DETECTION OF PATHOGENIC HUMAN ADENOVIRUSES AND ENTEROVIRUSES IN WATER SAMPLES COLLECTED FROM LAKE VICTORIA ALONG HOMA BAY TOWN, HOMA BAY COUNTY, KENYA

WASONGA MICHAEL OPERE

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DECLARATION

This thesis is my original work and has not been presented for degree or any other award in any other University or institution.

Michael Wasonga Opere
Department of Biochemistry, Microbiology and Biotechnology

Signature:..... **Date:** 04/07/2019

Approval by supervisors

We confirm that the work reported to this thesis was carried out by the candidate under our supervision.

Signature:..... **Date:** 4/7/2019

Dr. John Maingi
Department of Biochemistry, Microbiology and Biotechnology
Kenyatta University

Signature:.....**Date:** 5/7/2019

Dr. Omwoyo Ombori
Department of Plant Sciences
Kenyatta University

DEDICATION

This thesis is dedicated to my mother Rose Atieno Otieno for her undying love, encouragement, support and prayers throughout my journey towards achieving this goal. I also dedicate it to my sisters Hellen Opere, Pamela Opere, Monica Opere and Everlyn Opere for their support.

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ACRONYMS AND ABBREVIATIONS

A	Absorbance
AdVs	Adenoviruses
AV	Astrovirus
BAdV	Bovine adenovirus
BEV	Bovine enterovirus
CDC	Centres for Disease Control and Prevention
CBD	Central Business District
cDNA	Complementary deoxyribonucleic acid
CFU	Colony form unit
CPE	Cytopathic effect
CSOs	Combined sewer overflows
dsDNA	Double-stranded deoxyribonucleic acid
dNTP	Deoxynucleoside triphosphate
EC	Electrical conductivity
EDTA	Ethylene diamine tetra-acetate
ELISA	Enzyme-linked immunosorbent assay
EM	Electron Microscope
EtOH	Ethanol
EVs	Enteroviruses
FAO	Food Agriculture Organization of the United Nations
FCSV	Final concentrated sample volume
FEWSNET	Famine Early Warning System Network
GBEG	Glycine beef extract buffer
GuSCN	Guanidinium thiocyanate lysis buffer
HAV	Hepatitis A Virus
HAdV	Human adenovirus
HAstV	Human astrovirus
HEV	Hepatitis E Virus
IC	Interval of Confidence
KFSSG	Agriculture Livestock Sector Working Group of the Kenya Food Security Steering Group
KNBS	Kenya National Bureau of Statistics
LV	Lake Victoria
MW	Molecular Weight
NBS	Nation Bureau of Statistics
NLV	Norwalk-like viruses
OD	Optical density
OR	Odds ratio
PAdV	Porcine adenovirus
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
PEG	Polyethylene glycol
PEV	Porcine enterovirus
PFU	Plaque forming Unit
PVC	polyvinyl chloride

PTV	Porcine teschoviruses
RO	Reverse osmosis
RT-PCR	Reverse-Transcription Polymerase Chain Reaction
RV	Rotavirus
TAMRA	6-carboxy-tetramethylrhodamine
TBE	Tris borate EDTA
TDS	Total dissolved solids
LTM	Last Twelve Months
SLV	Sapporo-like viruses
ssRNA	Single stranded ribonucleic acid
UK	United Kingdom
UNICEF	United Nations Children's Emergency Fund
UPD	Upper respiratory diseases
WFP	World Food Programme

ABSTRACT

Lake Victoria is the main source of water in Homa Bay town as well as the surrounding community. Increase in population in the resent past has led to intensified human activities with a possible compromise on the sanitation standards around the town consequently resulting into increased fecal load to the lake through fecal pollution. Increased fecal contamination of the lake has consequently led to an increase in pathogenic microorganisms including waterborne enteric viruses. These viruses can affect both human and animals health by causing diseases such as gastrointestinal infections. Research on viral water quality in Lake Victoria is limited. The objectives of this study were to determine the occurrence of these pathogenic enteric viruses with respect to human adenoviruses and enteroviruses in Lake Victoria Waters. Factors that may have influenced the levels of contamination of the lake water by the viruses such as physical water parameters, seasonal variations, proximity to sewage effluent and pit latrines were assessed. Water samples were collected from six sites commonly used for domestic and commercial purposes spanning approximately 3 km along the shoreline for analysis for the presence or absence of the two groups of enteric viruses over a seven month period. A total of 216 water samples were analysed for possible contamination with the viruses using nested PCR method. Paired *t* test, ANOVA, Odds Ratios, Correlation and Regression analysis (STATA ver.13.0) were performed to determine factors associated with the virus contamination of the waters. P-values<0.05 were considered significant at 95 % confidence interval. Analysis showed that the lake is contaminated with adenoviruses and enteroviruses which were discovered in 11(5.09 %) and 7(3.24 %) of the samples, respectively. The presence of the enteric viruses was strongly associated with the distance from possible sources of contamination (odds ratio 20.28 and 4.86, confidence interval 2.42, and 0.95) for pit latrines and the sewage treatment plant respectively. Neither wet season nor dry season was associated with the prevalence of the viruses. Of the 72 samples collected from the sites (L5 and L6) closer to the sewage effluent points 13(18 %) tested positive for the two types of viruses. This research clearly signifies that waste water discharge and wastewater-impacted surface waters along the shores of Lake Victoria contain some enteric viruses. This contamination may be due to the fact that the enteric viruses can highly persist in the environmental waters due to their ability to be resistant to environmental conditions. This viral analysis will provide the much needed information in controlling the source of pollution of the lake, such as untreated sewage effluents. It will be useful in ascertaining health risks from the analysis of viral exposure. Further research and analysis is recommended to ascertain the true position concerning the public heath implication and whether the contamination may be as a result of ineffective waste water treatment.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Lake Victoria (LV), the second largest fresh water lake in the world is a source of livelihood for many people around the Eastern part of Africa. Over 20 million people depend on it for very many uses including drinking, recreational and industrial activities (Odada *et al.,* 2004). The lake's catchment area is one of the most densely populated regions in Africa and the lake is economically significant to this population (Kessides, 2006). The most important economic activities at the lake includes but not limited to water transport and fishing activities.

Over the last five decades, LV ecosystem and the water quality in general has been deteriorating (Odada *et al.,* 2004). The deterioration of the ecosystem and the water quality are as results of eutrophication, invasive species and acidification all of which can be associated to human impacts as a result of increase in population (Odada *et al.,* 2004). Populace development and the exercises required to support the expanding populace has led to expanded inflows of pollutants and supplements to the lake and waterways from the catchment leading to contamination of the lake and water-related general medical issues especially in low socio-economic activity areas such as Homa-Bay region (Obudho, 1995). Every day thousands of litres of crude sewage and refuse stream into the lake from households and enterprises found in many towns around the lake. Untreated sewage from Homa Bay town sewage treatment system for example was discharged into the lake directly since the treatment plant was out of order.

The increased fecal pollution of the nearshore lake waters might be related to the increased instances of water-borne illnesses and other water related medical conditions and maladies. These may include looseness of the bowels, intestinal worms, cholera, typhoid, diarrhoea and the enteric viruses' related cases (Otula, 2005). The volume of neighbourhood sewage discharge and urban overflow to the lake has transformed into an important general public health stress in the area. Increased contamination of the lake is debilitating its sustainable utilization, an imperative resource asset for Kenya, Uganda and Tanzania (Otula, 2005). Developing interest in global markets for fresh water fish species such as Nile Perch (*Lates niloticus*) and Tilapia (*Oreochromis niloticus*) from this lake has prompted the increase in the number of fishermen into the lake. Many fishing water crafts are out on the lake every night to supply Capital fish processing industry in the town with the raw materials. The large number of fishermen equally increases the chances of contamination by different species of microorganisms including enteric viruses as a result of improper disposal of human waste products (WHO/UNICEF, 2014).

Poor management of microbial contamination of aquatic environments presents a potential general public health hazard (Otula, 2005). Surveillance of most waterborne diseases worldwide shows that of the many cases recorded for untreated surrounding water, 28.7 % are of an obscure etiology and are responsible for intense gastroenteritis (Sinclair *et al.,* 2009). In recreational epidemiological studies, diarrhoea and respiratory ailments are the most common reported health outcomes (Colford *et al.,* 2007), with a belief that there is a relationship between these ailments and different enteric viruses contaminations.

Enteric viruses' presence in environmental waters will definitely affect human health as a result of poor biological water quality. Most of these microorganisms mainly originate from sewage discharged to surface waters such as Lake Victoria. Information regarding microbial activities in water ecology and their transmission in the environment varies (Kiulia *et al.,* 2014). The part played by enteric viruses in the lake ecosystem is less known in comparison to other microbes such as bacteria and protozoa, potentially because of the troubles related with recognizing the viral agents in the aquatic environment and lack of comprehensive research in that area. Infections due to enteric viruses have been linked to water at certain times and to various other sources such as consumption of contaminated food. Water related outbreaks with different sources have been documented in some countries including of utilization of polluted water from borehole or well (Lawson *et al.,* 1991; Beller *et al.,* 1997; De Serres *et al.,* 1999) and contamination of the county water supply with sewage effluent (Kaplan *et al.,* 1982).

Enteric viruses in the water supply may also bring about infections through food borne transmission as a result of an after-effect of people involved with food preparation becoming contaminated (Brugha *et al.,* 1999). Diseases propagated by contaminated water may be transmitted by drinking of contaminated drinking water, through contact with recreational water or inhalation (Haramoto *et al.,* 2018). The impact on human health brought on by these microorganisms has been reported comprehensively by various researchers including reviews on those diseases related to contact with recreational waters. Various waterborne disease outbreaks may be well documented of course with the exception of hepatitis A virus (HAV); however the disease transmission

through taking water has not been exhaustively demonstrated (Van Puijenbroek *et al.*, 2015).

