CRYPTOSPORIDIUM SCREENING OF SELECTED WATER RESOURCES OF THE CUVELAI-ETOSHA AND KUNENE BASINS AND IMPLICATIONS FOR WATER MANAGEMENT

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS OF THE DEGREE OF MASTERS OF SCIENCE IN WATER RESOURCES MANAGEMENT

OF

THE UNIVERSITY OF NAMIBIA

BY

Muhenje Swanu

200970925

2019

Main Supervisor: Dr Heike Wanke

Co-Supervisors: Professor Joanne Cable

Dr Billy McBenedict

Table of Contents

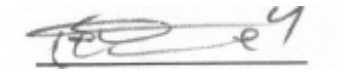
Abstract

In the Cuvelai-Etosha and Kunene Basins, a remarkable percentage of the population depends on a variety of unprotected water resources for both domestic and livestock purposes, which includes Earth dams, wells, boreholes and streams. Waterborne transmission of *Cryptosporidium* could be prevalent in water basins were water resources have no protection against direct-microbial contamination. However, little is known about the influence of management of water resources on *Cryptosporidium* prevalence. The main objective of this study was to assess the prevalence of *Cryptosporidium* in selected water resources of the Cuvelai-Etosha and Kunene basins and assess the implications of findings on water management. On-site parameters of conductivity, dissolved oxygen, temperature and pH were measured from a total of 47 water samples from 47 sampling sites were screened for *Cryptosporidium* at the end of the rainy season by quantitative polymerase chain reaction (qPCR). The results showed a prevalence of 4.3 %, all from unprotected water resources which suggest that management (protection) of water resources from direct access to animals and direct-runoff is essential to lower the risk of waterborne infection of cryptosporidiosis. We recommend source protection of water resources in the basins to lower the risk of *Cryptosporidium* contamination. Further longitudinal studies screening for *Cryptosporidium* should be conducted to assess prevalence.

Keywords: Cuvelai-Etosha Basin, *Cryptosporidium*, Kunene basin, Management practices, water resources, Source protection.

Declarations

I, Swanu Muhenje, hereby declare that this study is my own work and is a true reflection of my research, and that this work, or any part thereof has not been submitted for a degree at any other institution.

Muhenje Swanu  ……

Name of Student **Signature** **Date**

List of Abbreviations and/or Acronyms

CEB	Cuvelai-Etosha Basin
DO	Dissolved Oxygen
EC	Electric Conductivity
GIS	Geographical Information Systems
km²	Square kilometer
MAWF	Ministry of Agriculture, Water and Forestry
mg/L	Milligram per liter
mm/a	Millimeter per annum
mS/m	Milli-Siemens per meter
NamWater	Namibia Water Corporation Ltd
pH	Potential of hydrogen
qPCR	Quantitative polymerase chain reaction
SPSS	Statistical Package for Social Sciences
SDG	Sustainable Millennium Development Goals
UNAM	University of Namibia
WHO	World Health Organization

List of Tables

List of Figures

Acknowledgement

This work forms part of a M.Sc. study undertaken at the University of Namibia. It was partly funded by Cardiff University and molecular analysis was done there. I would like to express my sincere gratitude towards Dr Rhidian Thomas, Dr Anna Paziewska-Harris and the entire team at Cardiff who were involved in this work as well as the local communities for their cooperation during sampling. I would love to thank God almighty, who gave me the strength through it all. Professor Frank Kavishe who worked tirelessly and got me a bursary from MAWAF: “May your oil never run-out”. I also want to thank my Supervisors, especially my main supervisor Dr Heike Wanke who never wavered from the Begging. Professor Jo Cable, your timely response from Cardiff via email is appreciated very much, as well as Dr. Billy McBenedict. Rhidian Thomas whom we worked with during sampling, Anna Paziewska-Harris and everyone who was involved during the laboratory work, Thank you! Ministry of Agriculture, water and Forestry (UNESCO water sector support project), my studies would not be possible without your financial support.

Dedication

I dedicated this work to the memories of my late Sister (+Hivirikua Patricia Tjindunda).

_____Continue to rest in eternal peace_____

CHAPTER 1

Introduction

1.1 Background of the study

Cryptosporidium is an enteric protozoon that causes diarrheal illness, especially common in immune compromised individuals [2, 12, 14, 18, 19]. It is the second most important causative agent of diarrhea after rotavirus, and therefore a major death threat in Sub-Saharan Africa [16]. According to the WHO/UNICEF (2017) as cited by [2], rural communities are less likely to have access to improved water, which makes them more susceptible to waterborne diseases. Unfortunately, no published reports are available on the detection of *Cryptosporidium* species in Namibia. Chronic infection by *Cryptosporidium* affects cognitive development and often leads to wasting and stunting in children. Nitazoxanide is the only available drug for cryptosporidiosis; however, this drug is ineffective in immune-compromised individuals. Most regions in both Cuvelai-Etosha basin and Kunene are dominated by communal farming where cattle graze freely and have direct access to water resources which are also used by people. Free-grazing farming-systems pose a risk to water quality including contamination with *Cryptosporidium* especially in catchments without best protection management [7]. Protection of water resources through infrastructure development and practices is therefore crucial in zoonotic transmission of *Cryptosporidium.*

Most previous studies on water-borne diseases in the two basins relied more on *Escherichia coli* and total coliforms [21], additionally, the Namibian-bulk water supplier (NAMWATER) relies on *E. coli* and total coliforms as an indicator of fecal contamination, however, enteric protozoa of health importance such *Toxoplasma gondii*, *Cyclospora cayetanensis*, *Entamoeba histolytica*, *Blastocystis hominis* and *Cryptosporidium* species maybe be present in the absence of these commonly used fecal indicator-organisms [8].

Cuvelai-Etosha and Kunene basins water resources in rural areas are either surface water or groundwater whose water quality is often influenced by surface water due to inadequate source protection [21], and most is consumed directly without disinfection. For the purpose of this study, protected water resources would infer water resources where there are practices and infrastructure improvements which could prevent direct *Cryptosporidium* contamination.

1.2 Statement of the Problem

Cryptosporidium species have a variety of host including domestic and wildlife [19], these protozoa can be introduced into water, especially in water resources without any form of management in terms of infrastructure improvements and practices (unprotected) [7], posing a risk of cryptosporidiosis, a major death threat in Sub-Saharan Africa responsible for about 30-50 % of child mortality [18, 19].

1.3 Aims and Objectives

The main aim of the study is to assess the prevalence of *Cryptosporidium* in water-points in the Cuvelai-Etosha and Kunene basins and implications for water management. To achieve this, the following Specific objectives were formulated as follow:

- Screening for *Cryptosporidium* in water samples
- Observation of water resources for management practices/infrastructure improvements at each sampled point.

1.4 Hypothesis of the study

H_0: There is no significant difference in the prevalence of *Cryptosporidium* between protected and unprotected water resources.

Hi: There is a significant difference in the prevalence of *Cryptosporidium* between protected and unprotected water resources.

1.5 Significance of the Study

Cryptosporidium screening from water resources helps with the awareness (Knowledge) of the prevalence of this enteric protozoa and could be used in settings of water management practices and infrastructure improvements to mitigate *Cryptosporidium* spread [5, 10, 18, 22].

1.6 Limitation and Delimitation

Water quality studies information on *Cryptosporidium* does not exist as no studies were done on the prevalence of *Cryptosporidium* in Namibia. In addition, both the Cuvelai-Etosha and Kunene Basins are very complex basins with variable precipitation which might mean the seasonality of *Cryptosporidium* could be different within these basins. Therefore, the prevalence from this study might not be a true reflection of the actual prevalence in the study area.

1.8 Study Area

1.8.1 Location and Climate

The study area is in the western part of the Cuvelai-Etosha and upper north of the Kunene basin (Figure 1 and coordinates given in Appendix C). The mean annual rainfall of the study area ranges between 300 and 500 mm/a. The main farming system common in the two basins is communal farming and the water resources are used for domestic and livestock purposes. The highly variable rainfall diminishes from an average of 400-120 mm/a, from the west to the east of the study area as shown in figure 1.

