done on a regular basis and only when outbreaks occur: reactive management is used by the government to stop or eliminate the problem.

Table 5: Activities seen around the selected river water source sampling sites

River site	Description of observed activities
Mutale	Laundry, bathing, car wash, cattle drink from the river
Sambandou	Laundry, cattle and donkeys drink from the river, animal grazing, people fetch water for construction purposes and other household duties, animal faecal matter
Tshinane	Laundry, bathing, dumping of chicken feathers
Mutshundudi	Laundry, bathing, littering
Madadzhe	Agriculture, domestic sewage disposal, car wash
Luvuvhu (Mhinga)	Laundry, car wash, swimming, bathing, fishing
Luvuvhu (Mutoti)	Laundry, bathing, fishing, car wash, cattle drink from the river, people fetch water for construction purposes
Luvuvhu (Tshino)	Cattle grazing, fishing,
Nzhelele	Car wash, people fetch water, swimming, agriculture
Dzindi	Agriculture

4.2 Physical Parameters of Surface Water Sources

Several studies have noted some degrees of positive correlation between physical and chemical parameters and pathogen occurrence in surface waters. According to Rico et al. (2016), a well-balanced correlation exists between the physical and chemical parameters and biological composition of rivers. However, indiscriminate sewage disposal, industrial waste and various anthropogenic activities disturb the state of equilibrium.

The recordings in Figure 10 and Figure 11 show the parameter readings of water collected from the respective rivers for both winter months (June–August) and summer (October–December) for the year 2016. According to the South African Water Quality Guidelines, the target water quality range for pH of raw surface water sources is between 6.5 and 8.5 (DWAF, 1996). The acceptable pH range for surface water in accordance of WHO guidelines is between 6.0 and 8.5 (WHO, 2002; Jordaan & Bezuidenhout, 2012). In this study, there was not much difference between the pH for winter (7.2–8.4) and summer (6.8–8.2), and the recordings were all within the range between 6.0 and 8.5. It follows that all rivers were generally within the acceptable pH range for surface waters and thus unlikely to cause health effects.

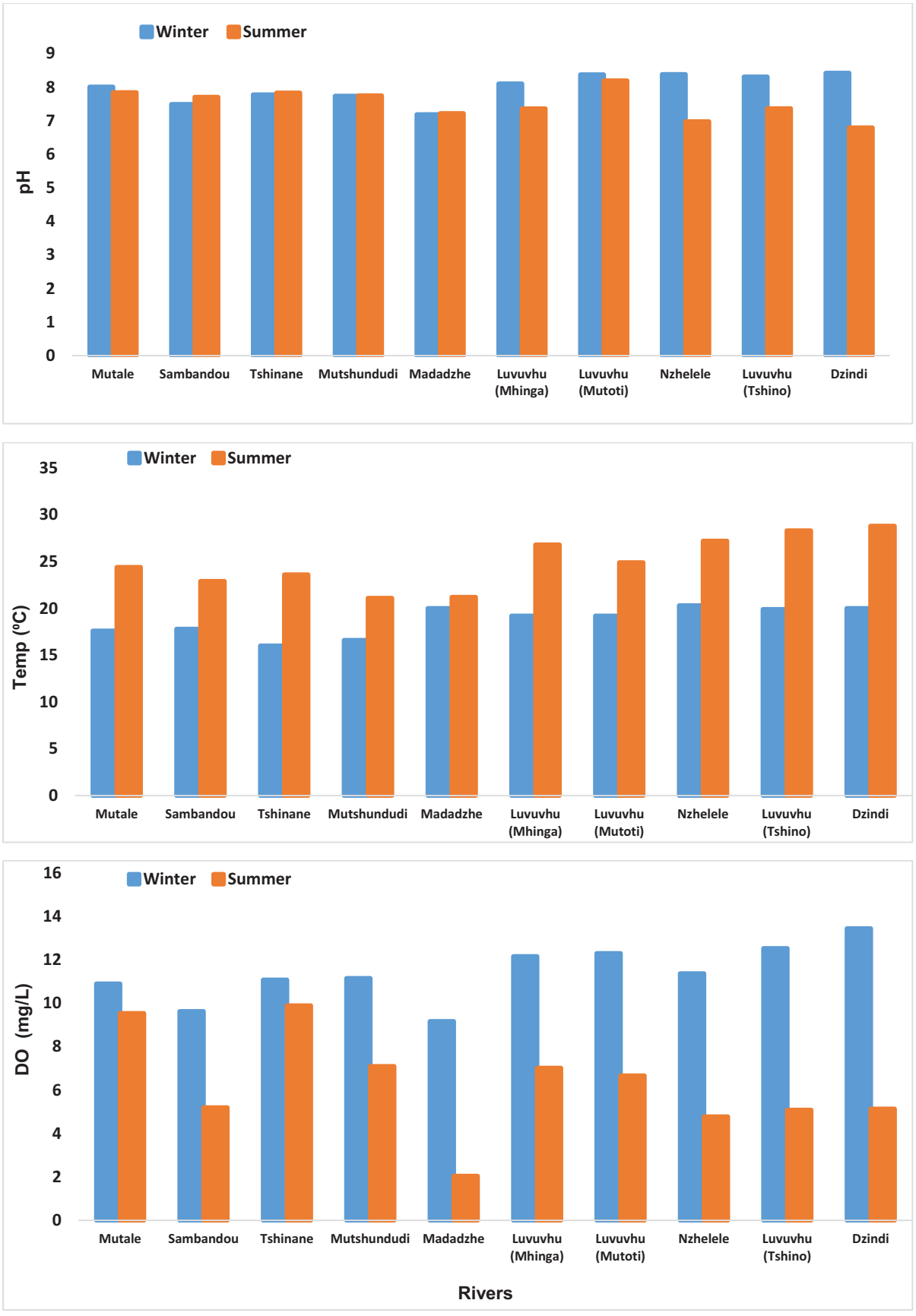


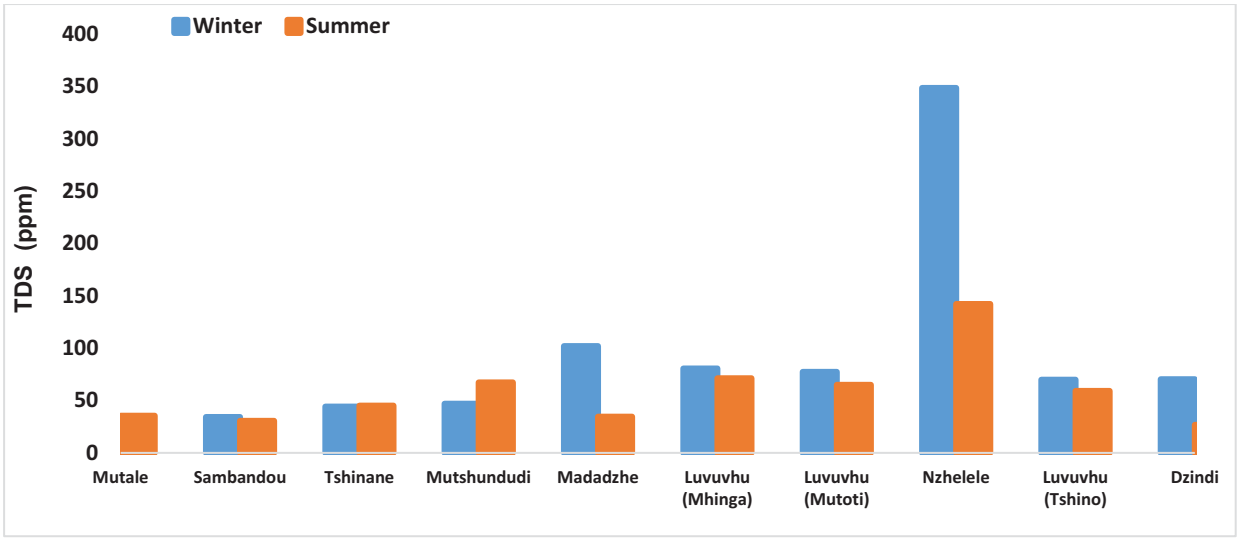
Figure 10: Assessment of pH, temperature and DO in various river water sources