Presence of viruses in water environment is understated in light of the fact that those agents which cause most enteric sickness cannot be developed by typical culture techniques. The main impetus for getting more data on viruses in an aquatic environment is generally a requirement for expanded knowledge adding to better general wellbeing. More knowledge of the behaviour of these viruses in water will encourage the provision of proper guidance to the general population and the authorities on utilization and treatment of water.

The process of analysing water for the isolation of enteric viruses is ordinarily a two-step process majorly due to the fact that the viruses are a few in numbers for a direct identification. Therefore the samples are normally concentrated in smaller volumes, generally under 10 ml to enhance detection. There are different types of water matrices in aquatic environments which are normally concentrated by different level of microbial and physical matter. Water is said to be of good quality if it contains no pathogenic microorganisms though may contain some physical impurities (Mans *et al.,* 2013). The different types of water matrices if polluted with fecal matter are likely to get contaminated with microorganisms such as human enteric viruses. The sewage effluent after treatment may contain varying concentrations of viruses which are normally being determined by a number of factors. These factors may include the type viruses circulating in that matrix at any given time, the season and the efficiency of the treatment techniques

during the sewage treatment. This means that discharge of sewage effluent into fresh water bodies such as Lake Victoria may lead to the viral contamination of the water enteric viruses.

Numerous sicknesses like gastroenteritis, keratoconjunctivitis, respiratory diseases and hepatitis are related with enteric viruses. These viruses are normally found in natural specimens like lake water, groundwater, surface water, sewage, costal water, shellfish and even the tap water thereby causing major health conditions in both rural and urban pollutions. (Enriquez and Gerba, 1995) and (Laxmivadana *et al.,* 2013).

With documented research about the presences of enteric viruses in water, it is important to be vigilant and to routinely monitor for the emergence and presence of these types of viruses especially in areas with high percentage of immunocompromised individuals (Haramoto and Kitajima, 2017). Despite the fact that availability of safe drinking water is viewed as a human right, many families experience the ill effects of deficient water supply (Asami *et al.,* 2016). This study was therefore carried out to analyse water samples from the most commonly visited shores of the lake around Homa Bay town for contamination with adenoviruses as indicators and enteroviruses as important pathogens in this part of the country using molecular based-detection assays.

1.2 Statement of the problem

The contamination of Lake Victoria is an important general concern both regionally in relation to the public health effects (Twesigye *et al.,* 2007). This is as a result of the

increased cases of water related infections reported from the communities around the lake (de Man *et al.,* 2014). The lake is an enormous system supplied by waterways that begin from distant regions. It therefore has components of both on location and off-site contamination. For example, agro chemicals (non point sources of contamination), industrial agro based pollution, straight discharge of untreated sewage effluents to the lake and contact as a result of swimming and bathing are some of the sources of pollution. The lower water quality of the lake may be as a result of the impact of contamination due to poor waste disposal mechanisms. This is evident by current algal bloom related to eutrophication and decomposition of the water hyacinth (*Eichhornia crassipes*) which is associated with waterborne diseases (Muyodi *et al*., 2009).

Poor waste transfer including fecal waste may lead to higher probability of the lake being subjected to microbial contamination. This is likely to be scenario in Homa Bay region being one of the regions within low socio-economic zones in the Country. Many kinds of microbial agents may form part of the contamination such as: parasites, protozoa and enteric infections which are the fundamental etiology of water related illness in the Lake Victoria area (Ouma, 2010). The latest WHO figures for diarrhoea related deaths per year worldwide for children under 5 years is estimated to be one million which is about 17 % of total deaths with majority in Sub-Saharan Africa (World Health Report 2012). In Western Kenya, drinking of the lake water and sharing of pit latrines by several households have been identified as some of the reasons that increase the risk of diarrhoea (Onyuka *et al.,* 2011). There are numerous pit latrines along the lake which are shared by several households and the public. The presence of dilapidated sewage treatment plant

within the vicinity of the lake may be a risk factor for viral contamination through fecal pollution. The untreated sewage from the conventional County Government wastewater treatment plant in the town discharged directly into the lake.

Inadequate sanitary facilities such low pit latrine coverage for proper disposal of fecal matter and absence of proper enlightenment to the surrounding community may encourage the utilization of bush as an option for fecal disposal as reported by UN-HABITAT (2010). This is a very unhygienic practice that is likely to increase chances of enteric viruses being washed down the lake thereby exposing the surrounding community to the risk of viral contamination. Garbage, fish cleaning waste, liquid and solid waste from eating areas, markets and households end up in the lake (Odada *et al.*, 2004).

1.3 Justification of the Study

There is a critical need to comprehend viral water quality and the characterization of pathogens in the environmental waters in the region including Homa Bay. Understanding of the viral water quality is expected to provide an effective system for accomplishing the new Sustainable Development Goals coordinated toward advance in sanitation and drinking water safety.

Research has not been reported in Kenya particularly on the presence or absence of viruses in Lake Victoria. Byamukama *et al.* (2005) carried out research on various forms of contamination and pollution on LV waters along Kenya and Uganda, which was

focused on the fecal coliforrm and not the viral contamination. Limited work has been carried out on genetic characterization and evaluation on the persistence of the enteric viruses in water and their infectivity in the natural hosts (Kiulia *et al.,* 2010). Studies have attempted to identify reliable indicators like existing bacteria in an attempt to demonstrate the likelihood of a viral contamination in the water, yet these remain unreliable (Haramoto *et al.,* 2018). It has also not been easy to get a reasonable literature on the issue of viral infections since "outbreaks" of waterborne infections can be sporadic and are not always reported. Enteric viruses have been shown to be resistant to environmental conditions and can spread over a longer distance than bacteria. It is therefore not appropriate to use bacterial indicators for viral contamination as viruses are frequently found with no bacterial pointer for fecal contamination.

No in-depth viral water quality-monitoring system has been put in place; subsequently data on the lake's viral water quality is pegged on limited data reported by researchers mostly on occasional information. Enteroviruses and adenoviruses were chosen among the other groups of enteric viruses for this study on the basis of a number of reasons. For example, enteroviruses have been utilized as a parameter for assessing environmental viral contamination, in that majority of them can be isolated and evaluated as PFU in cell culture (Caro *et al.,* 2001). Because of its predominance as a component of human vaccine, poliovirus which is one of the most common serotypes of enteroviruses has been suggested by many researchers for viral contamination monitoring though it is not always detected in wastewater. Adenoviruses on the other hand are the only human enteric viruses that contain DNA (Puig *et al.,* 1994). As for the choice of the study site, the main

motivation for its selection is that it is the most commonly visited shoreline along the lake in Homa bay town with intense economic activities.

1.4 Hypotheses

i. The physical water quality parameters of Lake Victoria waters along Homa Bay town significantly influence the occurrence of enteric viruses and are within the WHO acceptable levels for domestic use.

ii. Lake Victoria waters along Homa Bay town are contaminated with pathogenic human adenoviruses and enteroviruses.

iii. Wet and dry seasons influence the occurrence of human pathogenic adenoviruses and enteroviruses in Lake Victoria waters along Homa Bay town.

iv. Proximity of the dilapidated sewage treatment plant and pit latrines to the shore of Lake Victoria along Homa Bay town has an influence on the viral contamination of the lake waters.

1.5 Objectives

1.5.1 General objective

To verify the presence and seasonal distribution of pathogenic human adenoviruses and enteroviruses in Lake Victoria waters along Homa Bay town, and to assess if there is an association between point sources of human fecal pollution and the virus presence or absence.

1.5.2 Specific objectives

i. To determine whether the physical characteristics of water in Lake Victoria along Homa Bay town influence the occurrence of the enteric viruses and whether they are within the WHO acceptable levels for domestic use.

ii. To determine the occurrence of human adenoviruses and enteroviruses in Lake Victoria waters along Homa-Bay town.

iii. To determine whether wet and dry seasons have an influence on the occurrence of pathogenic human adenoviruses and enteroviruses in Lake Victoria waters along Homa Bay town.

iv. To assess if proximity of the defunct sewage treatment plant and pit latrines to the shore of Lake Victoria along Homa Bay town have an influence on the viral contamination of its waters.

1.6 Significance of the study

Findings from this study present an interesting source of understanding on the contamination of the lake water with enteric viruses, the role played by those viruses as human and animal pathogens and the risks inherent from waterborne exposure. It will be useful in guiding decisions concerning the use of different enteric viruses in lake water microbial quality evaluation. The findings will be helpful in assessing the health risks and measurement of the sources of contamination in a watershed because of their host specificity such as human pathogenic viruses. The results will be useful in understanding the relationship between changes in seasons and the viral contamination. This will in turn be helpful in documenting the sources and patterns of contamination of surface waters

with enteric viruses and consequently suggest viable recommendations on public waste management to the relevant authorities.

Data regarding the presence of enteric viruses in lakes will be helpful in surveying the potential danger of disease through waterborne transmission. Assessment of the viruses found in the samples collected from around the dysfunctional sewage treatment plant may be useful as a pointer to the kind of microbial contamination in the community (Katayama *et al.*, 2008). The local community will be enlightened and empowered to understand and participate in the improvement of sanitary conditions in their surroundings. Proper understanding and management of contamination source will help to protect the ecosystem of the lake.

CHAPTER TWO

LITERATURE REVIEW

2.1 Enteric viruses

Enteric viruses are a group of viruses that may be present in the gastrointestinal tract and are responsible for certain diseases and infections (Sibanda *et al.,* 2013). There are many kinds of these viruses that inhabit the human intestine; replicating in the epithelial cells. Some of such viruses cause local infection of the gastroenteritis and may result in other related diseases. Since the enteric viruses are discharged with the human waste they are normally present in the sewage although being obligate intracellular viruses they cannot multiply in the environment. Some of the common enteric viruses include picornaviruses, rotaviruses and noroviruses, which are non-enveloped RNA viruses, and two groups with double stranded DNA viruses that is adenoviruses and polyomaviruses (Donaldson *et al.,* 2002).