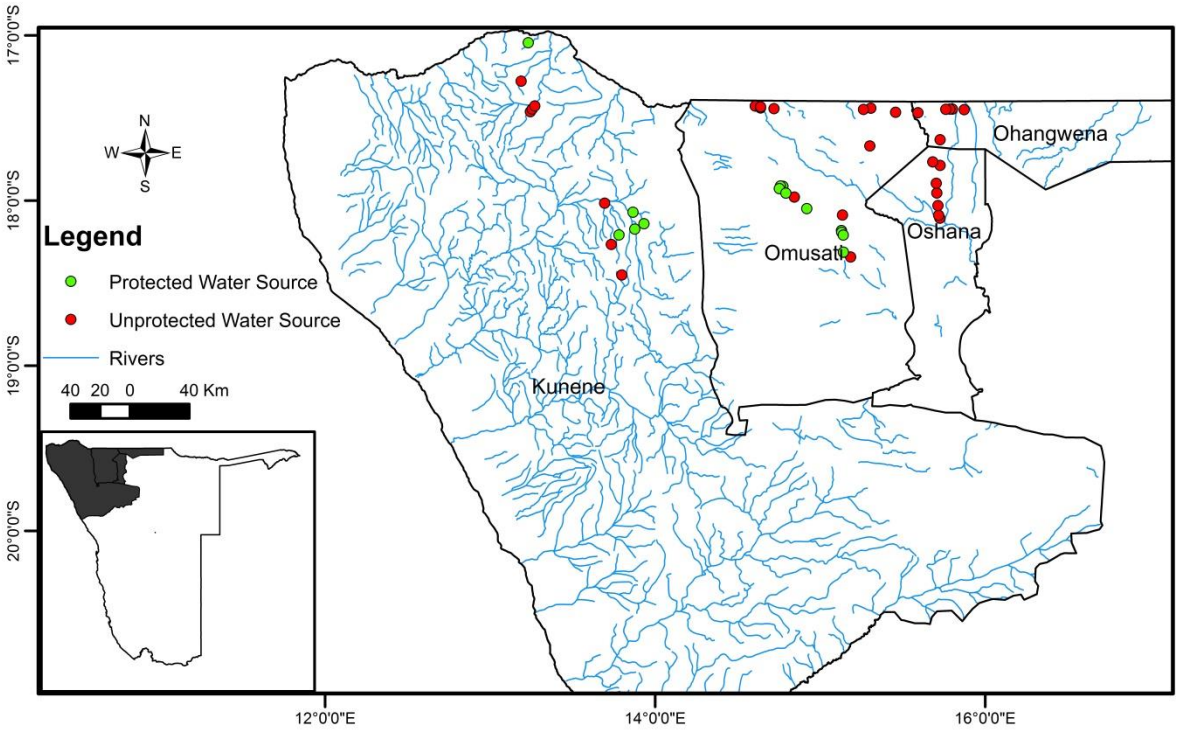


Figure 1: GIS Map depicting the sampled points in the study area classified into protected and unprotected water resources.

CHAPTER 2

2. Literature Review

2.1 Water sources in Kunene and Cuvelai-Etosha Basins

Rural communities in the Kunene and Cuvelai-Etosha basins continue to rely heavily on unsafe water with no water treatment and in some cases, with no protection to prevent animals from defecating in the water [21]. In addition, freshwaters in Cuvelai-Etosha Basin are at risk of contamination due to inadequate sanitation and waste disposal facilities [13]. These water resources include earth dams, uncultured springs and captured springs, hand-dug wells and boreholes and they are mostly used for domestic and livestock purposes. *Cryptosporidium* species have been reported in domestic animals such as cattle, goats, dogs and sheep [18], which are the most common domesticated animals in the Cuvelai-Etosha and Kunene Basins. In addition, the protozoa have been detected in 30% of children under the age of twelve years in Angola [18], an upstream country where most surface and ground water in the two Basins originates from. UNICEF Namibia reports incidence of diarrhea due to the consumption of untreated water [20].

2.2 Factors which influence the occurrence of *Cryptosporidium*

All animals' species are said to be potential hosts of *Cryptosporidium* species, hence people who live in close contact with animals are said to be at high risk of cryptosporidiosis' infection [18, 17]. The immune system of the host plays a significant role in the prognosis of the disease, healthy individuals only experience mild symptoms and normally get rid of the parasite in 3

weeks while immune compromised individual on the other hand are more prone to frequent diarrhea which can be fatal [19]. Precipitation is said to be a seasonal driver of *Cryptosporidium* as high prevalence is reported at the onset of rainfall season in some areas while at the end of the rainy season in others. The water supply system source of water in the community also play a major role as it was reported that *Cryptosporidium* was more prevalent in calves that relies on tap water as their source of water supply [14].

2.3 Methods of Analysis of *Cryptosporidium* from samples

Morphological identification of *Cryptosporidium* species is the standard form of diagnosis; however, these methods are time consuming, have low sensitivity and need a high-level of expertise for a better chance of oocysts recovery [14]. Molecular methods are normally used in developed nations as they are fast and can process numerous samples in a short period of time [10, 14]. In addition, such methodology can reveal the species/subspecies which tells us more about the epidemiology of cryptosporidiosis [2, 9, 13]. Concentration of oocysts during sample collection is normally done using sedimentation and flotation techniques with simple gravity sedimentation being the most cost effective and relatively easy to perform [1, 2, 9]. Preservation of samples is also important because of the time before analysis and the commonly used preservatives include: 10 % formalin, potassium dichromate, sodium acetate-acetic absolute ethanol, poly-venile alcohol and acid-formalin fixative [2]. All in all, recovery efficiency depends on the amount of water used (larger volumes produce better results) and the EPA method 1623 only said to have an average of 40% detection; consequently, in reality, the concentration of oocysts is always higher than detected.

CHAPTER 3

3.1 Sampling and methods

Water samples were collected from 47 sites in May 2018 as shown in Table 1 and Appendix A. The water resources were classified into two categories (protected and unprotected) based on whether animals have direct access to them. Physical parameters which were measured includes pH, electrical conductivity (EC), oxygen content (O) and temperature (T) using Hach field portable instruments (HQ 11d pH meter, HQ 14d conductivity meter, HQ 40d multi-meter). Convenience sampling technique was used as samples were taken from different sources (Surface water, Boreholes, and wells) based on accessibility to the site [9]. Samples were taken from 48 water resources in the CEB and Kunene basin and were classified into different categories according to the Namibian water quality standards based on the defined onsite parameters [15], as shown in Table 2.

Table 1: Samples taken from differently managed water resources.

Water Samples	Number of samples
Protected water resources	15
Unprotected water resources	32

3.2 Research design

The research work was based on both qualitative and quantitative research methods and the diagram below gives an outline of the research design:

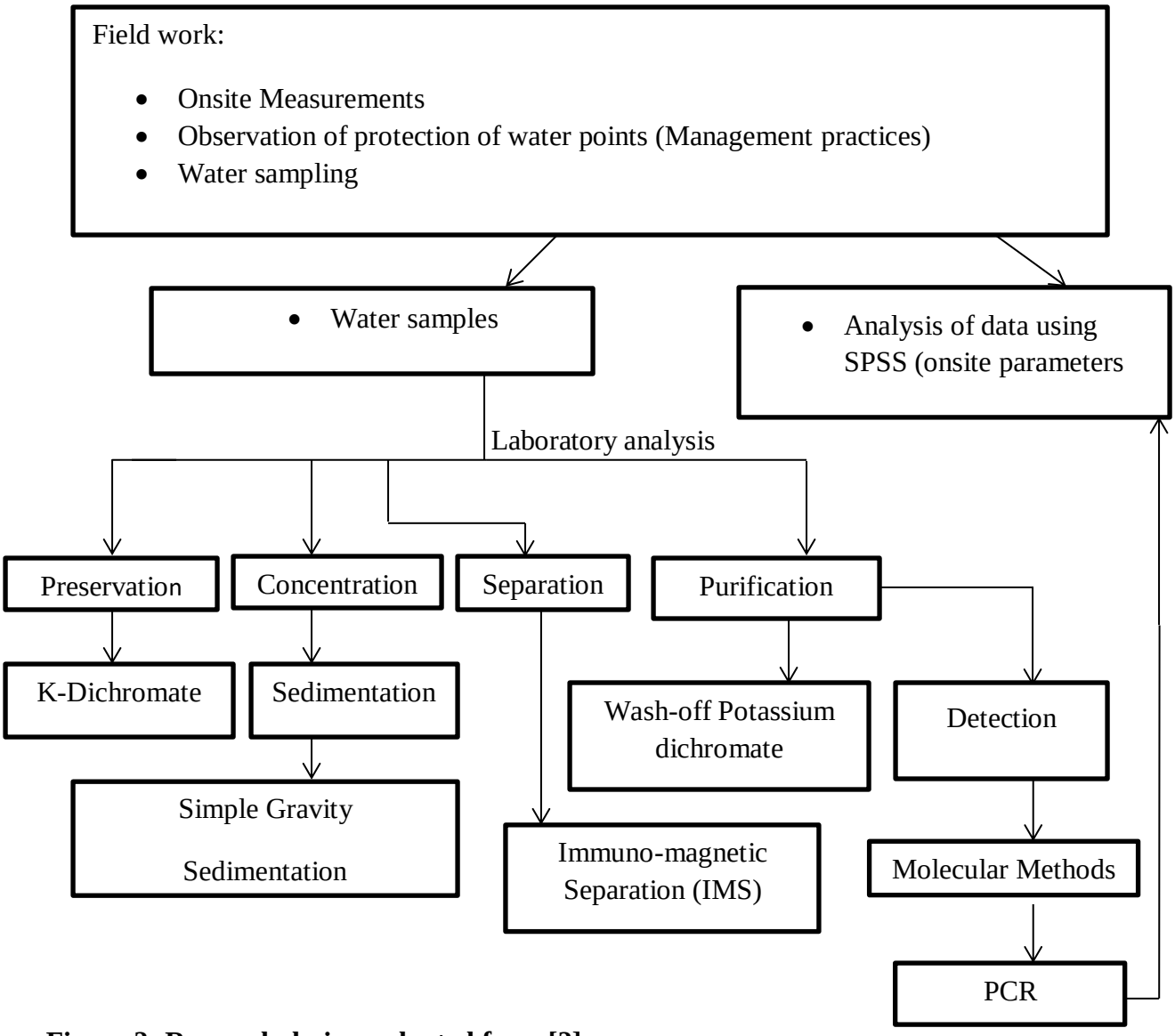


Figure 2: Research design, adopted from [2].

Figure 3: Examples of differently managed water resources. A) Protected water resource. B) Unprotected water resource

3.3 Research Instruments

- Potassium dichromate (2.5%) as a preservative.
- Water collection vessels (5 L)
- Sterile sample tubes (50 ml falcon tubes)
- 10 or 25 ml pipettes (strippettes).
- pH meter
- Dissolved oxygen meter
- Electric conductivity meter
- Temperature meter

3.4 Procedures

Five (5) liters of water were collected in a container and poured into a bucket through a 100 µm pre-filter (metal sieve) and stored in a 5 liters container. This was label and stored for later use. The five (5) liters water samples' were later shaken well and poured into two (2) different containers and allowed to settle for about 12 hours. This was done to ensure concentration of oocysts (if any) by simple gravity sedimentation. Some samples formed sediment (depending on turbidity) and *Cryptosporidium* oocysts were assumed to settle on top of the sediment layer.

Samples were then re-collected using 10 ml manual squeezable-pippete just above the sediment layer. For the samples without sediment at the bottom of the container, a sample was collected from the bottom of the container. The water sample will then be transferred into pre-filled falcon tubes with 25 ml of potassium dichromate at 2.5 percent.

Samples were washed to remove the PCR inhibitor (Potassium-dichromate) before a series of steps of molecular analysis were done (Appendix C).

3.5 Data analysis

Water resources were divided into two groups based on whether they are protected against direct access to animals or not. Statistical analysis such as mean, range, test of normality, median and hypothesis tests were done using SPSS version 24.

3.6 Research ethics

Research activities in this study were bound to ethical considerations by upholding ethical standards with respect to human lives, the environment, the University of Namibia as research permission was granted by the University of Namibia and ethical consideration was adhered to according to the research ethics of the university and community members were well acquainted with the research objectives and the information gathered from the research will only be used for academic purposes.

CHAPTER 4

4. Results

4.1 *Cryptosporidium* prevalence

A total of 47 samples were collected and grouped into categories of protected and unprotected water resources and two (2) samples were positive for *Cryptosporidium* based on qPCR (Appendix D) as shown in Table 2.

Table 2: Frequency table for *Cryptosporidium* Analysis:

	Frequency	Percent	Valid percent	Cumulative Percent
Negative samples	45	95.7	95.7	95.7
Positive samples	2	4.3	4.3	4.3
Total	47	100.0	100.0	100.0

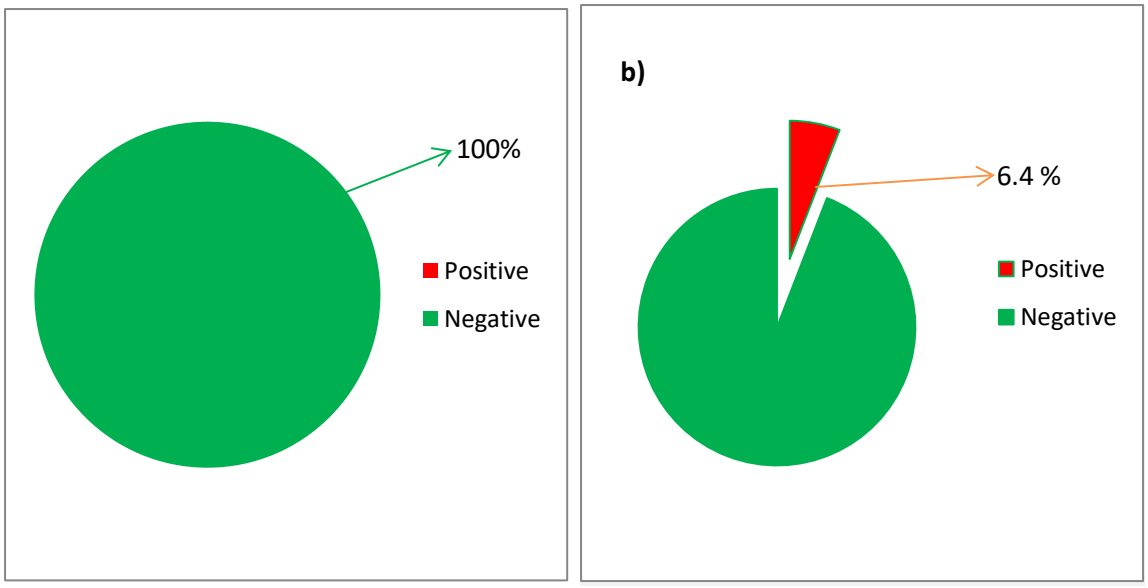


Figure 5: *Cryptosporidium* prevalence: a) Protected Water resources.

b) Unprotected water resources.

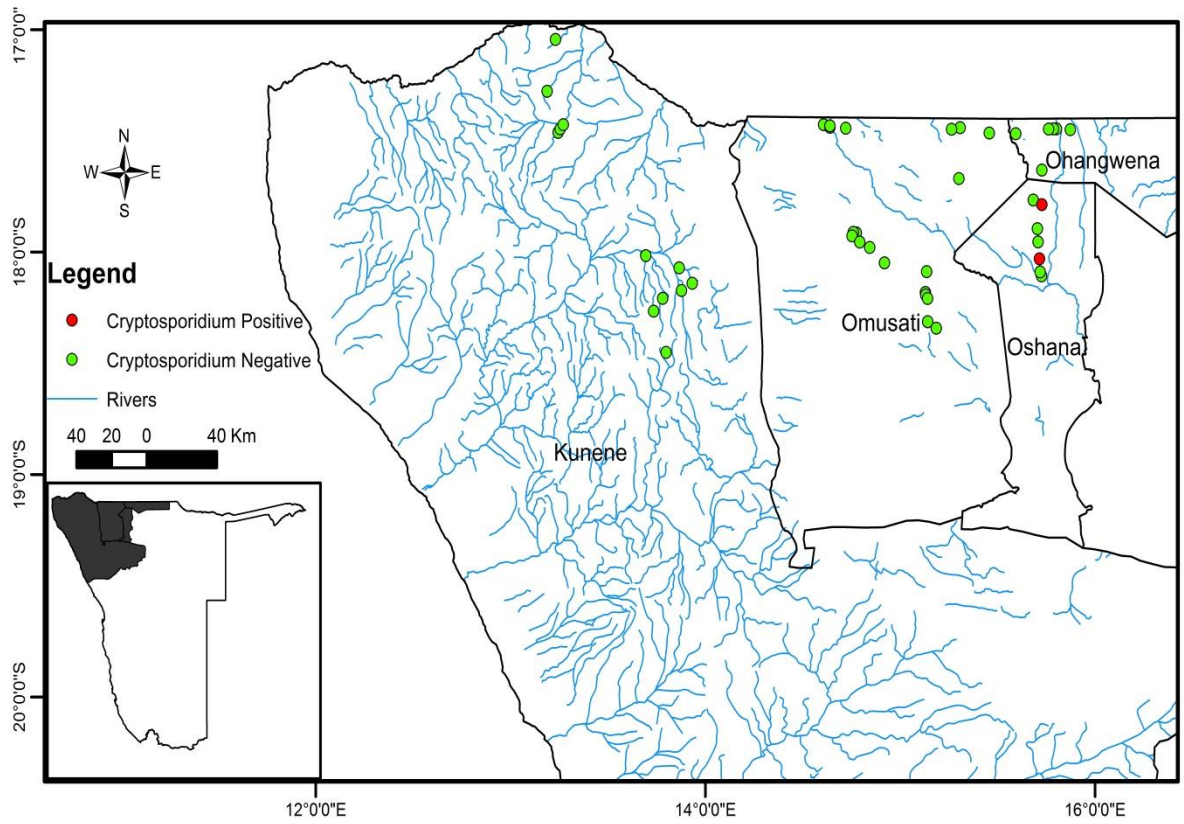


Figure 6: GIS map depicting the location of *Cryptosporidium* positive sites.