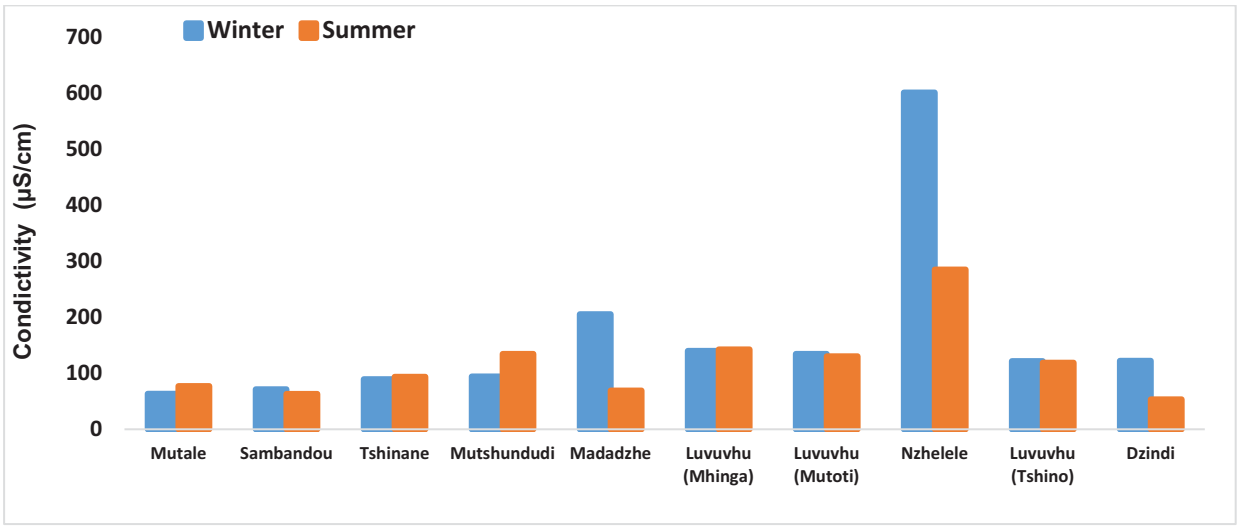
The minimum water temperature recorded was 16°C (winter) and 21.1°C (summer) while the maximum recorded water temperature was 20.3°C (winter) and 28.8°C (summer). The recorded temperatures for both seasons were from 0–30°C, which is the range supportive of good quality for surface waters (Chapman, 1996). According to literature, warmer temperatures during summer coincide with *Salmonella, Campylobacter* or *E. coli* infections (Kovats et al., 2004; Fleury et al., 2006) and low winter temperatures favour viruses such as rotavirus and noroviruses (Levy et al., 2009; Keller et al., 2010).

DO characterises the freshness of surface water, which is directly related to the amount of oxygen dissolved. This is supported by a positive correlation between biological oxygen demand and bacterial counts observed by Borade et al (2015). At the same time, Aiyesanmi et al. (2006) classified water of DO > 6.2 mg/L as acceptable quality while DO < 6.0 mg/L is classified as polluted water. In addition, Rao (2005) commented that DO concentration in unpolluted water normally ranges between 8 mg/L and 10 mg/L and concentration less than 5 mg/L adversely affect aquatic life. In the present study, DO was higher in winter than in summer (Figure 10). The minimum recorded DO was 9.16 mg/L in winter and 2.04 mg/L in summer, while the maximum DO was 13.43 mg/L and 9.88 mg/L respectively.

Both winter conductivity and TDS showed a noticeable difference. The minimum conductivity was 63.3 µS (Mutale River) while the maximum was 601 µS (Nzhelele River). The Nzhelele River showed the highest readings in conductivity and TDS among all ten rivers (Figure 11). The minimum TDS was recorded in Mutale River (29.9 ppm) while the maximum recorded TDS was noted in Nzhelele River (348.5 ppm).

Salinity did not differ much in most of the rivers. The differences were within 0.01 psu units. The Madadzhe and Nzhelele rivers showed a greater deviation with the rivers recording 0.10 psu and 0.25 psu respectively. The occurrence of *V. cholerae* in aquatic environments is primarily favoured by temperature and salinity (Singleton et al., 1982). However, salinity favourable for *V. cholerae* growths are found in inland coastal areas and estuaries; the optimum salinity for *V. cholerae* growth is between 5 psu and 25 psu (Sedas, 2007). In this study, none of the ten rivers had salinity in that range, which could be a possible explanation for the low detection of *V. cholerae* as discussed later in this chapter.





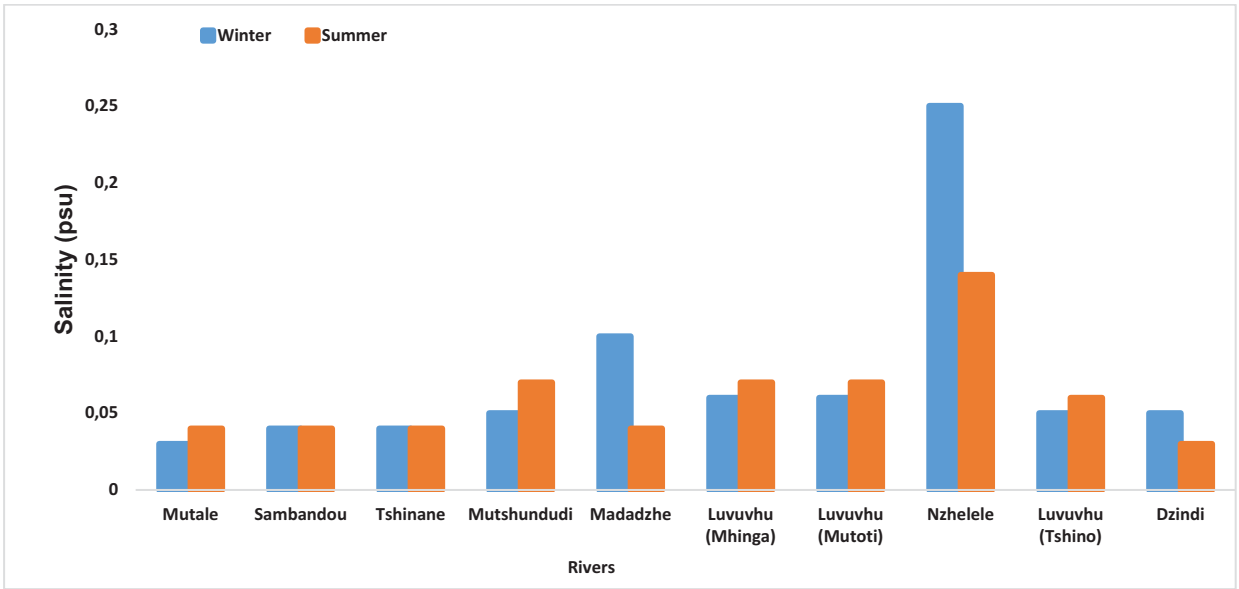


Figure 11: Assessment of TDS, EC and salinity of various river water sources

4.3 Microbial Quality of Surface and Groundwater Sources and Safety

4.3.1 Presence of indicator bacteria in groundwater sources

Figure 12 indicates the occurrence of *E. coli* and TC in boreholes. The results show a high risk (89%) for TC counts and only an 11% risk level for *E. coli* counts. The result obtained does not correlate with findings from the study conducted by Abia et al. (2017), which showed a high level of contamination in the boreholes assessed. However, the findings correlate with previous reports on the quality of borehole waters that are considered to be the 'safest sources' of all drinking waters due to their low level of microbial contaminants (Gwimbi, 2011; Palamuleni & Akoth, 2015).