Most enteroviruses form a distinct taxonomic group (including poliovirus, echovirus and coxsackievirus) and can thrive in readily available cell cultures of primate origin. Their viability in cell culture makes this group of viruses to be well characterized and distinguishable from other gastro enteric viruses. The other groups of gastro enteric virus are the heterogeneous virus which includes the astroviruses, rotaviruses, adenovirus 40/41, human caliciviruses and hepatitis A and E. These cannot be grown easily, if at all, in a culture. Their uniqueness lies in their ability to cause distinct gastrointestinal disease or hepatitis. Enteric viruses are normally introduced to aquatic environments through human related activities such as leaking sewage and septic systems, urban runoff, agricultural runoff, and in the case of estuarine and marine waters vessel wastewater

discharge, but they may however also be present naturally (Calgua *et al.,* 2008). As Griffin *et al.* (2003) posits, human and animal wastes harbor over 100 types of pathogenic viruses that are transported in the environment via groundwater, estuarine water, seawater, aerosols from liquid waste management plants, drinking water as well as private wells through groundwater pollution (Boehm *et al.,* 2009).

The main mode of transmission of these enteric viruses is the fecal-oral route; primarily infecting and replicating in the host's gastrointestinal tract. When discharged from the host's body through faeces, the viral load ranges between 105 and 1011 virus particles per gram of stool (Griffin *et al.,* 2003). Most of the enteric viruses belong to the families *Picornaviridae* (enteroviruses, polioviruses, echoviruses, coxsakieviruses and hepatitis A virus), *Caliciviridae* (noroviruses, astroviruses, caliciviruses and small round-structured viruses), Adenoviridae (adenoviruses) and Reoviridae (reoviruses and rotaviruses) (Maunula *et al.,* 2012). Enteric viruses that are pathogenic and waterborne include circoviruses (torque tenovirus and torque tenovirus-like virus).

Tenoviruses are non-enveloped viruses that are single-stranded circular DNA; making them resistant to heat inactivation. This classification is usually based on their molecular structures that make them resistant to any water treatment processes. Other enteric viruses include picobirnaviridae, parvoviruses (the smallest known enteric viruses, with single stranded RNA and highly heat resistant) (Dierssen *et al.,* 2008). *Picobirnaviridae* are small nonenveloped viruses with bisegmented double strands of RNA that make them extremely resistant to UV light (Dierssen *et al.,* 2008). As described by Haramoto *et al.*

(2010), polyomaviruses include JC virus, BK virus and simian virus 40; which are usually non-enveloped double-stranded DNA viruses are very stable to heat but less resistant to chlorination.

Most adenovirus serotypes as reported by Jiang *et al.* (2001), are difficult to culture in regular cell lines. This explains their presence in polluted water as well as their role in gastroenteritis which have been underestimated for a long time (Hemming *et al.*, 2013). Kishida *et al.* (2014) refer to subgenus F of adenovirus (serotypes 40 and 41) as "fastidious" due to the difficulty in isolation, and its role in causing infantile gastroenteritis. Main modes of transmission of enteric viruses are; food, water, fomites and cross contamination. Enteric viruses are also of public health concern due to their low infectious dose as explained by Fong *et al.* (2010). A study by Colford *et al*. (2007) found that the probability of infection from exposure to one rotavirus is 31 % and no more than 1 PFU is required to cause infection in 1 % of healthy adults with no antibody to the virus. According to Boehm *et al.* (2007) risk of infection from consumption of viruses in drinking water is 10 to 10,000 times higher than pathogenic bacteria at similar exposures (Verhougstraete *et al.*, 2010). This therefore makes enteric viruses in water a particular concern.

Enteric viruses according to Wade *et al.* (2008) are the main causative agents of most nonbacterial gastroenteritis outbreaks. A report by Girones *et al.* (2010) indicates that only a fraction of waterborne disease incidences caused by enteric viruses are ever reported. Enteric viruses have the ability to survive for longer periods in the fresh water

environment consequently increasing the probability of human exposure (Lodder *et al.*, 2010). World over, most public health stakeholders rely mainly on bacterial indicators such as fecal coliform and total coliform bacteria and enterococci to assess water quality (Zachos *et al.,* 2016). However, bacterial load is not the best indicator for the risk from many medically important pathogens, such as viruses, stressed pathogenic bacteria (viable but non-culturable) and protozoa as posited by Wade *et al.* (2010). One of the major drawbacks in using fecal coliform bacteria and other traditional indicators such as enterococci in assessing water quality is their availability in both human, animal feces and in natural soils. In addition, fecal coliforms have the ability to regrow in soil after excretion from their host (Verhougstraete *et al.,* 2010). Viral pathogens according to Kiulia *et al.,* (2010) have been suggested as an effective parameter for examining the sources of fecal contaminants in aquatic environments because of their host specificity. They may also be used alongside bacterial indicators to assess water quality and for disease surveillance (Gibson and Schwab, 2011). Pathogenic viruses are more resistant to chlorination than bacteria and are also resistant to lipid solvents and UV inactivation (Li *et al.,* 2009). Sinclair *et al.* (2009) found that enteric viruses can also survive under a wide pH range (pH 3 to 10) for extended periods at low temperatures for up to 130 days in seawater; 120 days in freshwater and; for 100 days in soil at a temperature of about 25 °C (Rodriguez *et al.,* 2009).

Enteric viruses also have an obligate host requirement as there is no potential for regrowth in the environment. Therefore, enteric viruses show great potential of water quality indicators to assess the risks related to infectious virus transmission and in

identification of the dominant source of fecal contamination in waters. Enteric viruses' contaminations cause major waterborne diseases to both man and animals although this impact is often underestimated. Enteric viruses are usually stable in the environment and may survive wastewater treatment as explained by Baggi and Peduzzi (2000) and Carter, (2005). Although the prevalence of certain enteric viruses such as HAstV and HAdV in diarrhoeal stool specimens has been documented in Kenya (Kiulia *et al.,* 2007; Magwalivha *et al.,* 2010) the contribution of waterborne transmission by enteric viruses in Kenya has not been investigated.

2.1.1 Enteric viruses in water

Quite a big number of different viruses excreted by human have been found in sewage and polluted waters (Hot *et al.,* 2003). Over one billion people worldwide lack access to clean water supplies and a further two billion plus lack adequate sanitation (WHO, 2012). It has been estimated that close to two million deaths per year are related to water contamination (Jiang *et al.,* 2005). Due to shortcomings in the epidemiological studies, the impact of waterborne diseases is difficult to assess and the health impact underestimated (Kajon *et al.,* 2007). Viruses transmitted by waterborne route are usually enteric viruses such as viruses which primarily infect the gastrointestinal tract and are shed in large numbers in the faeces of the infected individuals (Miagostovich 2009). Enteric AdVs for example have been shown to be transmitted by recreational waters (He and Jiang, 2005). There is therefore the potential of viruses that may not primarily replicate in the gastrointestinal tract to be transmitted by water (Jiang *et al.,* 2009).

Because enteric viruses are often found in fecal polluted water, Wong *et al.* (2009), suggests that AdVs be used as molecular index for test of human enteric viruses where infectivity need not to be established. Fecal bacteria are not effective indicators of viral load in recreational waters (Miagostovich *et al.,* 2008) due to their lack of correlation with viral perseverance in the environment (Boehm *et al.,* 2009). Fecal bacteria's ability to grow in sediments (Byappanahalli and Fujioka 1998), sand (Whitman *et al.,* 2003), algae (Verhougstraete *et al.,* 2010) and water (*He et al.,* 2005) also explain their ineffectualness in use as indicators for viral contamination (Verhougstraete *et al.,* 2010). To mitigate these challenges, molecular tools are now being used to identify and estimate human enteric viral pathogens in various environmental waters. Kiulia *et al.* (2010) studied the human enteric viral load at Mbooni, Mbagathi and Mtoine rivers in Kenya. The research established that enteric viruses are diverse and may be useful fecal indicators for open waters. The study also established that the occurrence of viruses in a river system was influenced directly by sewage and CSOs.

2.1.2 Epidemiology of enteric viruses in water

Enteric viral load is normally high in raw water sources and inadequately treated water supplies (Hamza *et al.,* 2009). The enteric viruses are ubiquitous in an environment with huge contamination with human feces and sewage (Wade *et al.,* 2010). Their occurrence in treated drinking water and river water has been described previously in a number of studies (da Silva *et al.,* 2007) and their potential risk widely recognised (Ngaosuwankul *et al.,* 2013). Risk based analyses indicate enteric viruses levels between 0.01- 0.001 in potable water could lead to an illness rate between 8.3/ 100 - 8.3/1000 (Haramoto *et al.,*

2009). In developing countries drinking water is one of the main sources of pathogenic contamination and enteric viruses have been detected even in treated drinking water (Fong *et al.,* 2007). Detection of these viruses in drinking water sources is not limited to the developing world only as similar studies have detected this in developed parts of the world such as in USA (Fong *et al.,* 2007). They have been detected in recreational beaches and have been responsible for a number of swimming pool - related out brakes (Hatherill *et al.,* 2004). In most cases, HAdVs are considered the most important enteric viruses implicated in environmental contamination (Avello *et al.,* 2001) but there is very little information regarding this in Kenya especially around the Lake Victoria region.

2.1.3 Enteric viruses as pathogens and the public health implications

The viruses have been linked to waterborne outbreaks worldwide with their infection patterns being influenced by the virus serotype, population group and type of exposure (Van Heerden *et al.,* 2004). Clinical manifestations of these viruses' infections in humans are usually determined by their viral type specificity and confection with different strains (Guimara˜es *et al.,* 2008). Some known serotypes of these viruses have been isolated in immunocompromised individuals while other serotypes have been identified to cause illnesses to general human population (Rock *et al.,* 2010). They have been found to cause quite a number of diseases in both humans and animals. These diseases are mainly caused by enteroviruses and adenoviruses (Allard *et al.,* 1992). Enteric viruses such have been reported as being the causative agents associated with sporadic and endemic diseases that result in not only death but also high economic losses (Meqdam *et al.,* 2007). Infections by HAdVs for example have been reported to occur throughout the year with outbreaks

majorly being reported in late winter, spring up to early summer with respiratory infections outbreak always being both epidemic and endemic (Fong *et al.,* 2007).