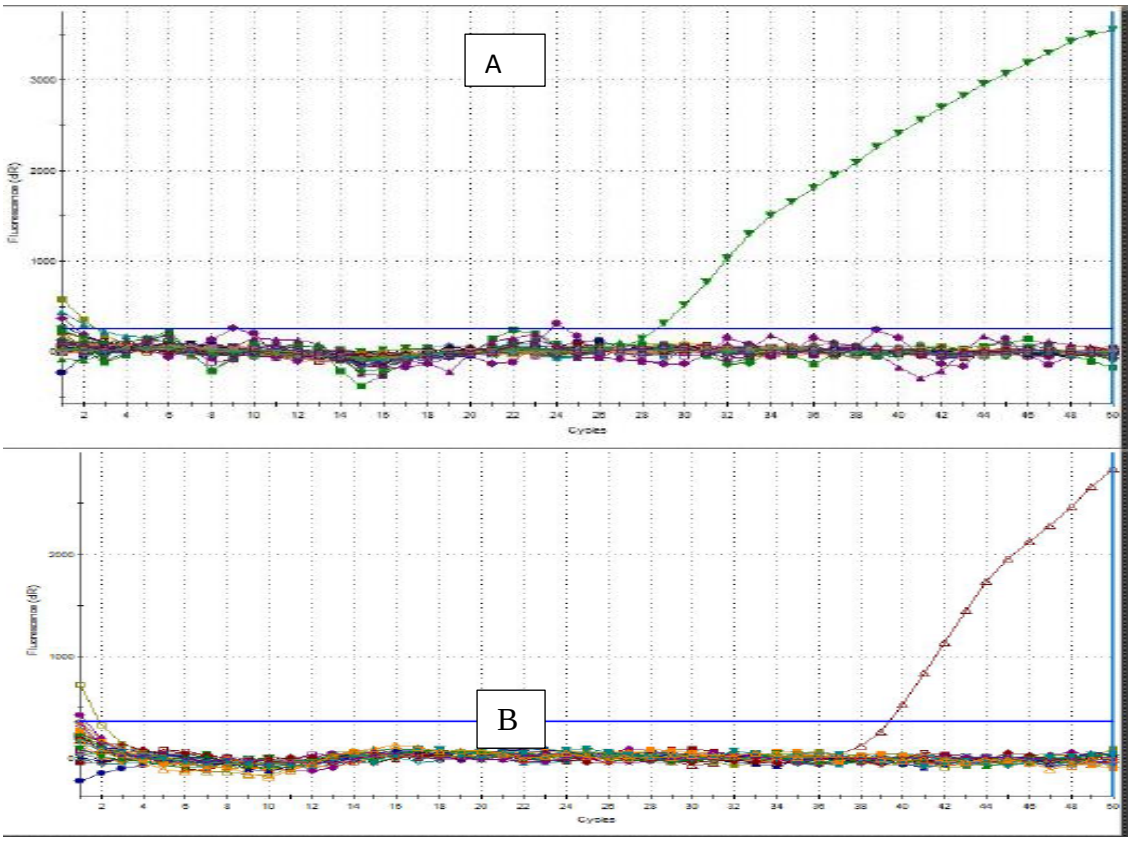


Figure 7: qPCR Amplification plots of the positive samples. A) Represents sample 1. B) Represents sample 12.

From the total of 47 samples, only Sample 1 and 12 showed the presence of oocysts presence as shown in Figure 7 and the threshold points were used to calculate the oocysts number in the samples with each having a threshold cycle of 29 and 39, respectively as shown in Figure 7.

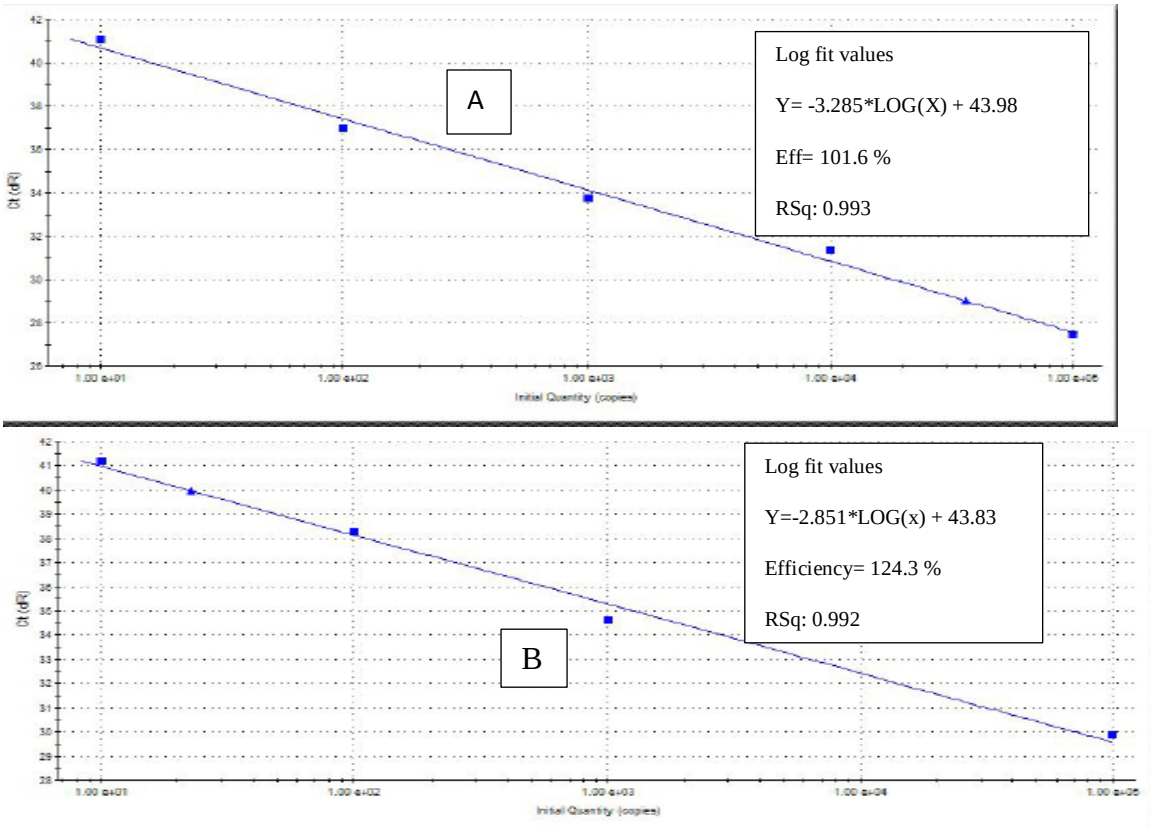


Figure 8: Standard curves of the positive samples done. A) Sample 1 B) Sample 12

The standard curves were done using the same volumes as samples (in terms of DNA extraction, DNA elution). Sample one (1) was done from about 2 liters equivalence while sample 12 was from about 2.5 liters equivalence. The Ct values for sample 1 and 12 were measured from figure 5 for each of the positive sample and were found to be 29 and 39, respectively.

1. Formulae: $N_n = 10^{n-b/m}$

Where n= Ct Value

y= mx + b

a). Sample 1: y= -3.285 × log(x) + 43.98

$N_{n1} = 10^{29-43.98/-3.285}$

=36318 oocysts/ 2 liters.

=18159 oocysts/l.

Concentration of oocysts above 10 oocysts/l may cause infection.

b). Sample 12: $N_{n12} = 10^{39-43.98/-2.851}$

y= -2.851×Log(x) + 43.83

=56 Oocysts/2.5 liters.

=22.4 oocysts/ Liter, this concentration may cause infection.