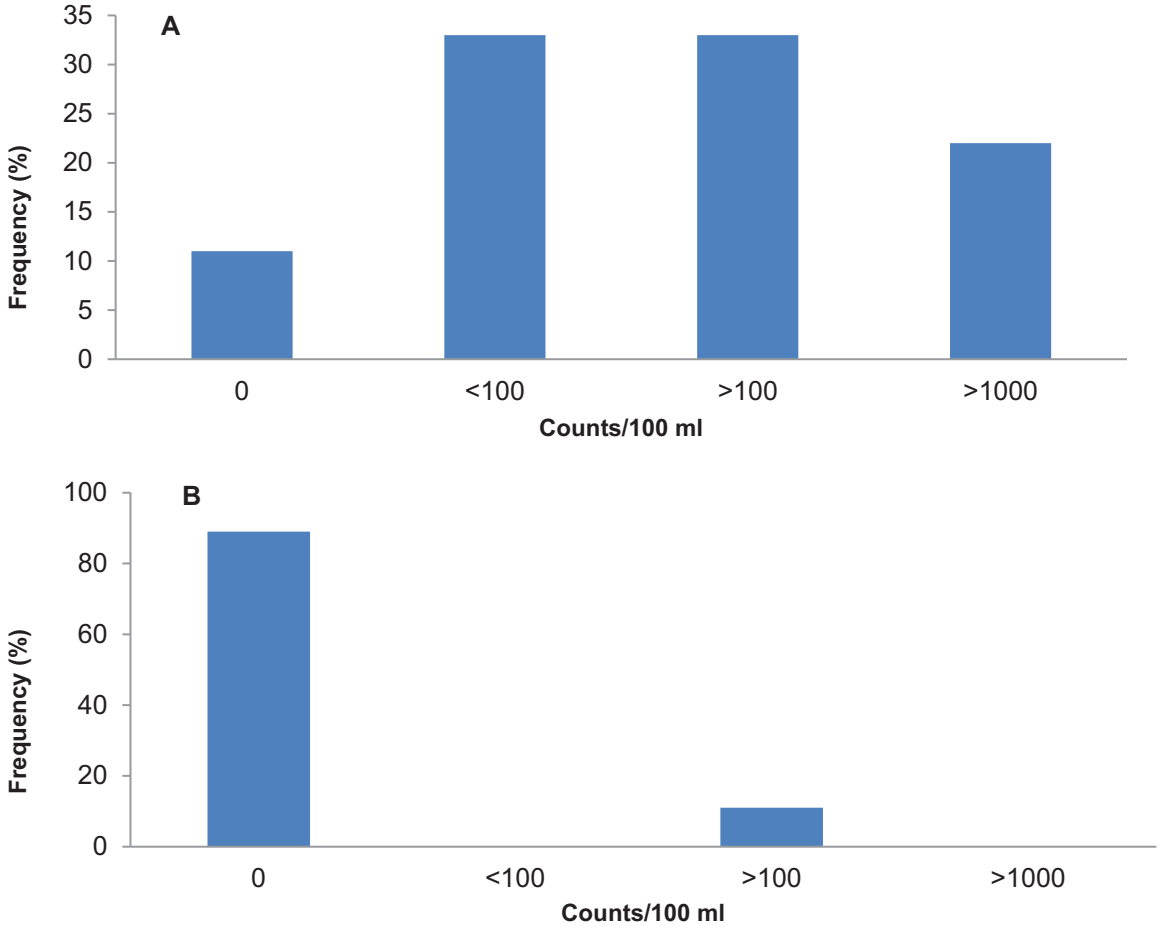


Figure 12: Risk category for TC (A) and *E. coli* (B) counts in waters collected from boreholes

4.3.2 Presence of indicator bacteria in household storage containers

The TC counts in households located in Muraga were found to be the highest where 60% of households have values above 1000/100 ml while only around 40% of households in the Tshikonelo and Basani villages have values above 1000/100 ml (Figure 13). Post-collection contamination in the household often compromises water quality (Brick et al., 2004; Wright et al., 2004). This may increase the proportion of people drinking unsafe water. The *E. coli* count was high for households located in the Muraga village while those in Basani and Tshikonelo had low *E. coli* counts (Figure 13). The high number of *E. coli* counts might be caused by poor water source protection, poor sanitation conditions and practices. This is supported by a study conducted by Gwimbi (2011) that demonstrated that cleaning behaviours and habits of the people at or near the water sources play a role in water contamination. In this study it was observed that water was collected in dirty containers or handled with dirty hands.

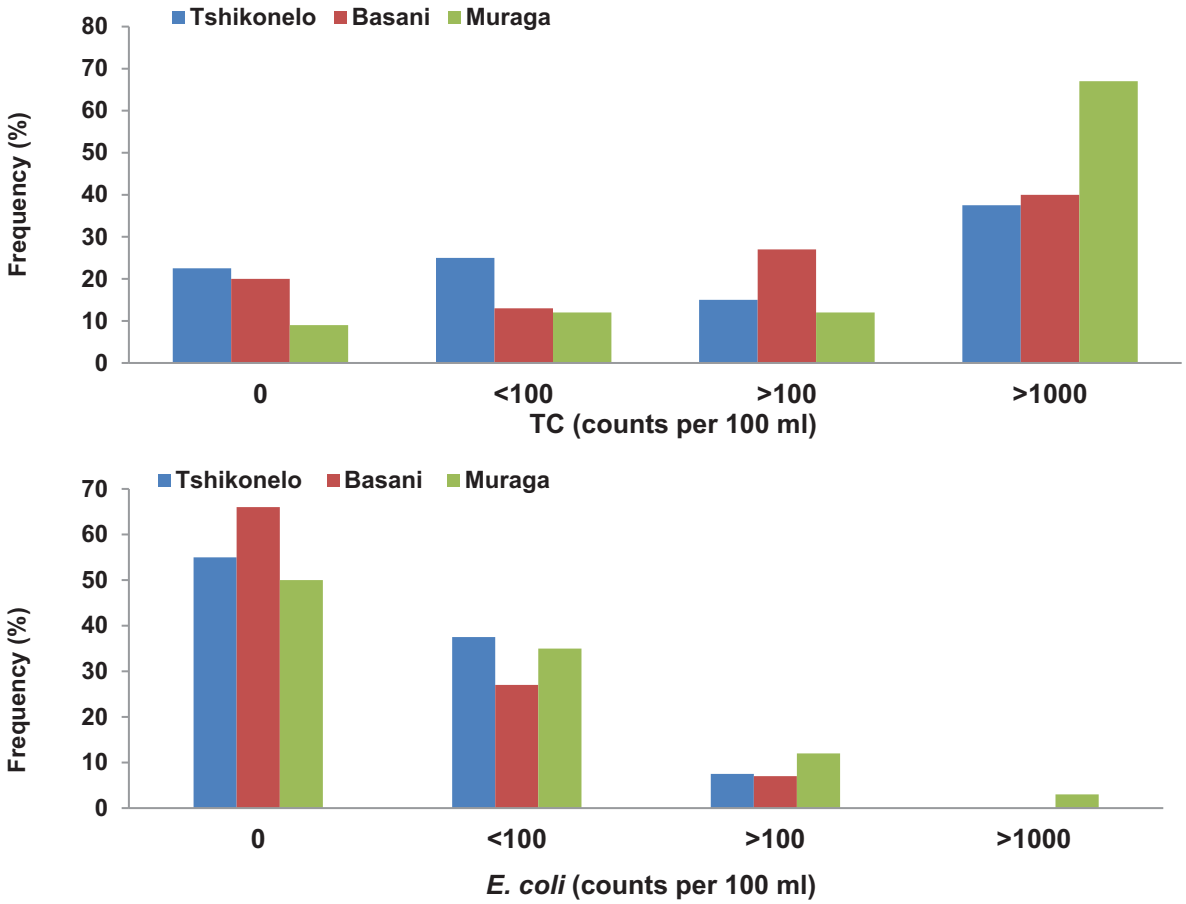


Figure 13: Distribution of TC and *E. coli* counts in household storage containers

4.3.3 Presence of indicator bacteria in surface water sources

The indicator concept rests on the assumption that its presence signals the presence of faecal pollution and the most likely presence of pathogens (Savichtcheva & Okabe, 2006). Coliforms and *Clostridium* bacteria are used worldwide as indicators of faecal contamination and to determine the presence of parasitic protozoans such as *Giardia* and *Cryptosporidium* oocysts (Briancesco & Bonadonna, 2005).