One of the common causes of diarrhoea in infants is enteric viruses (Basu *et al.,* 2003) which are normally brought about by poor water quality especially in developing countries. The water quality situation in the developing world has become a major cause of numerous public health problems contributing to high mortality rates majorly in immunocompromised persons, children and highly susceptible individuals (Fong *et al.*, 2007). Enteric virus infections besides the diarrhoea in infants are closely linked to quite a number of other infections and diseases such as self-limiting gastroenteritis, conjunctivitis, respiratory infections, meningitis, hepatitis, encephalitis and paralysis (Fong *et al.,* 2010), as well as chronic diseases such as myocarditis and insulin dependent diabetes (Girones *et al.,* 2010).

Evidence from reliable sources reveals that these waterborne viruses are much more prevalent in our environment although limited attention has been accorded to them as far as precaution is concerned (Muscillo *et al.,* 2008). Viruses like noroviruses, hepatitis A and E, enteroviruses, rotaviruses, adenoviruses, and astroviruses, as well as avian influenza (or H5N1 - the “bird flu”) have not only been detected from surface water sources such as rivers and lakes from the developing countries, but also from developed countries around the world (Shah *et al.,* 2017). One of the possible reasons for the presence of these viruses could be as a result of contamination of water ways by raw sewage. Homa Bay town, for example, has a large amount of untreated sewage

discharged every day to the lake. Over 20 other small towns and cities around Lake Victoria equally dump thousands of litres of raw sewage through by-passes and sewage overflows. Most sewage treatment instances are likely to be only for minimal treatment in many towns around the lake and no treatment at all as evident by the Homa Bay case. Cases of poor water treatment leading to diseases outbreaks by waterborne viruses have been reported worldwide (Bofill-Mas *et al.,* 2006). World Health Organization (WHO) in 2012 documented many cases of outbreaks, particularly in the third-world countries (Bofill-Mas *et al.,* 2000).

2.2 Enteroviruses

These are very small single stranded RNA viruses and one of the genera in the family Picornaviridae. Species beforehand grouped in the genus Rhinovirus have as of late been moved into the genera Picornaviridae (da Silva *et al.,* 2007). Enteroviruses have an icosahedral capsid ranging from 20 to 30 nm in diameter with a protein coat. Green and Lewis (1995) found that about 70 % (77 serotypes) and 30 % of non-poliovirus enteroviruses are associated with human and animal infections respectively. Due to the increased, consistent and effective use of vaccines, Poliovirus has been contained in the developed world (Fong *et al.,* 2010). This however is not the case in the developing world as it is still being reported in various parts of the world such as in Africa (Anis *et al.,* 2013). Non-polio enteroviruses however still cause various diseases in the developed world. They are among the most common causative agents of varied types of diseases in the developed countries besides the rhinoviruses which cause the common cold. Interestingly, rhinoviruses and enteroviruses share a lot of similarities. Enteroviruses

infections have been reported mostly in children unlike rhinoviruses which is common in adults as well. According to Boehm *et al.* (2009), enteroviruses cause about 10-15 million symptomatic infections a year worldwide.

2.2.1 Classification of enteroviruses

The genus enteroviruses belong to the family of Picornaviridae consisting of over 100 serotypes (Wylie *et al.,* 2015). There are 8 groups of species that have been identified (Table 2.1).

Table 2.1: List of enteroviruses serotypes

Species	Number of Serotypes
Poliovirus	3
coxsackievirus A	1
coxsackievirus B	5
Human enterovirus C	11
Human enterovirus A	12
Human enterovirus B	36
Human enterovirus D	2
Echoviruses	13
Bovine enterovirus	2
Porcine enterovirus A	1
Porcine enterovirus B	2

There are at least 61 human pathogenic non-polio enteroviruses. They include 23 Coxsackie A viruses, 6 Coxsackie B viruses, 28 echoviruses and 4 other enteroviruses.

2.2.2 Morphology of enteroviruses

A typical enterovirus virion is about 30nm in diameter consisting of a single-stranded RNA genome. It is non-enveloped and is isometric (icosahedral) in shape. It is made up of 60 units (protomers) each consisting of one molecule of each of the three surface coat

proteins and a fourth capsid protein. Shinozaki *et al.* (1991) found out that these virions have more surface features than most other picornaviruses, and a raised area at the fivefold axis engulfed by a groove into which the host cellular receptor binds. The virions of these viruses have a sedimentation coefficient of 160S in sucrose with buoyant density of about 1. 34 g/cm3 in $CsCl_2$. The four structural proteins are a salient feature that is common to all enteroviruses (Hamza *et al.,* 2009).

2.2.3 Genomic properties of enteroviruses

Enterovirus genom has a monopartite positive sense single-stranded RNA of about 7.3-7.4 kb in length. At the 5'-terminus there is a genome-linked protein (VPg) and a 3'-polyA tail (Shen *et al.,* 2008). A single polyprotein which is subsequently processed into the functional products is encoded by the RNA which contains 7,391 nucleotides.

2.2.4 Enterovirus stability

Various germicides, such as phenol, cresol, formaldehyde and sublimate can kill the enterovirus under conditions in which other enteroviruses are partially or not inactivated at all. However, the EVs are resistant to common preservatives such as chlorine (Tani *et al.,* 1995), which is the chemical of choice commonly used for water treatment. Studies have indicated that enteroviruses are however more sensitive to methanol and Ultra violet light (UV) as compared to adenovirus (Tani *et al.*, 1995).

2.2.5 Enteroviruses as human pathogens

EVs cause a wide range of manifestations, including asymptomatic infection and serious disease and fatality (Bergelson *et al.,* 1997). The presence of enteroviruses in the environment is a public health concern even at small levels (Arola *et al.,* 1996). Although most enteroviruses are transmitted by the fecal-oral route, their clinical outcomes extend beyond gastroenteritis. Some of them have the ability to travel from the intestinal tract to other organs of the body. These viruses are of major public health importance and have been associated with clinical infections such as gastroenteritis, upper respiratory diseases (UPD) and conjunctivitis in humans (Crabtree *et al.,* 1997). Besides the respiratory system infections, Coxsackieviruses for example have been associated with insulin-dependent diabetes and cardiac diseases, such as myocarditis and pericarditis (Gerba, 1990). Echoviruses are normally associated with common cold and respiratory diseases though they are less infectious compared to other enteroviruses. From patients with bronchitis, conjunctivitis, meningitis and paralysis resembling poliomyelitis the numbered enteroviruses (enterovirus types 68 to 71) have been isolated although these viruses have not been studied extensively (Lipp *et al.,* 2001).

Enteroviruses cause a number of symptoms in infected persons most of which are not severe, disappearing without treatment within a short period albeit with severity in younger persons (Lambertini *et al.,* 2008). Common symptoms in infected infants may include sepsis (bacterial infection of the blood) with high fever and lethargy, cold, headache, sore throat and cough. New-borns may though rarely develop infection of other organs such as liver and heart (Kiulia *et al.,* 2014). Other symptoms of enterovirus

infection include fever, muscle aches and rashes that are characterized by small, flat red dots on the skin of the chest and back with individual small sized lesions, vomiting and diarrhoea (Kollaritsch *et al.,* 2015). The vomiting and diarrhoea are occasionally associated with abdominal pain as well as ulcers in the mouth (Karafillakis *et al.,* 2015). One or all of these symptoms may manifest in a child with an enterovirus infection (Lee *et al.,* 2002).

Coxsackievirus A is normally associated with hand, foot and mouth disease which is normally common in children though can also be caused by enterovirus 71 (Xagoraraki *et al.,* 2007). This is a minor condition that quickly disappears by itself just like most other enteroviruses infections. Another rare condition for patients infected by enteroviruses is the aseptic or viral meningitis which again is normally short lived. There is very minimal or no symptoms at all in most adults infected with an enteroviruses (Dierssen *et al.,* 2008). The viruses have not only been isolated from the stool of an infected person but also from the respiratory secretions such as saliva, sputum, and nasal mucus. Bernhard *et al*. (2003) observed that enteroviruses cause about four infections per child during early years on average. However the children get immuned against most of these enteroviruses in most cases upon the disappearance of the illness. Enterovirus infections are more likely to occur along Lake Victoria in Kenya since these areas are prone to water contamination with human waste. Poliovirus has been virtually eliminated from the developed countries as a result of proper use of vaccines (Fong *et al.,* 2010). Non-polio enteroviruses however are still present as no vaccine has been developed at the moment.

2.2.6 Enteroviruses as animal pathogens

Some animal diseases resulting into diarrhoea, reproductive failure and neurological disorders have been linked to animal specific enteroviruses (Benhard *et al.,* 2003).Three animal specific enteroviruses have been identified namely: Bovine enteroviruses (BEV) which has two serotypes, Porcine enteroviruses (PEV) consisting of three serotypes, Porcine teschoviruses (PTV) consisting of eleven serotypes 10 of which were formerly classified as porcine enteroviruses and one Ovine enterovirus. Bovine enteroviruses have been identified in cattle where they have been associated with diarrhoea and abortions though they are usually non-pathogenic (Haramoto *et al.,* 2004).

2.3 Adenoviruses

These are double stranded DNA viruses belonging to the family *adenoviridae*. Adenovirus is possible candidate as an index virus as they are Ubiquitous and have been isolated in nearly every class of vertebrates (Shimizua *et al.,* 2007) and are species specific (Logan *et al.,* 2006). They were named after adenoid, the lymphoid tissue from which they were first isolated (Shanks *et al.,* 2010). AdVs have been recognised and associated with quite a number of clinical symptoms. They are associated with both sporadic and endemic diseases that may result in significant economic losses and death especially among populations in closed settings (Colford *et al.,* 2012.) such as the Homa-Bay case. Adenovirus has been reported in most environmental waters such as polluted rivers, lakes, coastal and other inland waters (Griffin *et al.,* 2003).