4.3 Onsite Parameters

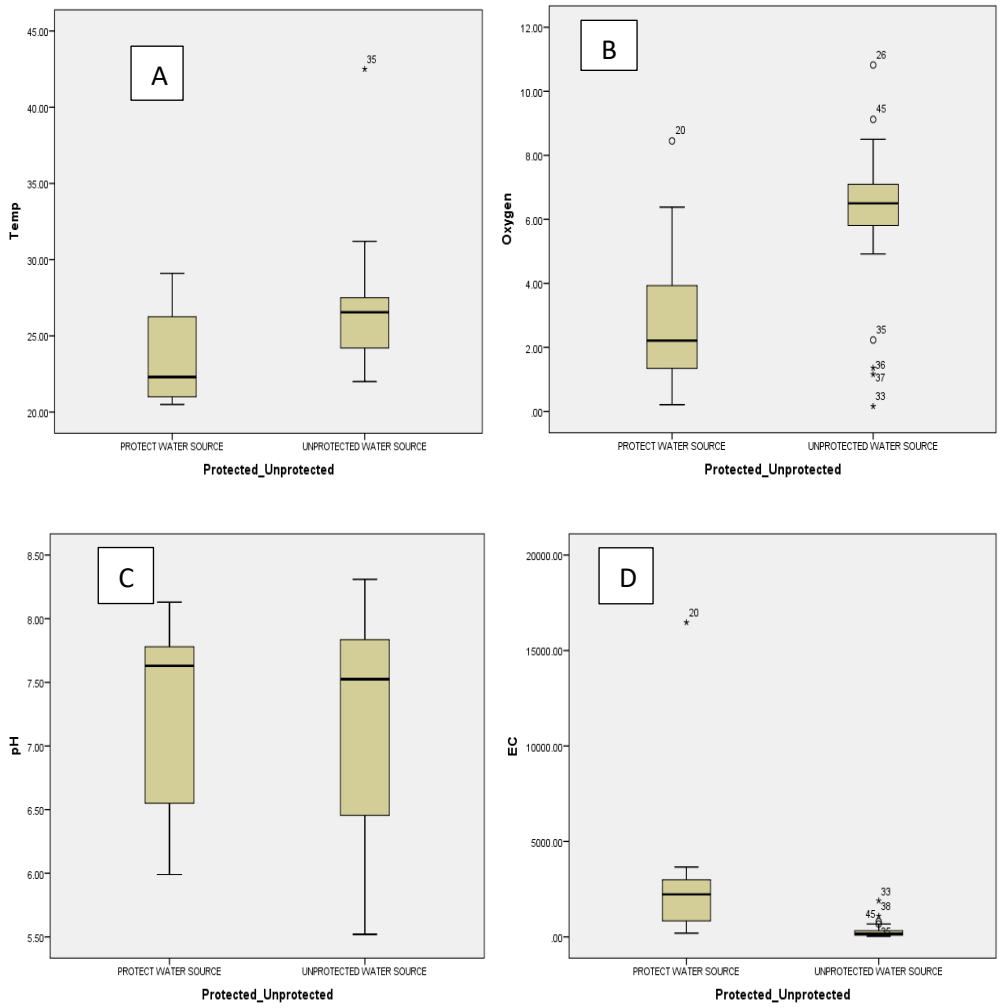


Figure 9: Box plots of the measured onsite parameters for protected and unprotected water resources: A). Represents Temperature, B). Represents DO, C). Represents pH while D). Represents EC.

Generally, the readings from physical parameters are skewed with outliers (Figure 9), rendering the arithmetic mean less suitable for measuring central location thereby making the median more appropriate.

4.3.1 Temperature

The temperature was measured on site and there was one hot spring which is the only outlier in the unprotected water resources category (Site 35 in Figure 7A). With the exclusion of the hot spring, the average temperatures of unprotected water resources was 25.9 ˚C while for protected water resources are 23.7 ˚C (Table 3).

4.3.2 Dissolved Oxygen

The amount of dissolved oxygen in the water is influenced by the temperature and salinity of the water. Fresh water normally has more DO holding capacity than salty and warm water. The dissolved Oxygen data used in the research was measured in milligrams per liter (mg/L). Protected and unprotected waters had DO of 0.21 mg/l and 0.16 mg/l respectively as shown in Table 3.

4.3.3 Potential Hydrogen (pH).

The pH values of protected and unprotected water resources had no outliers (Figure 8: A) and all were within reasonable range of use as stipulated by the Namibian water quality standards [14]. The average electrical conductivity was 2801.91 mS/m and 298.97 mS/m for protected and unprotected water resources, respectively (Table 3).

Table 3: Minimum, median, average and maximum values of the onsite parameters for protected and unprotected water resources. EC is in µS/cm, temperature in ºC and Oxygen in mg/l.

	Protected water resources				Unprotected water resources			
	Temp	**EC**	**pH**	**O_2**	**Temp**	**EC**	**pH**	**O_2**
Mean	23.6	2802	7.22	2.94	25.9	299	7.18	6.08
Maximum	29.1	16470	8.13	8.45	31.2	1892	8.31	10.82
Minimum	20.5	199.7	5.99	0.21	22	40.5	5.52	0.16
Median	22.3	2230	7.63	2.21	26.4	156	7.53	6.5
Range	8.6	16270	2.14	8.24	9.2	1852	2.79	10.66
N	15	15	15	15	27	27	27	27

Table 4: Classification of the samples based on pH and EC, based on the Namibian water quality standards [15].

Sample number	Water type	EC	pH	Water Quality category	Cryptosporidium's result
1.	River	81.6	5.72	B	Positive
2.	Canal	45.9	5.86	B	Negative
3.	Canal	42.2	5.85	B	Negative
4.	Hand dug well	42.9	5.53	B	Negative
5.	Floodplain Monitoring station	104.3	5.52	B	Negative
6.	Floodplain Monitoring station	77.1	5.57	B	Negative
7.	Floodplain Monitoring station	58.5	5.77	B	Negative
8.	Floodplain Monitoring station	38.3	6.16	A	Negative
9.	Floodplain Monitoring station	145.9	6.4	A	Negative
10.	Surface water	675	7.06	D	Negative
11.	Surface water	293	7.27	B	Negative
12.	Surface water	167.7	7.83	B	Positive
13.	Surface water	204.3	8.03	B	Negative
14.	Floodplain Monitoring station	119.8	8.27	A	Negative
15.	Hand dug well	169.2	7.65	A	Negative
16.	Hand dug well	3660	7.76	D	Negative
17.	Hand dug well	225	8.13	B	Negative

18.	Hand dug well	2230	7.68	D	Negative
19.	Hand dug well	199.7	8.09	B	Negative
20.	Hand dug well	16470	7.82	D	Negative
21.	Hand dug well	453	8.31	D	Negative
22.	Floodplain Monitoring station	141.1	7.86	A	Negative
23.	Floodplain Monitoring station	155.7	7.73	B	Negative
24.	Floodplain Monitoring station	117.6	7.82	A	Negative
25.	Canal	40.5	7.83	A	Negative
26.	Dam	55.8	8.05	A	Negative
27.	Dam	57.2	7.84	A	Negative
28.	Canal	41.5	8.16	A	Negative
29.	Hand dug well	3040	6.89	D	Negative
30.	Hand dug well	2940	7.23	D	Negative
31.	Hand dug well	3500	7.79	D	Negative
32.	Hand dug well	2330	7.77	D	Negative
33.	Hand dug well	1892	7.60	D	Negative
34.	Hand dug well	2860	7.63	D	Negative
35.	Surface water	804	6.7	D	Negative
36.	Hand dug well	304	5.89	C	Negative
37.	Hand dug well	345	6.3	C	Negative
38.	Surface water	1096	7.17	D	Negative
39.	Hand pump well	989	6.32	D	Negative
40.	Hand pump well	1292	6.25	D	Negative
41.	Engine operated borehole	941	6.19	D	Negative
42.	Hand Pumped well	744	5.99	D	Negative
43.	Earth Dam	156	6.51	B	Negative
44.	Spring	608	6.78	D	Negative
45.	Spring	678	7.11	D	Negative
46.	Earth Dam	199.4	7.45	D	Negative
47.	Earth Dam	298	7.64	C	Negative

4.4 Hypothesis testing

Hypothesis test of paired samples were done using SPSS version 24 to test for prevalence of *Cryptosporidium* and water management. In addition, hypothesis tests were done to test the relationship between onsite parameters and water management categories.