Figure 14 to Figure 16 show the results for all three indicator organisms (TC, *E. coli* and *C. perfringens*) assessed in river waters. The Madadzhe River is generally subject to domestic sewage contamination as it flows through residential areas. This is evident with the results indicated in Figure 14 with the highest *E. coli* count above 2400 MPN[2]/100 ml recorded in winter and a count of about 1300 MPN/100 ml recorded in summer. Madadzhe water is mainly used for irrigation purposes by the people around the catchment. The literature has highlighted the role of sewage-contaminated irrigation water in the incidence of diarrheal diseases due to the consumption of raw fresh produce (Cifuentes, 1998; Wachtel et al., 2002; Allende & Monaghan, 2015). In Sweden, lettuce irrigated by water from a small stream was incriminated in the outbreak due to infection by verotoxin-producing *E. coli 0157*, which was also identical to the isolates from cattle at a farm upstream from the irrigation point (Söderström et al., 2008). Although not proven in this study, in most rural and peri-urban communities, cattle were seen defecating at various points of the rivers (Table 5).

[2] Most probable number

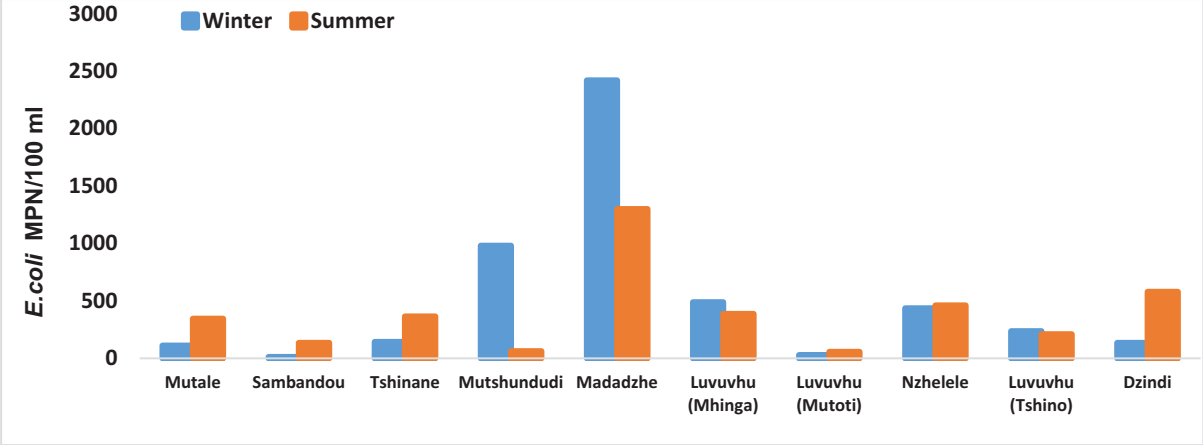


Figure 14: Prevalence of *E. coli* in various river water sources

TC are considered to be of faecal origin and include bacteria from the genera *Escherichia*, *Citrobacter*, *Enterobacter*, *Klebsiella*, *Serratia* and *Rahnella*. However, some bacteria in this group are not of faecal origin (DWAF, 1996; WHO, 1996). The significance of TC in water quality studies is that they generally give an overview regarding the hygienic quality of water (DWAF, 1996). According to DWAF (1996), the target water quality guideline range for TC is 0–5 counts/100 ml, which has a negligible risk of infection. Counts greater than 100 counts/100 ml are a significant threat to human health. In the present study, all rivers had high TC counts above 1700 MPN/100 ml throughout the seasons. The winter recordings for TC ranged from 1700–2500 MPN/100 ml while the summer recordings for TC ranged from 1900–2500 MPN/100 ml (as shown in Figure 15).

The present results show that the river waters are not safe for consumption since the presence of TC may also indicate the possible presence of enteric bacterial pathogens such as *Salmonella* spp., *Shigella* spp., and *Vibrio* spp. (DWAF, 1996). A study by Traore et al. (2016) on rivers in the Vhembe District also reported high counts of TC above 1000 MPN/100 ml, which are similar to the present study. However, a study by Potgieter et al. (2009) on river waters and communal taps in the same district reported TC counts below 1000 cfu[3]/100 ml. The present study also differs from studies done in both Nigeria (Oyhakilome et al., 2012) and Ghana (Frimpong et al., 2015) on dam waters and stream waters respectively. These two studies recorded high TC counts in the ranges of 300–1000 counts/100 ml.

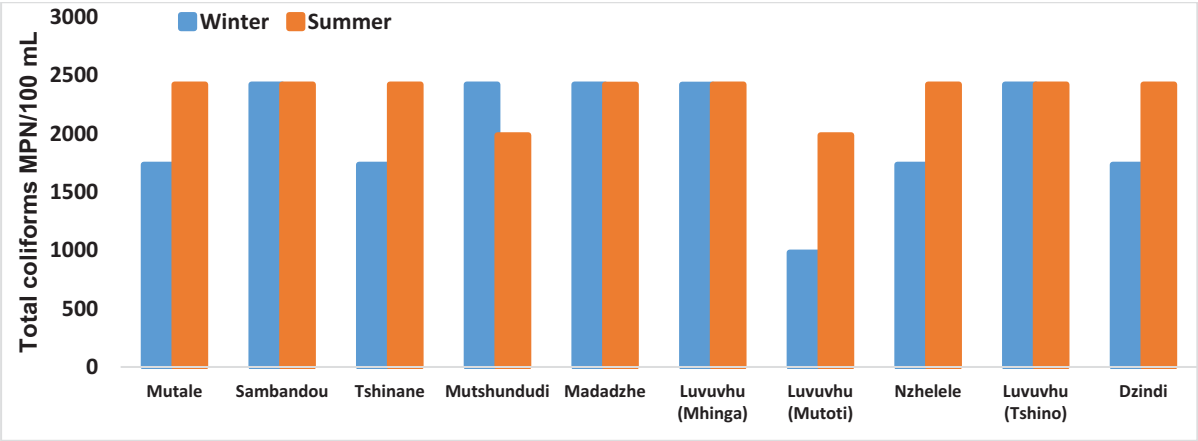


Figure 15: Prevalence of TC in various river water sources

[3] Colony forming units

According to Desmarais et al. (2002), *C. perfringens* normally indicate remote or old faecal contamination due to its extended viability in water bodies. Generally, *C. perfringens* was more prevalent in summer than winter since its detection was noticeable in nine of the ten (90%) rivers in summer relative to the seven of the ten (70%) rivers in winter. According to Savichtcheva & Okabe (2006), the detection of large numbers of faecal anaerobes such as *C. perfringens* in water samples indicates recent or extensive faecal contamination. The winter results would suggest that the Madadzhe River was contaminated with sewage. *C. perfringens* was not detected in the Tshinane, Tshino and Mutoti rivers during winter; however, *C. perfringens* was detected in summer in the Tshinane and Tshino rivers.