2.3.1 Classification of adenoviruses

Adenovirus belongs to the *Adenoviridae* family of viruses which consists of five genera. The five genera are: Genus Atadenovirus whose species type is Ovine adenovirus D, Genus Aviadenovirus whose species type is Fowl adenovirus A, Genus Ichtadenovirus whose species type is Sturgeon adenovirus A and Genus Mastadenovirus which includes all human adenoviruses. *Masterdenoviridae* species type includes Human adenovirus C. The other genus is Siadenovirus whose species type is Frog adenovirus. Serologically distinct viruses are defined as serotypes and these are grouped into species. Species are defined depending on a number of characteristics including calculated phylogenetic distance, DNA hybridization, restriction fragment length polymorphism analysis possibility of recombination, host range and degree of DNA homology (Fong *et al.,* 2010). Over 50 human adenovirus serotypes classified in six subgroups have been identified (Table 2.1) (Dong *et al.,* 2009).

2.3.2 Morphology of adenovirus

According to Brashear *et al.* (1982) a typical adenovirus virion has no envelop with a diameter ranging between 90-100 nm (Figure 2.1). They display a characteristic icoshedral capsid structure and are composed of 252 capsomeres: 240 sub units of the trimeric hexon protein (hexons) which form the facets and 12 peptons comprising the pentameric peptone base protein and the externally projecting trimeric fibre, forming the vertices (Brashear *et al.,* 1982).

2.3.3 Genomic properties of adenovirus

Adenovirus genome is a double-stranded (ds) DNA ranging about 26 to 45 kbp in length. Structurally it is linear and non-segmented and can carry about 22 to 40 genes. The genome has a terminal 55 kDa protein which is linked to the dsDNA 5' ends where they are used as primers during the replication (Chaberny *et al.*, 2003).

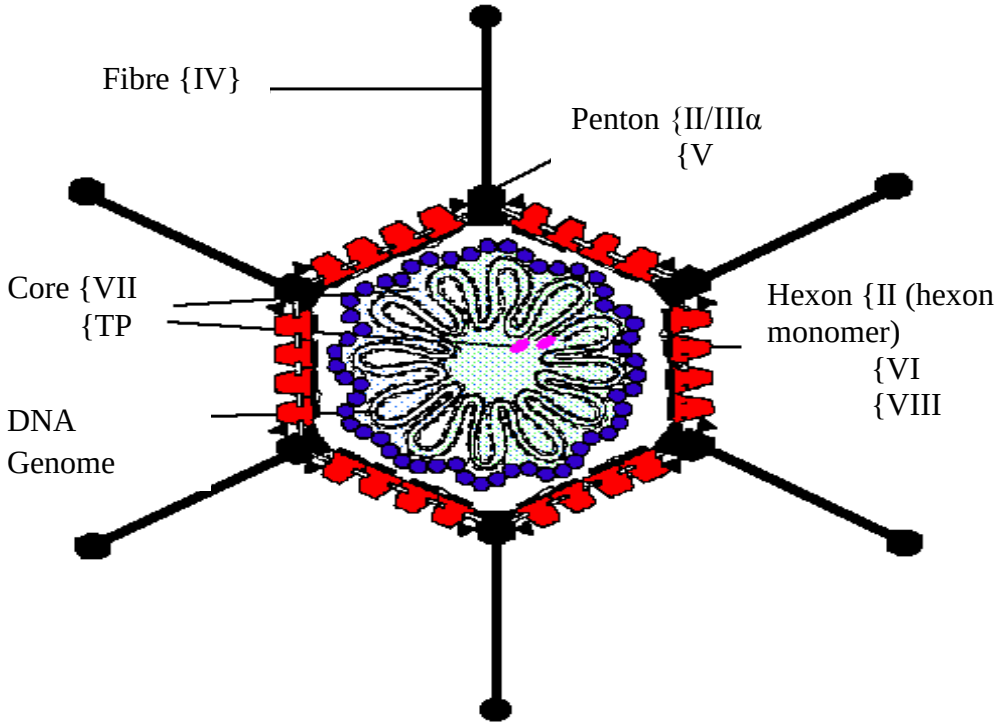

Figure 2.1: A section of mastadenovirus (Brashear *et al.*, 1982).

2.3.4 Adenovirus stability

In comparison to RNA viruses adenoviruses being double stranded can withstand UV radiation more. The double stranded feature is important for the virus stability as it through the use of the host cell's enzymes, helps in repairing damaged DNA (Kasza, 1966). They have been reported to be stable at -70 ^{o}C as well as being insensitive to lipid

solvents (McQuaig *et al.,* 2009). They are deactivated by heat, but their sensitivity to heat varies according to the genera. Mastadenovirus for example are inactivated by heating at 56 ^{o}C for 10 min (Singh, 2007). Substances that have been found to deactivate the virus are formaldehyde or bleach (sodium hypochlorite) (Dong *et al.,* 2009).

2.3.5 Adenoviruses as human pathogens

Adenoviruses are one of the essential causes for wide range of symptoms such as diarrhoea, vomiting, abdominal pain and febrile related ailments in children. These symptoms normally last for a short period of about 10 days before disappearing though the infections can be fatal in neonates and immune-compromised individuals. They cause symptomatic infections in several organ systems. Among the organs and organ systems affected are the respiratory system (pharyngitis, acute respiratory disease and pneumonia), eye (conjunctivitis), gastrointestinal tract (gastroenteritis), central nervous system (meningoencephalitis) and genitalia (urethritis and cervicitis) (da Silva *et al.,* 2007). Gastroenteritis in children for example has been linked to HAdVs type 40 and 41.

Adenoviruses prevalence is high in environments where contamination of water sources with human faeces or sewage can occur (Jalal *et al.,* 2005). Human adenoviruses (HAdVs) are transmitted through fecal-oral route and thus are likely to pose serious threat health wise to human beings through water consumption (Chmielewicz *et al.,* 2007). They can also be transmitted through inhalation of aerosols and contact with contaminated fomites (Lodder and Husman, 2005). Both enteric and non-enteric adenoviruses can be discharged in faeces and have both been isolated from aquatic

environments. They may be naturally present in such environments. Non- enteric adenoviruses such as adenovirus type 5 have been reported to account for about 11 % of clinical cases to WHO. Adenovirus infections occur throughout the year worldwide majorly affecting the children (Bitler *et al.,* 2013). Adenovirus types 40 and 41 have been associated with 5 % - 10 % of acute diarrhoeal cases in young children. The diarrhoeal illness can be prolonged in case of infants (Cunliffe *et al.,* 2010). Like enterovirus infections, adenovirus infections are not only less serious but also self-limiting within a short period. The infections rarely lead to death and treatment is therefore just supportive (Brown *et al.,* 1996), however in cases of severe illness and in immunocompromised individuals, antiviral therapy with cidofovir may be recommended (Lambertini *et al.,* 2008). Asymptomatic infections are also common, particularly in children owing to the fact that these viruses are likely to be discharged in faeces at a later time following primary infection (Ogorzaly *et al.,* 2010). Prevention of transmission may be achieved through control from primary sources of infections (Carter, 2005) like open domestic water sources along the shores of the lake.

2.3.6 Adenoviruses as animal pathogens

There is a wide range of species of animals that can be infected by animal-specific adenovirus. The species include birds which are infected by the genus Aviadenovirus, reptiles, amphibians and fish. Porcine adenoviruses (PAdV) have been associated with encephalitis and pneumoenteritis in pigs. Some bovine adenoviruses (BAdV) have also been isolated from cattle albeit with mild or no clinical symptoms. In calves however serious clinical symptoms such as keratoconjunctivitis, acute febrile condition and

pneumonenteritis have been reported and linked to some serotypes of BAdV (Cook, 1974).

2.4 Effects of environmental factors on enteric viruses survival

Certain aquatic environmental parameters such as dissolved oxygen, water temperature, turbidity and nutrient concentration among other factors may contribute significantly on the occurrence and the survival of viruses. These factors have been extensively studied and are important considerations when accessing possibility of viral contamination in environmental waters. Studies have shown that viruses may survive relatively longer in lower temperature conditions for example, though significant differences were not observed in their survival between seasons in natural environmental waters (Wait and Sobsey, 2001).

2.5 Other enteric viruses in water

2.5.1 Norwalk-like viruses (NLV)

Norwalk-like virus is one of the groups of enteric viruses from the genus Human calcivirus from the family *calciviridae*, a non-enveloped, single-stranded, positive sense RNA virus with a single capsid protein. Their morphology is not well defined, characterised by a tattered particle form (Fields *et al.,* 1996). The NLVs were formerly denoted as SRSVs in the UK (Lambden *et al.,* 1993; Jiang et al., 2001) and it has two geno groups (Kaplan *et al.,* 1982). Norwalk-like viruses have been associated with gastroenteritis among adults. Through the use of immune electron microscopy about six antigenic types have been identified (Lewis *et al.,* 1995). The NLVs like other

enteroviruses are shed in large numbers in faeces and therefore end up getting transported in sewage systems to open water bodies such as lakes and rivers. The virus is persistent in the in the environment and this makes it one of the agents that draws a lot of public health concerns (Lees *et al.,* 1995). There is plenty of literature concerning sewage contamination of drinking water sources leading to outbreak of NLV (Lawson *et al.,* 1991; Teunis *et al.,* 2008). Recreational contact related infections and outbreaks have been however less reported and this relationship has always been pegged on conjectural or epidemiological circumstances (Gray *et al.,* 1997).

The other group of human calciviruses is Sapporo-like viruses (SLV) which were formerly referred to as 'classic calciviruses'. Like the NLVs, SLVs have been divided into 2 geno groups (Berke *et al.,* 1997; Hale *et al.,* 1999; VinjeÂ *et al.,* 2000). Unlike NLVs their Morphology is well defined with less amorphous surface outline. They are shed in faeces though their potential for waterborne related infections remains uncertain since they majorly infect only the young children as adults are immuned. They have also been associated with gastroenteritis though this has majorly been reported in children who are less than one year old (VinjeÂ *et al.,* 2000).