Table 5: Onsite parameters and water management

Null Hypothesis	Test	Sig.	Decision
1. The distribution of EC is the same across categories of protected and Unprotected.	Independent Samples Mann-Whitney U Test	0.001	Reject the null hypothesis
2. The distribution of pH is the same across categories of protected and unprotected.	Independent Samples Mann-Whitney U Test	0.576	Retain the null Hypothesis
3. The distribution of dissolved oxygen is the same across categories of protected and unprotected	Independent Samples Mann-Whitney U Test	0.001	Reject the null hypothesis
4. The distribution of Temperature is the same across categories of protected and unprotected.	Independent Samples Mann-Whitney U Test	0.010	Reject the null hypothesis

Based on Table 5, the only onsite parameter which remained the same across categories of protected and unprotected water resources is pH as the null hypothesis was retained.

In general, the parameters which showed correlation are temperature and dissolved oxygen , they showed a negative correlation as observed in Table 6.

In addition, there is also a negative correlation between EC and Temperature for hand-pumped wells' and Earth Dams' water resources as observed in Table 6.

Table 6: Correlation-coefficients for onsite parameters.

Parameter	Test	EC	pH	Oxygen	T
EC	Pearson Correlation	1	0.153	-0.037	-0.317*
pH	Pearson Correlation	0.153	1	0.237	-0.396**
Oxygen	Pearson Correlation	-0.037	0.237	1	0.109
Temp	Pearson Correlation	-.317*	-.396**	0.109	1

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant a the 0.005 level (2-tailed).

c. Listwise N=43

Generally, there is a negative correlation between EC and dissolved oxygen of -0.037 at 0.05 significant level as shown in table 6.

Table 7: Hypothesis test: *Cryptosporidium* prevalence and water management.

Null Hypothesis	Test	Sig.	Decision
1. The distribution of different values across *Cryptosporidium* Test and protected and unprotected water resources are equally likely.	Related-Samples McNemar Test.	0.001	Reject the null hypothesis.

Based on table 7 which shows the results of hypothesis testing using SPSS, the null hypothesis was rejected because the significance levels were less than 0.005. Therefore, it can be concluded that there is a significant difference between on-site parameters and differently managed water resources categorized into protected and unprotected water resources. This is based on the fact that Cryptosporidium positive samples all came from unprotected water resources.

5.1 Discussion

In general, unprotected water resources had more DO than protected water resources, an indication that protected water resources are more salty and warmer. The higher EC in protected water resources could be attributed to shallow regional aquifers from which they are tapping from [4]. Results in the Cuvelai-Etosha and Kunene Basins indicated that the median concentrations for protected water resources were Dissolved oxygen (2.2 mg/L); Electrical Conductivity (2230); Temperature (22.3), pH (7.63), while the medians concentrations for unprotected water resources were Dissolved oxygen (DO) (6.5 mg/L); Conductivity (156); Total dissolved solids (TDS) (878 mg/L); Temperature (26.4), pH (7.53) as shown in table 3. The presence of *Cryptosporidium* in the present study highlight the need for more research in the water basins in Namibia with a combination of different methods for effective oocysts detection which might be used in updating water treatment in the study area. In this study, only five (5) liters of water was used for concentration, while an amount of 10-1000 liters is normally recommended [17]. Characteristics of the water such as turbidity and algae plays a role in recovery efficiency and given the high turbidity observed in most water resources and the low number of samples taken at each side (One sample per side), the prevalence of *Cryptosporidium* could be more than the 4.3 % currently detected. In addition, Quantitative polymerase chain reaction (qPCR) is said to be unable to distinguish between signals 1, 2, 5 and 10 oocysts per liter; this implies that possible oocysts in this range were not detected in this study. Furthermore, different management strategies employed in water resources could be said to be helpful in the prevention of contamination with *Cryptosporidium* species because all positive results came from surface water without protection. This correlates with other studies that reported incidence of *Cryptosporidium* in surface water

without protection in Cameroon [6] which highlight the importance of the "protection of water resources through infrastructure improvements at water points and practices in prevention of *Cryptosporidium* contamination" [21]. Based on the results of this study, ground water is safe in terms of *Cryptosporidium* contamination as all positive results came from surface water. Given the fact that more than 50% of the population in the Cuvelai-Etosha basin relies on surface water for their daily needs, there is a need for more longitudinal studies on *Cryptosporidium* which could lead to the definition of *Cryptosporidium* level in the Namibian water quality standards. According to this study, pH and electrical conductivity may give wrong impression of the biological quality of water as the samples which were *Cryptosporidium* positive met the Namibian water-quality standards based on the measured values of pH and EC (Table 3). The higher prevalence and widespread of *Cryptosporidium* in Africa [18], calls for more frequent research of enteric protozoa as recent hydrological droughts could lead to increase waterborne infections. Other enteric protozoa of health concern which could be of interest in the study area include *Toxoplasma gondii*, *Cyclospora cayetanensis, Entamoeba histolytica, Gardia* species and *Blastocystis hominis.*

5.2 Conclusion and Recommendations

This study reveals the presence of *Cryptosporidium* in water resources in the study area and could be the cause of diarrhea in Namibia as reported [20], especially in children and immune compromised individuals and suggests possible contamination of these resources with wastewater. Water resources management especially in regards to source protection is crucial to the prevention of direct microbiological contamination as supported by the results which show that unprotected water resources are at risk, therefore, it is recommended that water resources be protected in order to prevent direct runoff and contamination. However, for the water in the two basins to be fit for human consumption according to the Namibian water quality standards, treatment is crucial. The study therefore recommends extension of pipelines or treatment of water of existing water resources in the study area to lower the risk of *Cryptosporidium* and other related enteric protozoa. Since the positive samples came from surface water and only from one part of the CEB, and given that more than 50% of the population in the CEB relies on surface water, more extensive studies are recommended on *Cryptosporidium* covering a wider area of the CEB with different methodologies for oocysts recovery.

References

[1] F.E. Adeyemo, G. Singh, P. Reddy & T.A. Stenström, "Methods for the detection of *Cryptosporidium* and *Giardia*: From microscopy to nucleic acid based tools in clinical and environmental regimes, ".*Acta Tropica*, Vol. 184, pp 15-28. 2018.

[2] S.A Ahmed & P. Karanis, "Comparison of current methods used to detect *Cryptosporidium* oocysts in stools," *International journal of Hygiene and Environmental Health*. Vol. 221, pp 743-763. 2018.

[3] F.A. Armah, B. Ekumah, D.O. Yawson, J.O. Odoi, A.R. Afitiri, & F.E. Nyieku, (2018). "Access to improved water and sanitation in sub-Saharan Africa in a quarter century," *Heliyon*, Vol. 4, no.11, e00931. 2018.

[4] A. Bittner, Desk study report: "Cuvelai-Etosha groundwater investigation". Windhoek. 2006.

[5] B.A. Bryan, J. Kandulu, D.A. Deere, M. White, J. Frizenschaf & N.D. Crossman, "Adaptive management for mitigating *Cryptosporidium* risk in source water: a case study in an agricultural catchment in South Australia," *Journal of Environmental Management*. Vol. 90, no. 10, pp 3122-3134. 2009.

[6]. T. Dalu, M. Barson, & T. Nhiwatiwa, "Impact of intestinal microorganisms and protozoan parasites on drinking water quality in Harare, Zimbabwe". *Journal of Water, Sanitation and Hygiene for Development*, Vol. *1, no.* 3), 153-163. 2011.

[7] K. Duhaime & D. Roberts, "Theoretical implications of best management practices for reducing the risk of drinking water contamination with *Cryptosporidium* from grazing cattle," *Agriculture, Ecosystems & Environment*, Vol. 259, pp 184-193. 2018.

[8] T.A., Edge, I.U., Khan, R. Bouchard, J. Guo, S. Hill, A. Locas, A., ... & R. Yang, "Occurrence of waterborne pathogens and *Escherichia coli* at offshore drinking water intakes in Lake Ontario," *Appl. Environ. Microbiol.*, Vol. 79, no.19, pp 5799-5813. 2013.

[9] I. Etikan, S.A Musa & R.S Alkassim, "Comparison of convenience sampling and purposive sampling," *American journal of theoretical and applied statistics*, Vol. *5*, no 1, pp 1-4. 2016.

[10] A.O. Hughes, C.C Tanner, L.A. McKergow, & J.P. Sukias, "Unrestricted dairy cattle grazing of a pastoral headwater wetland and its effect on water quality," *Agricultural Water Management*, Vol 165, pp 72-81. 2016.

[11] J. Kalinová, A. Valenčáková, E. Hatalová, O. Danišová, M. Trungelová & R. Hromada, "Occurrence of *Cryptosporidium* in the water basins of Nitra region, Slovakia," *Acta Tropica*, Vol. 179, pp 36-38. 2018.