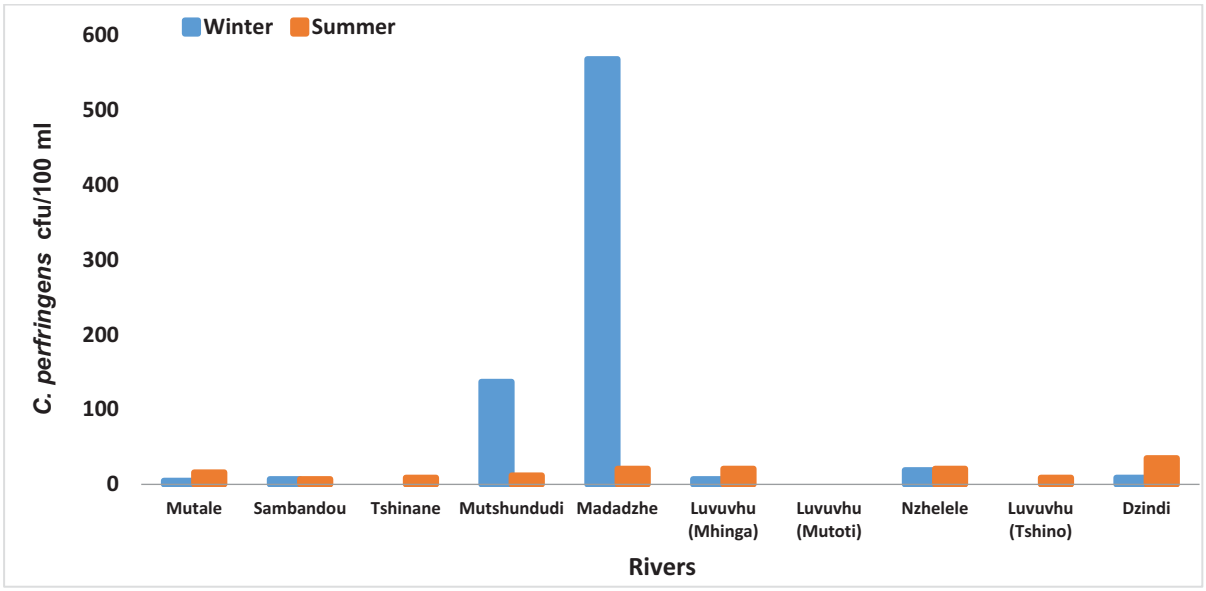


Figure 16: Prevalence of *C. perfringens* in various river water sources

C. perfringens is one of the faecal anaerobic bacteria recognised as indicators of faecal pollution. Its spores are readily detectable in numbers especially in sewage-contaminated water (Sorensen et al., 1989). *C. perfringens* has been used to assess waterborne pathogen levels because total counts in polluted waters strongly correlate with concentration of *Cryptosporidium* oocysts, enteric human viruses and *Giardia* cysts (Payment & Franco, 1993; Brookes et al., 2005).

An epidemiological study conducted at freshwater beaches in Germany noticed adverse health effects when average levels of *C. perfringens* exceeded 10 cfu/100 ml (Wiedenmann et al., 2006). However, *C. perfringens* standards have not yet been evaluated based on epidemiology studies on the acceptable risk associated with faecal pollution (Savichtcheva & Okabe, 2006). The detection of *C. perfringens* spores in this study might reflect the survival of spores in sediments (Potgieter et al., 2009). In addition, its presence indicated the possible presence of enteric viruses and parasites such as *Cryptosporidium* and *Giardia* (Briancesco & Bonadonna, 2005; Potgieter et al., 2009). *C. perfringens* has been considered in assessing waterborne pathogen levels because total counts in polluted waters strongly correlate with the concentration of *Cryptosporidium* oocysts, enteric human viruses and *Giardia* cysts (Payment & Franco, 1993; Brookes et al., 2005). The results of this study were in agreement with other studies. A study done by Potgieter et al. (2006) on the microbiological quality of groundwater sources used by rural communities in the Vhembe District also detected *C. perfringens*. Another study by Potgieter et al. (2009) in the Vhembe District also detected *C. perfringens* in river water samples.

4.3.4 Risk of diarrhoeal diseases

The ten rivers in this study were all contaminated with *E. coli* and thus may pose a risk to people who consume raw water. The WHO risk assessment criteria shown in Figure 17 suggest that (WHO/UNICEF, 2012):

- Counts less than 1 MPN/100 ml present no risk.
- Counts between 1 MPN/100 ml and 10 MPN/100 ml present a low risk.
- Counts between 11 MPN/100 ml and 100 MPN/100 ml present an intermediate risk.
- Counts above 100 MPN/100 ml present a very high risk.

For both winter and summer, none of the ten rivers were within the low risk criterion. According to Gruber et al. (2014), *E. coli* show a stronger association with diarrheal risk. In a study done by Luby et al. (2015) in Bangladesh, the risk of diarrhoea was shown to increase with even moderate increases in *E. coli* contamination, and indeed, an *E. coli* concentration between 1 MPN/100 ml and 1000 MPN/100 ml suggested that the respective dose showed a significant impact in causing diarrhoea.

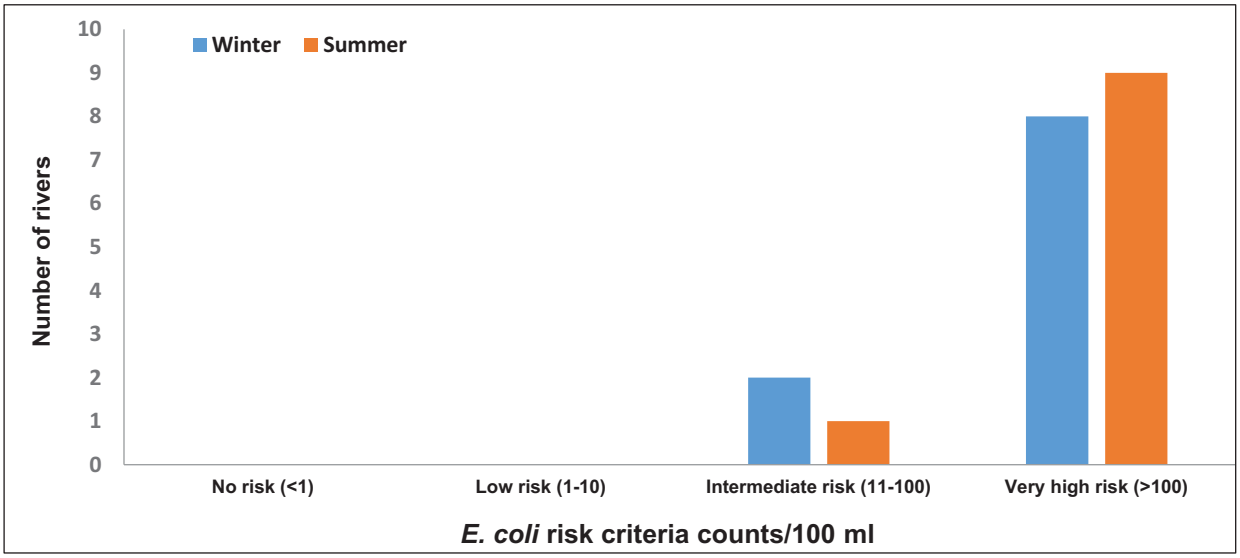


Figure 17: Risk assessment based on WHO/UNICEF (2012) criterion for *E. coli* indicator

4.4 Prevalence of Diarrhoea Pathogens in Surface and Groundwater Sources

4.4.1 Prevalence of pathogenic bacteria in surface water sources

4.4.1.1 E. coli

E. coli strains have been successively characterised with multiplex PCR (Omar & Barnard, 2014; Traore et al., 2016). Table 6 shows the results of the various *E. coli* strains identified. The *mdh* gene was detected in all samples. This gene serves to confirm the presence of *E. coli* in the case of zero detection of pathogenic genes (Omar & Barnard, 2014). The *eaeA*, *eagg* and *astA* genes were frequently detected as shown in Table 6. Generally, all five pathotypes were detected in this study. The current results are similar to results obtained in the study done by Traore et al. (2016) in the Venda region on rivers around the rural areas, which detected all five pathotypes. Another study by Ndlovu et al. (2015) in Western Cape, South Africa, on river water also detected pathogenic strains of *E. coli* such as EAEC, EIEC and EHEC. The latter study differs from the present study in that the *ipa*H gene was used for EIEC, while in the present study the *ial* gene was used for EIEC. In a study by Titilawo et al. (2015) on river water in western Nigeria, no *ipa*H gene was detected in pathogenic strains of *E. coli* (such as ETEC, EPEC and EHEC). In addition, a study done by Sidhu et al. (2013) in Austria on river water detected the *ipa*H gene for EIEC. The latter study detected *lt* and *st* genes infrequently, similar to results found in this study.