2.5.2 Astroviruses

Astroviruses (AV) is a positive sense single stranded RNA virus belonging to the family *Astroviridae.* The virion is 28 nm in diameter with a stellate surface structure which are about five to six in number. This virus has been associated with diarrhoea in but not limited to children (Willcocks *et al.,* 1992). These viruses are not easily cultured in

ordinary cell cultures though this has been successful when cultures are generated from intestinal tumours (Willcocks *et al.,* 1990; Wyn-Jones and Herring, 1991). Little has been reported about the occurrence of these viruses in aquatic environments. A few such reports are as described by Marx *et al.* (1995) and PintoÂ *et al.* (1996) in which the isolation was done from an area where there was a concurrent existence of gastroenteritis. Astrovirues just like the rotavirus and adenovirus are always persistent in different water matrices though they have been found to be slightly susceptible to higher temperatures (Abad *et al.,* 1997).

2.5.3 Rotavirus

Rotaviruses are double stranded RNA viruses from the family *Reoviridae* (Barardi *et al.,* 1999). The virion is 75nm in diameter with an icosahedral in structure with a triple capsid protein layer. The virion is about 75nm in diameter with a segmented RNA. The structure is icosahedral and enveloped in a triple capsid protein coat with a protrusion of 60 spikes (Mathew *et al.,* 2014). From an EM, an infectious rotavirus virus particle is characterised by a smooth surface that transforms to a rugged one upon the loss of the outer shell (Birch *et al.,* 1983). The rough particles which are non-infections are more environmentally persistent compared to the smooth complete infectious type and therefore are found in faeces in large numbers (Nakagomi *et al.,* 2013). About six serogroups (A-F) have been identified there are other basis of classification such as serotypes and genogroups which enhance monitoring and identification of the strains circulating in a given community (Linhares and Justino 2014). Rotavirus is one of cause

of gastroenteritis in infants. Little has been reported about waterborne transmission except on grounds of contamination of drinking water by sewage (Gerba *et al.,* 1996).

2 .5.4 Hepatitis A and E viruses

Hepatitis viruses are small non-enveloped single stranded RNA viruses with a symmetrical structure. Hepatitis A (HAV) belongs to the genus Hepatovirus of the family *picornaviridae.* One serotype has been recognised with human being as the only natural host. A lot has been reported as far as waterborne transmission of Hepatitis A (HAV) is concerned where they have been associated with water related diseases (Mackoviak *et al.,* 1976). However HAV has been fairly contained in developed countries where high levels of hygiene are observed (Maguire *et al.,* 1995), though a few cases still get reported. It is relatively persistent in the environment and has been reported to be heat resistant and has the ability to survive in low pH. Poor residential settings where hygiene is inadequate have provided good grounds for outbreaks of HAV in developing countries. Contaminated drinking water (Bloch *et al.,* 1990) and recreational waters (Mahoney *et al.,* 1992) have been associated with the outbreak. The HAV has been isolated from different environmental samples including water from the well (De Serres *et al.,* 1999), from (Jothikumar *et al.,* 1998), sewage sludge (Graff *et al.,* 1993) and in recreational waters. Hepatitis E virus (HEV) virus is about 30nm in diameter with icosahedral structure. It is classified under the family *Caliciviridae* as it shares a lot of features with a typical calcivirus. Waterborne transmission and outbreaks have been documented with infections similar to those of HAV (Jothikumar *et al*., 1993).

2.6 Concentration, recovery and detection of the viruses from water samples

There are two steps involved in isolation of viruses from water (Ahmed *et al.,* 2010). The first step involves application of efficient recovery and concentration procedures as viral particles in water are presumed to be low in number (Johnston *et al.,* 2010). These procedures are applied to reduce the volume of both much polluted water and viral concentrates from water samples with low pollution levels. There are quite a number of isolation and detection methods albeit with no standard procedures that can be applied depending on certain factors such as efficiency and the costs (Kilpatrick *et al.,* 2011). Other important factors to consider while selecting a suitable technique includes Virus recovery rate, ability to be completed within a short time, range of viruses concentrated, ability to process large volume of water and being easily repeated in the lab (De Paula *et al.*, 2007).

Adsorption-elution technique was the method of choice mainly because of its simplicity; availability of the instruments required and cost effectiveness (Ruggeri *et al.,* 2015). In this procedure, the sample containing the virus is brought into contact with an adsorbent such as electron - charged glass wool filter membrane. Under certain specific conditions of pH (normally 6-8) and ionic strength, the virus gets adsorbed to the glass wool filters. A buffer such beef extract buffer is used to elute the adsorbed virus at a higher pH (about 9.5) into smaller volumes so as to enhance the release of the adsorbed virus from the adsorbent.

The other three methods that have been applied in other studies are entrapment, ultracentrifugation and immuno-affinity columns and magnetic beads method (Verbyla and Mihelcic, 2015). Detection of enteric viruses from water environments has always used the methods from their clinical applications (Ahmed *et al.,* 2012). The nucleic acid is extracted from the recovered virus particles and then subjected to purification purposes of removing the PCR inhibitors. Once purified, the nucleic acid is stored safely under recommend temperatures. RNA must be handled with a lot of care as it is very prone to degradation compared to the DNA. Its purification must therefore be carefully executed.

Molecular biology technology has enhanced the process of identification of viruses in water. Use of PCR has transformed the field of water viral analysis by enhancing amplification of viruses which do not grow properly in ordinary cell cultures such as rotaviruses. The PCR technique addressed some of the problems that were associated with gene probe (Enriquez and Gerba, 1995) such as sensitivity, specificity, speed and efficiency (Arola *et al.,* 1996). PCR involves an enzymatic controlled amplification of a specific target DNA sequence in vitro.(Chigor and Okoh, 2012), (El-Senousy *et al.,* 2007). For RNA viruses, RT-PCR technique has been used for detection from environmental samples such as rivers, lakes and marine recreational waters (Kopecka *et al.,* 1993), ground waters (Abbaszadegan *et al.,* 1993), sludge (Straub *et al.,* 1995) and shellfish (Le Guyader *et al.,* 1994). Rotbart (1990) in details described the use of RT-PCR in detection of enteroviruses. It has also been described in the detection of adenoviruses (Puig *et al.,* 1994), HAV (Graff *et al.,* 1993), astrovirus (Marx *et al.,* 1995) and rotavirus (Cruz *et al.,* 1990). Other studies in which PCR has been extensively

discussed includes by Pallin *et al.* (1997) involving the comparison of the sensitivity of the PCR and the cell culture methods, multiplex RT-PCR for detection of enteroviruses from environmental samples and detection of polyomaviruses in sewage using RT-PCR (Bofll- Mas *et al.*, 2000).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

The study was carried out at the shores of Lake Victoria along Homa Bay town in Homa Bay County, Western part of Kenya (Figure 3.1). Homa Bay County has Homa Bay town as one of the administrative centre and lies between longitudes 34.30^{o}E and 34.20^{o}E and latitudes 0.30^{o}S and 0.35^{o}S and the altitude ranges between 3000 m and 3500 m above the sea level (Figure 3.1).

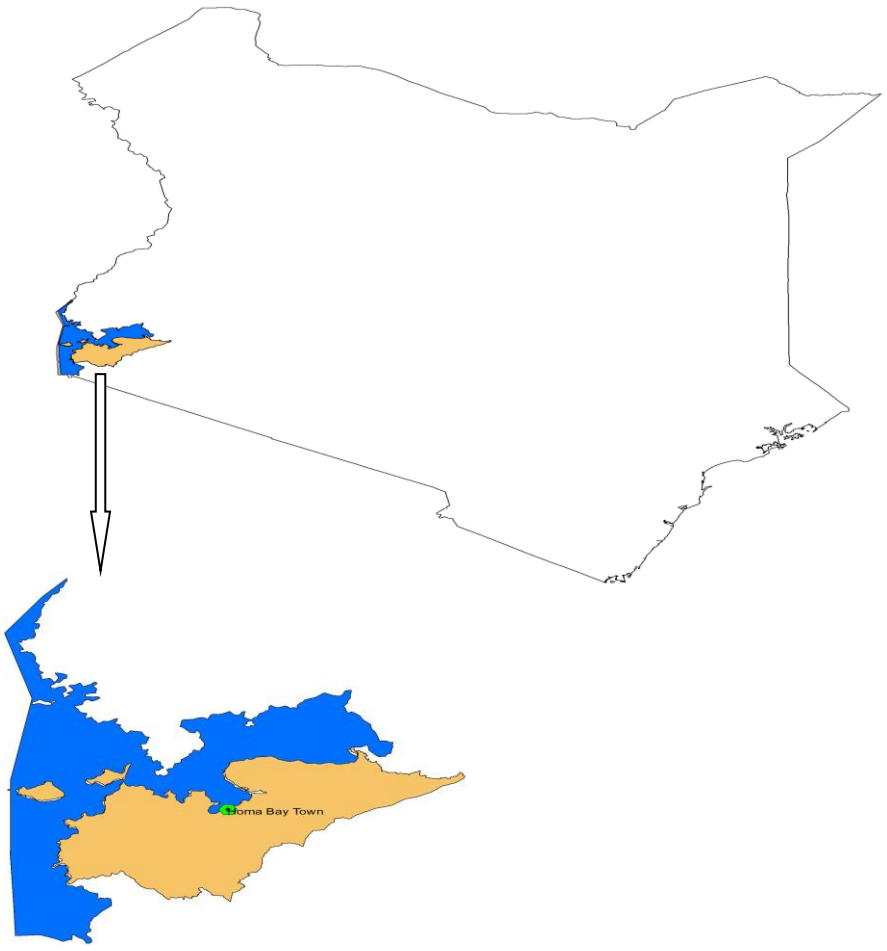

Figure 3.1: Map of Kenya showing Homa Bay County where the study was carried out
Source: Baker and Eric (2008).