[12] A Khan, J.S. Shaik, & M.E. Grigg, "Genomics and molecular epidemiology of *Cryptosporidium* species," *Acta Tropica*, Vol. 184, pp 1-14. 2018.

[13] T. Kluge, S. Liehr, A. Lux, P. Moser, S. Niemann, N. Umlauf, & W. Urban, " IWRM concept for the Cuvelai Basin in northern Namibia", *Physics and Chemistry of the Earth, Parts A/B/C*, *33*(1-2), 48-55.2008.

[14] M.R. Mahmoudi, J.E. Ongerth & P. Karanis, "*Cryptosporidium* and Cryptosporidiosis: the Asian perspective," *International journal of hygiene and environmental health*, Vol. 220, no.7, pp 1098-1109. 2017.

[15] NamWater, "Guideline for the evaluation of drinking-water for human consumption with regard to chemical, physical and bacteriological quality". Based on the Water Act, Act 54, 1956. 2011.

[16] N.J Ruecker, J. C. Matsune, D.R. Lapen, E. Topp, T.A. Edge & N.F Neumann, "The detection of Cryptosporidium and the resolution of mixtures of species and genotypes from water". *Infection, Genetics and Evolution*, Vol *15*, pp 3-9. 2013.

[17] S.E Staggs, E.M Beckman, S.P Keely, R. Mackwan, M.W Ware, A.P Moyer,., ... & E.N Villegas, "The applicability of TaqMan-based quantitative real-time PCR assays for detecting and enumerating Cryptosporidium spp. oocysts in the environment". *PloS one*, Vol *8. No* 6, e66562. 2013.

[18] S.A. Squire & U. Ryan, "*Cryptosporidium* and *Giardia* in Africa: current and future challenges," *Parasites & vectors*, Vol. 10, no.1, pp 195. 2017.

[19] S.A. Squire, R. Yang, I. Robertson, I. Ayi, & U. Ryan, "Molecular characterization of *Cryptosporidium* and Giardia in farmers and their ruminant livestock from the Coastal Savannah zone of Ghana," *Infection, Genetics and Evolution*, Vol. 55, pp 236-243. 2017.

[20] UNICEF Namibia, "Cholera Outbreak Report #1", 2014.

[21] H. Wanke, A. Nakwafila, J.T Hamutoko, C. Lohe, F. Neumbo, I. Petrus, I., ... & M. Quinger, "Hand dug wells in Namibia: An underestimated water source or a threat to human health?," *Physics and Chemistry of the Earth, Parts A/B/C*, Vol. 76, pp 104-113. 2014.

[22] W. Yan, K. Alderisio, D.M. Roellig, K. Elwin, R.M. Chalmers, F. Yang, F., ... & L. Xiao, "Subtype analysis of zoonotic pathogen *Cryptosporidium* skunk genotype," *Infection, Genetics and Evolution*, Vol. 55, pp 20-25. 2017.

Appendix A: Raw hydro-chemical data collected in the study.

Name	Water type	EC [µS/cm]	pH	Oxygen [mg/l]	Temperature [◦C]
Okatana river	River	81.6	5.72	5.17	*
Ehenye	Canal	45.9	5.86	7.5	*
Ogongo	Canal	42.2	5.85	7.13	27.5
Endola	Hand dug well	42.9	5.53	5.86	28.1
Engela	Floodplain (monitoring station)	104.3	5.52	5.76	28.3
Ouhongo	Floodplain (monitoring station)	77.1	5.57	6.85	31.2
Ohaingu	Floodplain (monitoring station)	58.5	5.77	7.03	*
Omufitu	Floodplain (monitoring station)	38.3	6.16	5.07	*
Okambebe	Floodplain (monitoring station)	145.9	6.4	6.33	27.2
**	Surface water	675	7.06	7.06	28.3
**	Surface water	293	7.27	7.15	27.8
**	Surface water	167.7	7.83	4.92	27
**	Surface water	204.3	8.03	6.83	27.5
**	Floodplain (monitoring station)	119.8	8.27	6.38	26
Omusati region	Hand dug well	169.2	7.65	6.21	24.5
Omusati region	Hand dug well	3660	7.76	1.04	20.7
Omusati region	Hand dug well	225	8.13	6.38	24.7
Omusati region	Hand dug well	2230	7.68	1.73	21.1
Omusati region	Hand dug well	199.7	8.09	0.54	20.9
Omusati region	Hand dug well	16470	7.82	8.45	21.4
Amarika	Hand dug well	453	8.31	6.22	24.1
**	floodplain (monitoring station)	141.1	7.86	6.62	24.3
**	floodplain (monitoring station)	155.7	7.73	5.4	23.1
**	floodplain (monitoring station)	117.6	7.82	8.5	27
**	Canal	40.5	7.83	7	26.4
**	Dam	55.8	8.05	10.82	27.3
**	Dam	57.2	7.84	7.85	26.8
**	Canal	41.5	8.16	7.5	25.1
Near Okahao	Hand dug well	3040	6.89	1	20.6

Near Okahao	Hand dug well	2940	7.23	3.12	22.5
Near Okahao	Hand dug well	3500	7.79	4.74	20.5
Near Okahao	Hand dug well	2330	7.77	5.55	22.1
Near Okahao	Hand dug well	1892	7.6	0.16	24.7
Near Okahao	Hand dug well	2860	7.63	0.21	22.3
Otjijandjasemo	Surface water	804	6.7	2.23	42.5
Otjijandjasemo	Shallow hand dug well	304	5.89	1.16	23.3
Otjijandjasemo	Shallow hand dug well	345	6.3	1.36	25.5
Otjijandjasemo	Surface water	1096	7.17	7.06	26.7
Epupa P.SC	Hand-pumped well	989	6.32	1.65	27.8
Okovingava	Hand-pumped borehole	1292	6.25	2.27	25.8
Ondore	Engine-operated borehole	941	6.19	2.21	27.3
Ondororundu	Hand-pumped well	744	5.99	2.11	29.1
Otutati	Earth Dam	156	6.51	6.94	23.9
Okorosave	Spring	608	6.78	3.05	26.7
Okorosave	Spring	678	7.11	9.12	22.2
Ondjete	Earth Dam	199.4	7.45	5.88	22.5
Ongarangombe	Earth Dam	298	7.64	5.88	22

*Not recorded due to instrument malfunctioning.

**Unknown

Appendix B: Location of Sample sites and field observations.

Date	Site number	GPS (S)	GPS (E)	Field notes
5/15/2018	1	17.78761	15.72766	Very dirty water, lots of rubbish.
5/15/2018	2	17.76658	15.68326	Slow moving water, canal (water from Angola) which led to treatment works), concrete all the way (sometimes underground)
5/15/2018	3	17.67025	15.30146	Upriver from Site 2, just where water comes out of long tunnel. Near agricultural college UNAM
5/16/2018	4	17.63211	15.72761	Near to floodplain, people drink from the water when other source dries up, man seen swimming in water
5/16/2018	5	17.45004	15.87340	Floodplain (water level monitoring station)
5/16/2018	6	17.44694	15.80255	Floodplain (water level monitoring station)
5/16/2018	7	17.44695	15.78691	Floodplain (water level monitoring station)
5/16/2018	8	17.44795	15.76197	Floodplain (water level monitoring station)
5/16/2018	9	17.46879	15.59330	Floodplain (water level monitoring station)
5/17/2018	10	18.10788	15.72603	Very rural area, but locals still use tap water. Animals present in water.
5/17/2018	11	18.08962	15.71882	Very rural area, but locals still use tap water. Animals present in water.
5/17/2018	12	18.03111	15.71496	Very rural area, but locals still use tap water. Animals present in water.
5/17/2018	13	17.95478	15.70800	Very rural area, but locals still use tap water. Animals present in water.

5/17/2018	14	17.89583	15.70439	Very rural area, but locals still use tap water. Animals present in water.
5/18/2018	15	18.08833	15.13604	with animal access, not currently in use (water level low).
5/18/2018	16	18.18240	15.12963	Proper HDW with steps leading in, cattle can't directly acces but faeces all around (easily washed in).
5/18/2018	17	18.19331	15.13153	Area with several wells (possibly one per) family?), as above.
5/18/2018	18	18.20939	15.14079	Proper HDW with steps leading in, cattle can't directly access but feces all around (easily washed in).
5/18/2018	19	18.31391	15.14187	Proper HDW with steps leading in, cattle can't directly access but feces all around (easily washed in). "
5/18/2018	20	18.31306	15.14152	Close to #19, but animals/people get sick from drinking from this well.
5/18/2018	21	18.34270	15.18555	No animals nearby, person washing clothes in the water.
5/19/2018	22	17.46584	15.45765	Floodplain (water level monitoring station).
5/19/2018	23	17.44136	15.30798	Floodplain (water level monitoring station).
5/19/2018	24	17.44865	15.26371	Floodplain (water level monitoring station).
5/19/2018	25	17.42809	14.60735	Upriver from Site 3, canal (water from Angola) concrete all the way, person seen washing baby in the water (people drink directly from this canal to avoid paying for water).
5/19/2018	26	17.43969	14.63961	Large dam, cattle grazing in the water
5/19/2018	27	17.43333	14.63957	Large dam, cattle grazing in the water, water being piped out.