Commensal *E. coli* was detected in only two rivers, namely, Mhinga and Mutoti during winter. However, summer results for the same two rivers showed presence of pathogenic *E. coli* strains such as EPEC, ETEC, EAEC, EHEC, and EIEC. This would probably mean that people are carriers of the pathogenic strains of *E. coli* and, according to literature, warmer temperatures during summer coincide with *E. coli* infections (Kovats et al., 2004; Fleury et al., 2006). The high prevalence of EHEC and other pathotypes in summer (60%) compared to winter (10%) would suggest that both humans and animals were major contributors to faecal contamination (Titilawo et al., 2015). EHEC is well-known for causing bloody diarrhoea; its presence in water would pose high risk of bloody diarrhoea. A study by Isaäcson et al. (1993) reported that the consumption of surface water in South Africa and Swaziland saw a large outbreak of bloody diarrhoea. In this study, both EPEC and EAEC were detected more frequently in summer and winter. A study done in India on surface water from shallow wells reflected that the consumption of water contaminated with EAEC was significant in causing diarrhoea (Hunter, 2003).

Table 6: Distribution of *E. coli* pathotypes in winter and summer

River site	Winter months		Summer months	
	Pathotype	**Detected genes**	**Pathotype**	**Detected genes**
Sambandou	EPEC (atyp)	*mdh, eaeA, astA, gapdh*	EPEC (atyp), EIEC, ETEC	*mdh, eaeA, ial, lt, gapdh, astA*
Mutale	EPEC (atyp), EAEC	*mdh, eaeA, eagg, astA*	EPEC (atyp), EHEC, EAEC	*mdh, eaeA, stx1, stx2, eagg, gapdh, astA*
Tshinane	EPEC (atyp), EAEC	*mdh, eaeA, eagg, astA*	EPEC (atyp), EPEC (typ), EHEC	*mdh, eaeA, bfp, stx1, astA*
Mutshundudi	EPEC (atyp), ETEC, EAEC	*mdh, eaeA, st, eagg, astA*	EPEC (atyp), EPEC (typ), ETEC, EAEC	*mdh, eaeA, bfp, lt, eagg, gapdh, astA*
Madadzhe	EHEC, EIEC, EAEC	*mdh, stx1, stx2, ial, eagg, astA*	EPEC (atyp), ETEC, EAEC	*mdh, eaeA, lt, eagg, gapdh, astA*
Luvuvhu (Mhinga)	COM	*mdh*	EPEC (atyp), EHEC, EIEC, ETEC, EAEC	*mdh, eaeA, stx1, ial, lt, st, eagg, astA*
Luvuvhu (Mutoti)	COM	*mdh*	EHEC, EPEC (atyp)	*mdh, stx2, eaeA,*
Luvuvhu (Tshino)	EPEC (atyp), EPEC (typ), EAEC	*mdh, eaeA, bfp, eagg, astA*	EPEC (atyp), EPEC (typ), EIEC, ETEC	*mdh, eagg, bfp, ial, lt, st, astA, gapdh*

River site	Winter months		Summer months	
	Pathotype	Detected genes	Pathotype	Detected genes
Nzhelele	EPEC (atyp), EAEC	*mdh, eaeA, eagg, astA*	EPEC (atyp), EPEC (typ), EHEC, EAEC	*mdh, eaeA, bfp, stx1, eagg, gapdh,astA*
Dzindi	EPEC (atyp), ETEC	*mdh, eaeA, lt, gapdh, astA*	EPEC (atyp), EPEC (typ), EHEC, ETEC, EAEC	*mdh, eaeA, bfp, stx1, stx2, lt, eagg, gapdh, astA*

Com = commensal, *E. coli,* EPEC (atyp) = atypical enteropathogenic *E. coli*, EPEC (typ) = typical enteropathogenic *E. coli*, EAEC = enteroaggregative *E. coli*, EHEC = enterohaemorrhagic *E. coli*, EIEC = enteroinvasive *E. coli* and ETEC = enterotoxigenic *E. coli*

4.4.1.2 Vibrio spp.

The outcomes from API-20E tests did not identify *V. cholerae*, although a doubtful presence of *V. cholerae* was confirmed in Luvuvhu, which turned out to be related species such as *V. fluvialis, V. parahaemolyticus* and *V. alginolyticus.* Using PCR, the results revealed the presence of *V. cholerae* by detecting the *sodB* gene (Ntema et al., 2010). This gene was detected in seven of the 11 (64%) winter isolates and in 14 of the 19 (74%) summer isolates. PCR was more specific in identifying *V. cholerae* unlike API-20E that assumed possibilities of *V. fluvialis* as well as other enteric bacteria listed in Table 7. Generally, *V. cholerae* was more prevalent in summer than winter since it was detected in nine out of ten (90%) and in five out of ten (50%) of the sources respectively.

Table 7: *Vibrio* spp. recovered from water sources used in rural communities of Venda

River site	Winter months		Summer months	
	API-20E	PCR (*sod*B)	API-20E	PCR (*Sod*B)
Mutale	*V. fluvialis, Aeromonas hydrophila*	*V. cholerae*	*V. fluvialis, V. alginolyticus*	*V. cholerae*
Sambandou	–	–	*Providencia rettgeri*	–
Tshinane	*V. fluvialis, Bibersteinia trehalosi*	–	*V. fluvialis*	*V. cholerae*
Mutshundudi	*V. fluvialis, Bibersteinia trehalosi*	*V. cholerae*	*V. fluvialis, V. parahemolyticus*	*V. cholerae*
Madadzhe	*V. fluvialis*	*V. cholerae*	*V. fluvialis, Proteus mirabilis*	*V. cholerae*
Luvuvhu (Mhinga)	–	–	*V. cholerae, V. parahemolyticus, V. fluvialis*	*V. cholerae*
Luvuvhu (Mutoti)	–	–	*V. fluvialis*	*V. cholerae*
Luvuvhu (Tshino)	*V. fluvialis, Bibersteinia trehalosi*	*V. cholerae*	*V. fluvialis*	*V. cholerae*
Nzhelele	*V. fluvialis, V. alginolyticus, V. parahemolyticus, Bibersteinia trehalosi*	*V. cholerae*	*V. fluvialis, Bibersteinia trehalosi*	*V. cholerae*
Dzindi	–	–	*V. fluvialis, Bibersteinia trehalosi*	*V. cholerae*

4.4.1.3 Salmonella spp. and Shigella spp.

Shigella spp. were not detected in any of the samples tested. However, the water samples from Mutale were positive for *Plesiomonas shigelloides*, which are also important enteric bacteria with similarities to some serotypes of *Shigella* spp. – specifically *S. flexneri, S. sonnei* and *S. dysenteriae* (Shimada & Sakazaki, 1978; Albert et al., 1993). API-20E confirmed most of the presumptive positives to be members of TC such as *Citrobacter spp.* and *Enterobacter spp*. Generally, the prevalence of *Salmonella spp.* was high in both summer (100%) and winter (90%). The high prevalence in summer might have been due to the surface run-off since it rained more frequently in summer (Jenkins et al., 2008; Robin et al., 2012). In addition, warmer temperatures in summer are favourable for *Salmonella* spp. (Fleury et al., 2006). The results for *Salmonella* spp. (Table 8) are in agreement with other findings. In the Limpopo Province, the studies of Potgieter et al. (2005) on surface water used as drinking water source also detected *Salmonella* spp. According to Smith et al. (2011), typhoid in South Africa remains endemic in some rural areas that lack a potable water supply.