This is an inland port connecting other important inland ports such as Kisumu and Mbita. Homa Bay town is a transportation and commercial hub that serves a huge population of the surrounding region in which commercial activities such as fishing, crop and animal husbandry thrive. The town serves as a manufacturing centre, with processed foods as the major products. The town has a population of about 59,844 according to the 2009 population census (KNBS, 2010). Homa Bay town was chosen because there are no documented studies on the prevalence of enteric viruses and also due to its geographical and demographical aspects. Homa-Bay is located on the Eastern side of the Olambwe Valley Forest. The town is along Kisumu, Mbita Point and Rongo road, slightly south of the equator (UN-Habitat, 2010). Homa Bay town is situated about 80 miles from Kisumu City to the South and about 248 miles from the capital city of Kenya, Nairobi. The surrounding region in Homa Bay town is mainly involved in Agricultural activities (both crop and animal husbandry). There is also trade and tourism due to Lambwe Valley Game Reserve and the Homa-lime Hot springs. However fishing still remains the largest economic pillar of the region.

Fishing activities take place both day and night in which fish is harvested mainly to supply the fish processing plant in the town as well as for the local market. The prevailing climatic condition is tropical wet with a binomial rainfall distribution pattern. The long rains normally occur around the month of April while the short rains normally occur around the month of November (FAO, WFP, FEWS NET and KFSSG 2012). Monthly mean temperature is normally about 11.54 °C with the annual potential range for evaporation being about 1180 mm to 1322 mm (Odada *et al.,* 2009).

3.2 Sanitation situation in Homa Bay town

The steep slopes make the area vulnerable to urban runoff to the lake during rainy seasons. Intense migration to the town from the surrounding communities in the recent past as well as population growth has resulted into the increased demand for amenities such as fecal disposal items like septic tanks, pit latrines, toilets and proper sewage systems. These facilities are in a short supply within the study area thereby increasing the exposure to fecal contamination (Odada *et al.,* 2004). There is inadequate clean water supply in most households in the town leading to heavy reliance of the poor quality lake water for various domestic needs.

3.2.1 Sewerage System

Homa Bay town has a sewage system whose coverage is not adequate as it is connected to a very small percentage of the municipal area (KNBS, 2002). The defunct sewage treatment plant is situated at the shore of the lake in which it discharges untreated sewage directly to the lake. This likely will result to high fecal contamination of the water in the lake. The stabilization ponds of the treatment system and the treatment equipment are in dilapidated state. Many household and units are also not connected to the sewer lines (UN-Habitat, 2010).

3.2.2 Septic tanks and soak pits

As an alternative to the inadequate sewage coverage, many residential and household units have resorted to the use of septic tanks and soak pits. Their popularity in the town

may also be attributed to the lack of adequate waste water treatment system (KNBS, 2002).

3.2.3 Pit latrines

Most of the residents of Homa Bay town dispose fecal waste by use of pit latrines. The pit latrines are cheap to construct and to maintain as well since they don't rely on use of water which is inadequate supply in the town. New ones are normally dug to replace the filled ones though treatment and exhaustion are also done especially in institutions. Despite the fact that this is the most popular fecal disposal method, still the latrine coverage in the area especially the surrounding community is inadequate leading to use of bushes around the lake as alternative for fecal disposal.

3.2.4 Storm water drainage

Homa Bay town lacks adequate storm water drainage system. The incomprehensive drainage system existing has been seriously hampered by solid waste blockage especially plastic bags. Open and broken slabs of manhole covers are a common site along the streets of the town. However there exists primary natural drainage system comprising of streams being that the town is downhill. This though has been affected by construction activities.

3.2.5 Wastewater disposal

There is no proper treatment for wastewater in the town leading to a possible contamination of the surface waters and the lake. The disposal methods for the wastewater include use of pit latrines, septic tanks, soak pits and sewerage system.

3.3 Sampling sites

The actual sample collection sites along the shoreline were located within a radius of about 3 km covering 2 public beaches in the study area (Figure 3.2). The main beach is located at about 10 meters from the pier while the second one is located about 3 km to the North of the first beach. The six sampling points along the shoreline were conventionally busy areas based on the observation of the level of human activities such as fishing and water transport. The surrounding potential sources of fecal contamination facilities that were observed at the area such as the defunct sewage treatment plant and the pit latrines were also taken into consideration while identifying the sites. The six sampling sites designated L1 to L6, were located approximately 2 to 3 km apart from one another (Figure 3.3).

Other that the sewage treatment plant, other facilities around the study area include capital fish processing factory, Water treatment plant, loading zone, hotels and an Open Air Market. There are numerous human activities going on around the sample collection area area which are likely to affect the occurrence and concentration of the viruses through water usage and commonness of infection within the service population

(Haramoto *et al.*, 2018). Some of the activities identified include fishing, animal and crop farming, washing, water transport, recreational activities and retail business.

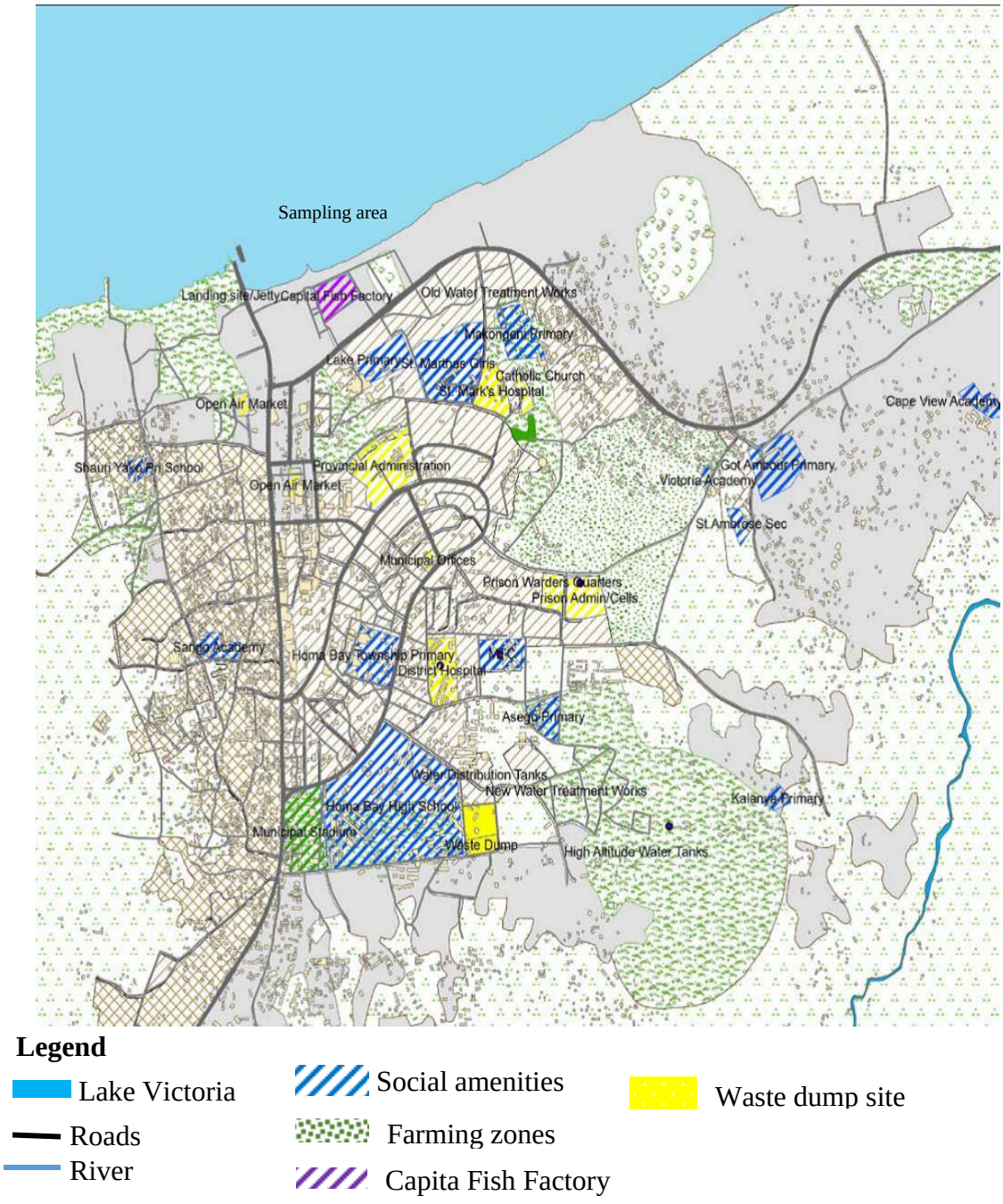

Figure 3.2: Map of Homa Bay town showing the sampling area. Source: UN-Habitat (2010).