5/19/2018	28	17.44404	14.72109	Middle of other sites (same canal).
5/20/2018	29	17.91443	14.77517	Don't drink directly from this one but cook with it (and bathe), get most of their drinking water from Okahao.
5/20/2018	30	17.91221	14.76010	Used as drinking water.
5/20/2018	31	17.92809	14.75356	New Hand dug well, built 2017.
5/20/2018	32	17.95558	14.79294	New Hand dug well, protected
5/20/2018	33	17.97988	14.84414	Filthy Hand dug well, mainly used for animals but people will drink from here if desperate. Elephants also come here to drink sometimes
5/20/2018	34	18.04913	14.91970	Lots of manure around, used for animals mostly.
5/24/2018	35	17.46236	13.24493	Hot Spring, people seen washing and bathing in the water.
5/24/2018	36	17.44688	13.25557	Very shallow hand dug well about 2.5 m deep.
5/24/2018	37	17.42782	13.27125	Very shallow hand dug well about 2.5 m deep.
5/24/2018	38	17.27767	13.18839	Spring, there is a moderate water flow. Both human and animals have direct access to the Water. No protection zone.
5/24/2018	39	17.04477	13.23155	Well with a concrete protection zone.
5/25/2018	40	18.07155	13.86627	Well with a concrete protection zone.
		18.17306	13.87784	Borehole protected with concrete.

5/25/2018	41			However animals drinking trough closer to the water source.
5/25/2018	42	18.14092	13.93332	Well protected with a concrete protection zone.
5/25/2018	43	18.01671	13.69503	Animals directly drink from the dam and humans drink from this water
5/25/2018	44	18.20882	13.7816	This water has a protection zone and human tap from this water.
5/25/2018	45	18.20882	13.7816	Filthy water, brown in color. Animals drink and defecate in the water.
5/25/2018	46	18.26635	13.73525	Water is brown (probably due to sedimentation). Both human and animals have direct access to the water. No protection zone.
5/25/2018	47	18.45134	13.7991	Water is brown (probably due to sedimentation). Both human and animals have direct access to the water. No protection zone.

Appendix C: Laboratory Procedures.

Separating oocysts from water sample (which was stored in 2.5% potassium dichromate):

1. Samples were centrifuged at 2000 g for 8 min
2. The pellet was then re-suspended in 10 ml of water.
3. To a flat sided L-10 tube,1ml 10 × SL buffer A and 1ml 10 × SL-buffer B were added (for 10ml water volume).
4. The sample was then transferred immediately to the L-10 tube containing the SL-buffer above.
5. Dynabeads were then vortexed for 10 s. Beads were re-suspended completely by inverting the vial.
6. 100 µl of Dynabeads were then added to the L-10 tube (for 10 ml water volume).
7. The L-10 tube was then affixed to the rotating mixer and rotated at 15-25 rpm (about half way between minimum and maximum setting) for 1 hour at room temperature.
8. The MPC/MPC-6 was placed with the flat side of the tube towards the magnet.
9. Without removing the tube from the magnet, the magnet side of the MPC-1 was placed downwards (tube is horizontal and above the magnet).
10. The tube top was gently rocked top to bottom (approx. 90°) tilting the cap end and base end of the tube up and down in turn for 2 min with approx. 1 tilt/s.
11. The magnet was then returned to upright position, tube vertical, with the cap at the top. The cap was removed and the supernatant was poured off into the waste jar.
12. The tube was removed from the magnet and the sample re-suspended in 1 ml 1 × SL Buffer A (100 µL Buffer A + 900 µL dH_2O).
13. The material was mixed gently to re-suspend all material in the tube.

14. All the liquid was then transferred and beads from the L-10 tube to a labeled 1.5 ml micro-centrifuge tube.
15. The micro-centrifuge tube was placed into the MPC-S with magnetic strip in place in the vertical position.
16. Without removing the micro-centrifuge tube from the MPC-S, the MPC-S was gently tilt back and forth 90° for 1 min with approx. 1 tilt/s.
17. The supernatant was then immediately aspirated from the tube and cap held in the MPC-S. For more than one sample processing, three 90° was conducted back and forth motions before removing the supernatant from each tube.

Dissociating the oocysts/cysts from Dynabeads:

1. The magnetic strip was removed from the MPC-S (pull strip to the left).
2. Addition of 50 µl of 0.1N HCl to the micro-centrifuge tube was done and then vortexed for 10 s.
3. The tube was placed in the MPCS without a magnetic strip in place and allowed to stand in a vertical position for at least 10 minutes at room temperature.
4. Vortex for 10 seconds and ensures all sample is at the base of the tube.
5. Place the micro-centrifuge tube in the MPC-S.
6. The magnetic strip was then placed in the MPC-S in the tilted position and the tube was allowed to stand undisturbed for approximately 10 s.
7. Transfer all fluid from the micro-centrifuge tube to another tube.
8. Add 50 µl of 0.1N NaOH to neutralize.
9. Top up with PBS to 1.5 ml.

10. Briefly vortex and centrifuge at 4000 × g for 8 min.
11. Remove all but bottom 100 µl of supernatant.
12. Store in 4°C for further analysis.

DNA extraction

1. The sample was froze-thawed 6 times using liquid nitrogen and 37°C heat block.
2. Add 1 ml L6 buffer to the sample and mix well (a sample prepared this way is stable for couple of days at room temperature, for couple of weeks in -4°C or for up to 6 months in -20°C).
3. Vortex the silica for 10 seconds until re-suspension and add 40 µl to the sample.
4. Mix the sample continuously for 5 minutes by vortex.
5. Centrifuge 30 s at 12000 × g and remove supernatant leaving the last 10 µl.
6. Add 1 ml L2 wash buffer to the silica deposit and vortex until a uniform suspension is formed.
7. Centrifuge for 30 s at 12000 × g and discard supernatant.
8. One milliliter of 70% ethanol was added to the silica deposit and vortexed until a uniform suspension was formed.
9. Centrifuge for 30 seconds at 12000 × g and discard supernatant.
10. Add 900 µl of acetone to the silica deposit and vortex until a uniform suspension is formed.
11. Centrifuge for 30 seconds at 12000 × g and discard supernatant.
12. The open tubes were dried with silica deposits in a heat block for 5 min at 56°C.

Fifty µl molecular grade H_2O was added to the silica pellets and vortexed until a uniform suspension was formed.

13. The tubes were incubated (closed) in a heat block for 5 min at 56°C.
14. Centrifuge for two (2) minutes at 12000 × g.
15. The supernatant containing DNA (and RNA) was transferred carefully into each corresponding new vial and silica was discarded (carefully done so not to transfer silica), stored at -20°C.

Reagent	**Stock concentration**	**Working concentration**	**Final concentration**	**Volume per tube**
Polymerase mix[1]	2 × concentrated	2 × concentrated	1 × concentrated	6.25 µl
Forward primer[2]	100 µM	10 µM	0.25 µM	0.33 µl
Reversed primer[3]	100 µM	10 µM	0.25 µM	0.33 µl
Probe[4]	100 µM	10 µM	0.12 µM	0.15 µl
Nuclease-free water				4.19 µl

[1]AmpliTaq Gold® 360 Master Mix, Applied Biosystems, ThermoFisher Scientific

[2] 5′- GAA ATA ACA ATA CAG GAC TTT TTG GTT TTG-3′

[3] 5′- TTA TTC CAT GCT GGA GTA TTC AAG GCA TAT-3’

[4] 5′ FAM [6-carboxyfluorescein]-TAC GAG CTT TTT AAC TGC AAC AA-XS-BHQ [Black Hole Quencher] 3′

Reagent mix which was used for qPCR:

1.25 µl of DNA should be added to 11.25 µl of a master mix (i.e. the mix of polymerase, primers, probe and water) to a total volume of 12.5 µl per reaction tube. The method was validated for DNA extraction according to SOP number CRYPTO/12.

Thermal profile of PCR:

50°C for 10 min

95°C for 10 min

50 cycles of:

95°C for 15 s

60°C for 1 min