Table 8: *Salmonella* spp. recovered from river sources used in rural communities of Venda

River site	Winter months		Summer months	
	API 20E	**PCR (*ipa*B)**	**API 20E**	**PCR (*ipa*B)**
Mutale	*Plesiomonas shigelloides*	*Salmonella* spp.	*Salmonella spp., Salmonella enterica spp. arizonae*	*Salmonella* spp.
Sambandou	*Enterobacter cloacae*	*Salmonella* spp.	*Salmonella spp., S. enterica spp. arizonae*	*Salmonella* spp.
Tshinane	–	–	*Salmonella spp., S. enterica spp. arizonae, Providencia rettgeri, Edwardsiella hoshinae*	*Salmonella* spp.
Mutshundudi	*C. freundii*	*Salmonella* spp.	*Edwardsiella hoshinae, Salmonella spp.*	*Salmonella* spp.
Madadzhe	*Proteus mirabilis, Serratia liquefaciens*	*Salmonella* spp.	*Salmonella spp., S. enterica spp. arizonae, Providencia rettgeri*	*Salmonella* spp.
Luvuvhu (Mhinga)	*Edwardsiella hoshinae, Proteus mirabilis*	*Salmonella* spp.	*Salmonella spp., S. enterica spp. arizonae, Proteus mirabilis*	*Salmonella* spp.
Luvuvhu (Mutoti)	*Enterobacter cloacae, C. freundii*	*Salmonella* spp.	*Edwardsiella hoshinae*	*Salmonella* spp.
Luvuvhu (Tshino)	*C. freundii*	*Salmonella* spp.	*Salmonella spp., S. enterica spp. arizonae, E. coli type 2*	*Salmonella spp.*
Nzhelele	*Enterobacter cloacae, C. freundii, Salmonella spp., S. enterica spp. arizonae*	*Salmonella* spp.	*Salmonella spp., S. enterica spp. arizonae, Pseudomonas aeruginosa, C. freundii*	*Salmonella* spp.
Dzindi	*Enterobacter cloacae, Yersinia enterocolitica*	*Salmonella* spp.	*Salmonella spp., S. enterica spp.*	*Salmonella* spp.

4.4.2 Prevalence of pathogenic *E. coli* in groundwater sources

Groundwater has been appreciated as the most abundant and cost-effective source of potable water supply in South Africa (Xu & Braune, 1995). However, the results of this study showed that groundwater is liable to contamination by *E. coli*, which is an indicator of faecal pollution. The present study detected pathogenic strains of *E. coli* (atypical and typical EPEC, EAEC and EHEC), which most likely threaten the health of the communities supplied by the boreholes (Taonameso et al., 2018). The study by Momba et al. (2006) on drinking water sources in Nkonkobe observed that *E. coli* was more implicated in groundwater sources (75%) than surface water (25%). A study done by Potgieter et al. (2006) in South Africa on groundwater established a possible cause of contamination of groundwater. It was observed that some boreholes were drilled next to sanitary facilities such as pit latrines and septic tanks. The results on the occurrence of pathogenic genes are similar to those obtained by Abia et al. (2017) with virulence genes such as *stx1* and *stx 2* for EHEC, *eagg* for EAEC, and *eaeA* for EPEC.

4.4.3 Prevalence of pathogenic *E. coli* in household water storage containers

Several pathogenic strains of *E. coli* were prevalent in the villages in the Vhembe District (Table 9). Abia et al. (2017) noted a positive correlation between the abundance of *E. coli* and the prevalence of virulence genes. In this study, more virulence genes were identified in Muraga and Tshikonelo villages and *E. coli* counts were very high in the two villages. A study done by Potgieter et al. (2006) observed that inadequate access to sanitary facilities in most households in rural villages is one of the risk factors for contamination of stored household water. According to Vannavong et al. (2017), members of households without toilet facilities often resort to open defecation without any hand-washing facilities. They are more likely to contaminate stored drinking water. Atypical EPEC, which is an important cause of diarrhoea (Trabulsi et al., 2002; Liebchen et al., 2011), was the most prevalent strain in all villages. Humans are reservoirs for typical EPEC; both animals and humans can be reservoirs for atypical EPEC (Trabulsi et al., 2002). Basani village, with only one strain detected, had improved sanitary facilities.

Table 9: Type of *E. coli* strains found in household storage containers from villages

	Muraga village	Basani village	Tshikonelo village
***E. coli* pathotypes detected in the village water containers**	COM, EPEC (typ), EPEC (atyp), EHEC, EAEC, ETEC	EPEC (atyp)	COM EPEC (typ), EPEC (atyp), EHEC, ETEC EAEC

EPEC (atyp) = atypical enteropathogenic *E. coli*, ETEC = enterotoxigenic *E. coli* EHEC = enterohaemorrhagic *E. coli*,
EPEC (typ) = typical enteropathogenic *E. coli*, EAEC = enteroaggregative *E. coli*, Com = commensal *E. coli*

4.4.4 Prevalence of viruses in surface water sources

Enteric viruses are transmissible via the faecal–oral route by ingesting contaminated water/food (DWAF, 1996). Total Culturable Virus Assay (TCVA) is generally recommended by USEPA to monitor viruses in surface water. The presence of viruses is registered as CPE in specific host cells (Chapron et al., 2000). Generally, epidemiologically important viruses are not detected by the TCVA method, specifically, wild type strains of HAV, human rotavirus, human astroviruses and noroviruses are difficult to culture in vitro (Formiga-Cruz et al., 2005; Greening, 2006). The South African Standard Guidelines for viruses in water based on the TID_{50}/10 L[4] of sample stipulate that no viruses be found in water, which is the target water quality range. Viral doses greater than 10 pose a very high risk (DWAF, 1996). Also, the SANS 241-1 (2015) standards imply that cytopathogenic viruses not be detected in water otherwise at least a viral dose of 2 poses human health risk. Table 10 shows the results obtained from cell culture for cytopathogenic viruses, direct PCR for enteric viruses and PCR-cell culture for enteric viruses.

[4] Total infectious dose

In this study, the cell culture results revealed that only reovirus showed a positive result on cyto-pathogenicity, which was detected only in Madadzhe and Nzhelele sites in winter and summer respectively (Table 10). The findings from this study on reovirus are in line with the findings by Matsuura et al. (1984) that reoviruses were the most commonly isolated viruses in CPE-positive samples. Reoviruses exhibit moderate clinical significance and studies regarding their detection in environmental water samples are still limited (Spinner & Di Giovanni, 2001). Despite negative results under TCVA, the results could be expected since most viruses do not show CPE. Indeed, according to Lee et al. (2005), analysis of enteric viruses by cell culture alone might underestimate the concentration of viruses in environmental samples. Hence, integrating TCVA and PCR guarantees conclusive results since more viral particles can be detected (Grabow et al., 2004; Lee et al., 2005). The integrated cell culture PCR results for the present study revealed that both enteroviruses and adenoviruses do not show CPE in cell cultures since PCR was able to detect their nucleic acid. Enteroviruses were detected in two of the ten (20%) samples only in winter while adenoviruses were detected in only one of the ten (10%) winter samples.

None of the epidemiologically important viruses like rotavirus and norovirus were detected using integrated cell culture PCR. This result actually agrees with the finding that the respective viruses are difficult to culture in vitro (Formiga-Cruz et al., 2005; Greening, 2006). Direct PCR proved to be the best method especially for detecting rotaviruses and noroviruses due to its high degree of sensitivity (Lee et al., 2005). Four out of ten (40%) samples were positive for rotaviruses only in winter; enteroviruses were present in only three of ten (30%) winter samples (Table 10). Both noroviruses and adenoviruses were detected in the Madadzhe River: noroviruses were detected in winter only; adenoviruses were detected in both winter and summer. This result indicates that noroviruses are associated with sewage contamination as it was detected in Madadzhe River, which is a sewage-polluted river (sighted during sample collection). This observation is supported by Calgua et al. (2013), in which noroviruses were detected in rivers known to receive domestic sewage discharge from urbanised areas in Rio de Janeiro, Brazil. Sewage-contaminated water used for irrigation of vegetables is a prominent means in which viruses can also be transmitted to humans when consuming raw produce (DWAF, 1996; Van Zyl et al., 2006). The Madadzhe River is used as an irrigation source by individuals to water their gardens; this could possibly contribute to the transmission of viruses to consumers of the fresh produce from gardens.

The low detection of viruses in summer unlike winter might be due to temperature effects. High temperatures accelerate the rate at which viral reduction takes place in water (Levy et al., 2009; Krauss & Griebler, 2011). It could also be due to the dilution effect of rain on river catchments. Other important viruses such as HAV, astroviruses, and sapoviruses were detected only in Madadzhe River during the winter season. The detection of viruses in water in this study shows that the water quality of some of the rivers is poor since no viruses are expected to be detected in water meant for domestic use (DWAF, 1996). According to a National Institute for Communicable Disease (NICD) (2014) report, a diarrheal outbreak has been reported in Bloemhof (North West Province, South Africa) in which multiple pathogens including enteric viruses such as astroviruses, adenoviruses and noroviruses were detected in the stools of patients. This highlights the importance of astroviruses in causing diarrhoea.

Table 10: Virologic assessment of water quality from various river water sources used in rural communities of Venda

River site	Winter months				Summer months			
	Rotavirus	**Norovirus**	**Adenovirus**	**Other endemic viruses**	**Rotavirus**	**Norovirus**	**Adenovirus**	**Other endemic viruses**
Mutale	–	–	–	Entero[3]	–	–	–	–
Sambandou	–	–	–	–	–	–	–	–
Tshinane	Rota[2]	–	–	–	–	–	–	–
Mutshundudi	Rota[2]	–	–	Entero[2]	–	–	–	–
Madadzhe	–	Noro GI[2], Noro GII[2]	Adeno[3]	Reo[1], HAV[2], Entero[2,3] Astro[2], Sapo[2]	–	–	Adeno[2]	–
Luvuvhu (Mhinga)	–	–	–	Astro[2]	–	–	–	–
Luvuvhu (Mutoti)	Rota[2]	–	–	–	–	–	–	–
Luvuvhu (Tshino)	–	–	–	Entero[2]	–	–	–	–
Nzhelele	–	–	–	–	–	–	–	Reo[1]
Dzindi	Rota[2]	–	–	–	–	–	–	–

– Not detected,
1 – Cell culture,
2 – PCR (direct),
3 – PCR (cell culture)

Rota – rotaviruses
Adeno – adenoviruses
Entero – enteroviruses

Sapo – sapoviruses
Noro GI – norovirus genotype I
Noro GII – norovirus genotype II

HAV – hepatitis A virus
Reo – reoviruses
Astro – astroviruses

4.4.5 Occurrence of parasites in surface water sources

According to the South African Guidelines for water quality, the target water quality range for cysts (*Giardia*) or oocysts (*Cryptosporidium*) in water for domestic use is 0 cysts/10 L or oocysts/10 L. Doses greater than 1 may be a risk of parasite infection (DWAF, 1996). The number of parasites required to induce infection is relatively low and the infectious dose (ID_{50}) has been estimated to be as low as 83–123 oocysts for *Cryptosporidium* (Chappell et al., 2006) and 19–50 cysts for *Giardia duodenalis* (Adam, 2001). Extrapolations from the dose response data analysed from the Milwaukee cryptosporidiosis outbreak indicated that ingesting 1–10 oocysts gives a discrete probability of infection for some individuals (Eisenberg et al., 1998).

In this study, parasites were detected in 30% of winter samples and 10% of summer samples (Table 11). This suggests that the quality of water from the four rivers (Sambandou, Madadzhe, Mutshundudi and Tshinane) is more likely a health hazard if the water is consumed continuously without treatment (DWAF, 1996). The result of 2 cysts(oocysts)/10 L found in this study is in agreement with results found in one of the rivers studied by Sigudu et al. (2014) in Gauteng Province (South Africa). *C. perfringens* results predicted that the Mutshundudi and Madadzhe rivers could have parasites (Savichtcheva & Okabe, 2006). *Giardia* cysts were detected only in these two rivers.

Table 11: Parasitic assessment of water quality from various river water sources used in rural communities of Venda

River sites	Winter months		Summer months	
	Cryptosporidium oocysts/10 L	Giardia cysts/10 L	Cryptosporidium oocysts/10 L	Giardia cysts/10 L
Mutale	–	–	–	–
Sambandou	–	–	–	1
Tshinane	2	–	–	–
Mutshundudi	–	2	–	–
Madadzhe	–	62	–	–
Luvuvhu (Mhinga)	–	–	–	–
Luvuvhu (Mutoti)	–	–	–	–
Luvuvhu (Tshino)	–	–	–	–
Nzhelele	–	–	–	–
Dzindi	–	–	–	–

- Not detected

5 CONCLUSIONS AND RECOMMENDATIONS

5.1 Summary of Findings and Conclusions

The aim of this study was to determine the prevalence of diarrhoea-causing pathogens in water used for drinking and domestic purposes in the rural and peri-urban communities in the Venda District. The results highlighted the occurrence of enteric pathogens in source and groundwater used by the local communities for domestic purposes. These findings suggest the need to draw up sustainable plans for strategies on water protection for rural and peri-urban communities where water treatment is not available. Access to and the availability of safe drinking water is a basic human right and essential for good health. Improving access to safe drinking water is essential and efforts should be made to ensure that drinking water provided to communities complies with national safety standards.

South Africa does have a Drinking Water Quality Framework to manage drinking water quality effectively and to protect the health of the public/consumers. To address key factors that govern drinking water quality, an integrated system of approaches and procedures is needed. In terms of the Constitution, municipalities are responsible for delivering services where the Water Services Act has refined a specific mandate that states "*municipalities are to ensure provision*". Policymakers did acknowledge that the provision of water is actually very complex and requires both technical and managerial skills (Smith, 2009). This simply implies that municipalities are expected to get cost-effective assistance where necessary. However, over the years, only poor levels of municipal compliance to water services in South Africa have been reported (Smith, 2009). In 2009, it was reported that 6% of the sites assessed across South Africa failed to meet the health requirements of the national standard: the affected sites were found to be in rural areas. The causes of the non-compliance included the deterioration of the reticulation network. It was pointed out that unless drastic measures were taken, the situation would gradually lead to a decline in water quality. In 2018, the Vhembe District has backlogs in service deliveries including the implementation of water, sanitation and hygiene services.

Water service authorities (WSAs) are responsible for monitoring the quality of drinking water provided to consumers, compare the results to drinking water standards (national and international), and to communicate any health risks to consumers and other stakeholders (DWAF, 1997). The Department of Health collects information regarding incidences of waterborne diseases such as diarrhoea and uses the information to facilitate interventions. Environmental health officers give health and hygiene education related to water and sanitation services and do routine drinking water quality monitoring at the point of use using indices of faecal contamination. Civil society (usually organised groups of citizens such as water committees in communities) engages with stakeholders to discuss problems they find. The framework has to build on existing "best practices". It is important that the framework is based on proactive preventable management and not on a system that promotes reactive management. A preventative risk management approach can only be successful when all role players or stakeholders, from government to the consumer, are supportive and committed.

The biggest problem facing South Africa today, especially in rural and peri-urban communities, is that many WSAs do not follow the required procedures for monitoring and managing drinking water quality. This is mainly due to a lack of capacity and resources. Effective and efficient drinking water service delivery is either non-existent or ineffective due to a lack of monitoring (such as limited number of water testing done; lack of basic lab equipment for testing; broken lab equipment for testing; and inexperienced staff), inadequate maintenance budgets and vandalism, which then affect the sustainability of programmes. Operational management (assessment of risk/hazards and events that compromise drinking water quality is not done. This monitoring should be done regularly from the catchment (sources) to the consumer (point of use) and back to the environment (grey water, sewage etc.) to target problems so that procedures can be followed for immediate preventative and corrective action to re-establish control of processes. Fast action will protect the consumer when drinking water quality guidelines have been exceeded.

5.2 Recommendations

Therefore, the following is recommended:

- More risk analysis in communities must be done and proactive management must be employed.
- Hazardous events, their causes and different scenarios must be identified and documented and the level of risk for each event should be estimated – the impact must be established and the intensity of the impact must be considered.
- Contingency plans for each community should be drawn up, which contain specific education plans, capacity building programmes, funding, specific management systems and management tools, must have regular monitoring times scheduled and must be acceptable to all role players (especially consumers).
- Preventative strategies for rural and peri-urban areas and communities must be established, water sources must be protected, and barriers to protect contamination must be provided.

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