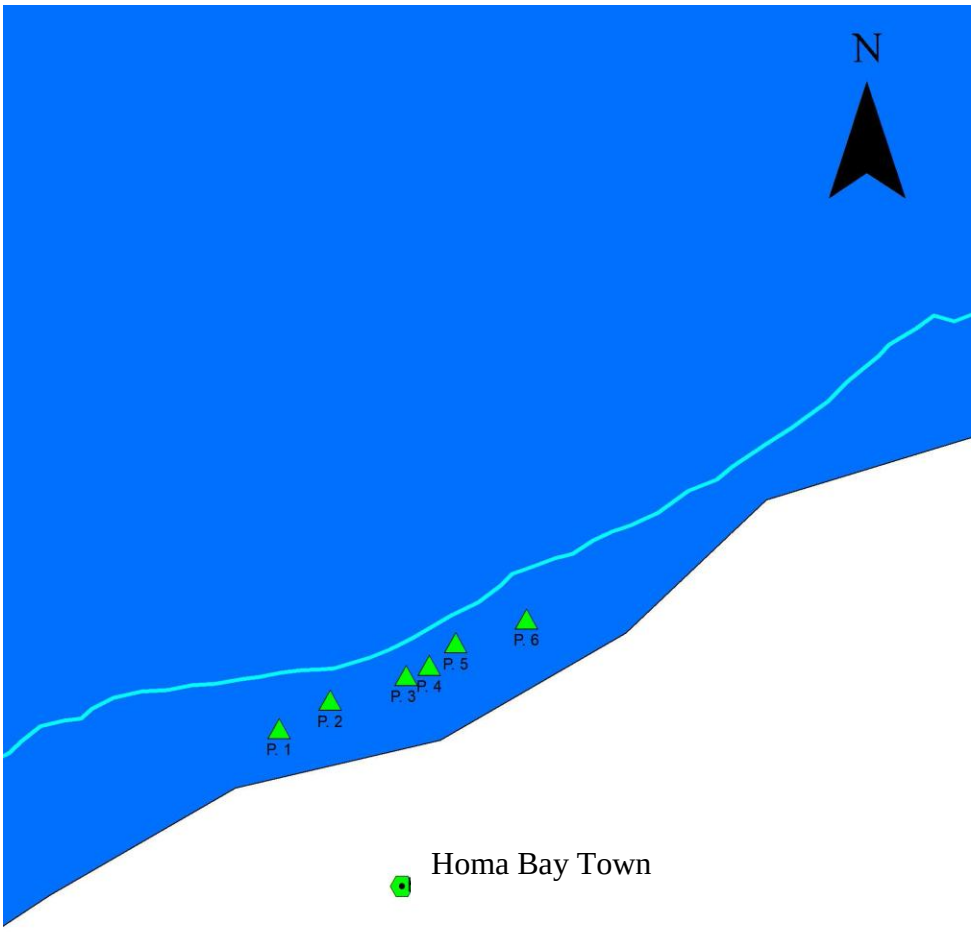

Figure 3.3: A GPS map showing the location of the sampling points along the shore line.

Site L1 was located next to the main first beach which also acts as the landing zone where boats used for fishing and transport anchor. This site was very busy with a lot of human activities including drawing water for domestic use.

There were a full-contact human activities, such as fishing, tourism, cargo loading and offloading and recreation activities like swimming in this site. Plate 3.1 shows the expansive water mass in the loading zone around site L1. This site was also closer to the dilapidated pier where ships used to dock (Plate 3. 2).

Plate 3.1: Landing site next to site L1.

Plate 3.2: Homa-Bay pier next to site L1.

Site L2 was located next to the loading area of Capital Fish factory (Plate 3.3). The waste from this fish factory was directly discharges into the lake. Wastes generated from the discharge of effluents from these facilities entered the lake through this point. Disposal of the remains of the fish at the plant was also not properly executed as they were dumped into a land fill which is about 3 km away from the main beach.

Plate 3.3: The runway leading to the loading area Capital Fish factory located next to site L2.

Site L3 was located next to an open air fish market where fish remains (*mgongo wazi*) are sold (Plate 3.4). A lot of human activities were taking place around this place since it is a busy fish market. Market women collect the remains of fish from the Capital fish factory which are rewashed and sold to locals here at a cheaper price. Public pit latrines are

found in this site which was mostly used by the fish mongers from the nearby fish market.

Plate 3.4: A fish vender prepares fish remains for sale next to site L3.

Site L4 was located at an open place where domestic animals grazed (Plate 3.5). The beach is at the background (Plate 3.5). One private latrine was observed near this site. This place is less polluted compared to other areas. Recreational activities such as swimming, laundry activities and fishing were also common at this site. This site was located mid-way between the two beaches.

Plate 3.5: Cows grazing next to site L4.

Site L5 was located directly opposite to the dilapidated Homa Bay municipal sewage treatment plant as shown in plates 3.6, 3.7 and 3.8. It is the closest of all to the dilapidated municipal sewage treatment plant; located just about 100 m from the plant. This is a highly polluted region with raw sewage directly entering the lake. Wastewater from the whole of Homa Bay town is normally discharged into the Lake through this point (UN-Habitat, 2010). The current dilapidated sewage treatment plant was constructed over 2 decades ago along with urban drainage system. Currently, the raw sewage flows away from this treatment plant and ultimately into the Lake untreated in that this plant stopped functioning more than a decade ago (UN-Habitat, 2010) . The system receives discharge wastes from the whole town but disinfection is not performed. This site is located close to the second beach.

Plate 3.6: Two stabilization ponds of the dilapidated sewage treatment system located near the lake and site L5.

Plate 3.7: Sewage treatment plant machines in a dilapidated condition next to site L5.

Plate 3.8: One of the wells of the sewage treatment plant and the lake at the background next to site L5.

Site L6 was also located closer to sewage plant but about 800 m from site L5. This site is close to the second beach. This was a highly polluted site with the raw sewage effluent that was discharged directly to the lake (Plate 3.9). It was located at a slightly sloppy ground.

Plate 3.9: Sewage effluent discharging into the lake next to site L6.

3.2 Study design

In this study, a longitudinal study design was used in which the samples were collected during the wet and dry on a monthly basis for a period of seven months. The study was carried out along the shoreline beaches of Lake Victoria at six designated locations in Homa Bay town between October 2011 and April 2012 with an attempt to correlate different factors influencing the lake water quality such as fecal pollution, seasons and physical water quality aspects.

3.3 Sample collection

Standard sampling guidelines and methods as described by the American Public Health Association (2005) and Onyuka *et al.* (2011) were followed. Simple random sampling was adopted as the sampling design of choice based on the need to increase accuracy level. Collection was carried out using sterilized clean plastic containers at a depth of about 50 cm. Sample collection was repeated in six different months during both rainy and dry seasons (3 rainy months and 3 dry months) (Table 3.1). The main aim of the samples being collected within different rainfall timelines was to determine whether there is a correlation between the presence of the viruses in question in the lake water with changes in seasons. The sampling rounds (monthly collections) had a total of 36 samples per trip, 6 samples for every site in a ten litre volume sterile container while taking precautions to ensure no air bubbles were present.

Table 3.1: Sampling periods and volume of water sampled

Site							
Sampling period	Season	L1	L2	L3	L4	L5	L6
Oct 2011	Wet	60	60	60	60	60	60
Nov 2011	Wet	60	60	60	60	60	60
Jan 2012	Dry	60	60	60	60	60	60
Feb 2012	Dry	60	60	60	60	60	60
Mar 2012	Dry	60	60	60	60	60	60
April 2012	Wet	60	60	60	60	60	60

* 60 –The volume of water collected in litres per site

Figure 3.3 shows a satellite image showing the distribution of the sampling points across the study area. The sample size of 6 per site on a monthly basis was determined based on the need for high precision since the concentration of enteric viruses in water is always very low (Haramoto *et al.,* 2018).

The containers used for water sample collection were treated by adding 10 ml of Sodium thiosulphate solution for sterilization to neutralise any possible residual chlorination effect and to prevent continued of microbicidal action. The $Na_2S_2O_3$ was prepared by dissolving 10.2 g of $Na_2S_2O_3$ in 1litre of distilled water. The containers were then thoroughly washed and rinsing first with distilled and then with the lake water. The containers were filled with the samples to the top to reduce chances of air bubbles formation to minimise chances of aerobic respiration during transit and then firmly sealed. The sampling was repeated for a period of six months resulting into a total of 216 water samples of 2160 litres. The samples were then transported on ice to the Enteric Viruses Research Group –Institute of Primate Research Laboratory in Nairobi for

analysis. The samples were kept at a temperature of 4 ºC until they were processed (Kiulia *et al.,* 2010).

3.4 Analysis of water physical quality parameters

Various physical characteristics of the water were measured and recorded on site to examine the quality of the water. The physical parameters considered include temperature, pH, dissolved oxygen, total dissolved solids, electrical conductivity and turbidity.

3.5 Analysis of the samples for viral contamination

The water samples were dichotomised into either the presence or absence of the viruses (adenovirus and enterovirus) being studied. The analysis of the samples for viral contamination process involves virus concentration from the environmental water samples followed by the recovery of the concentrated virus. The nucleic acids of the recovered viruses are amplified using PCR. The method of choice for the viral isolation and recovery was the glass wool adsorption-elution technique. The method adopted was that described by Woolfaardt *et al.* (1995) and Grabow *et al.* (2007).

3.5.1 Treatment and assemblage of perpex glass wool columns

The pesrpex tubes were first decontaminated by rinsing them using 3.5 % sodium hypochlorite solution. The tubes were then thoroughly rinsed using tap water before a final rinse using rinsed using distilled water. Oiled sodocalcic glass wool (Bourre 725QN; Saint Gobain, Isover-Orgel, France) was rinsed for 15 minutes with 18-mohm

reverse osmosis (RO) water. It was then washed with 1 M HCl for 15 minutes and then rinsed again with RO water. To raise the pH to 7.0, the glass wool was then was washed with 1 M NaOH for 15 minutes before being subjected to final rinsing using RO. The clean glass wool was stored in phosphate-buffered saline (PBS) at 4 °C until use. The prepared glass wool was then packed in the glass wool columns of fifteen grams wool/column (5X3/column) packed one at a time. The column is 20 cm long and has a diameter of 30 mm. Fifteen grams of the glass wools were stuffed in the glass wool columns to a density of 0.5 g cm^{-3} dry weight using a metal plunger. Five grams portion of glass wool were compressed into the columns on each other while separated using steel sieve gauze with size pore of about 1 mm^2 placed in between each of them. The packed columns were then flushed with PBS (pH 7.0) prior to running the sample. A different column of 16 mm with a diameter of 6.6 cm polyvinyl chloride (PVC) pipe was used for cases where the samples were slightly turbid leading to a low filtration rate.

3.5.2 Preparation of apparatus for the filtration process

Ten litre containers were decontaminated using 3.5 % sodium hypochlorite solution and left to stand for 1 hr before filling with the sample. Five millilitres of 3.5 % sodium hypochlorite solution was added to the containers and then rinsed with tap water. To remove the traces of the sodium hypochlorite solution the containers were further rinsed with distilled water. The recovery apparatus (The perpex column, a metal plastic coupling link attached to each end of the column connected to the waste collecting flask and the 10 litre water reservoir containers which had earlier been decontaminated by using 3.5 %

sodium hypochlorite solution and rinsed with distilled water) were then mounted on a clamp stand (Plate 3.10).

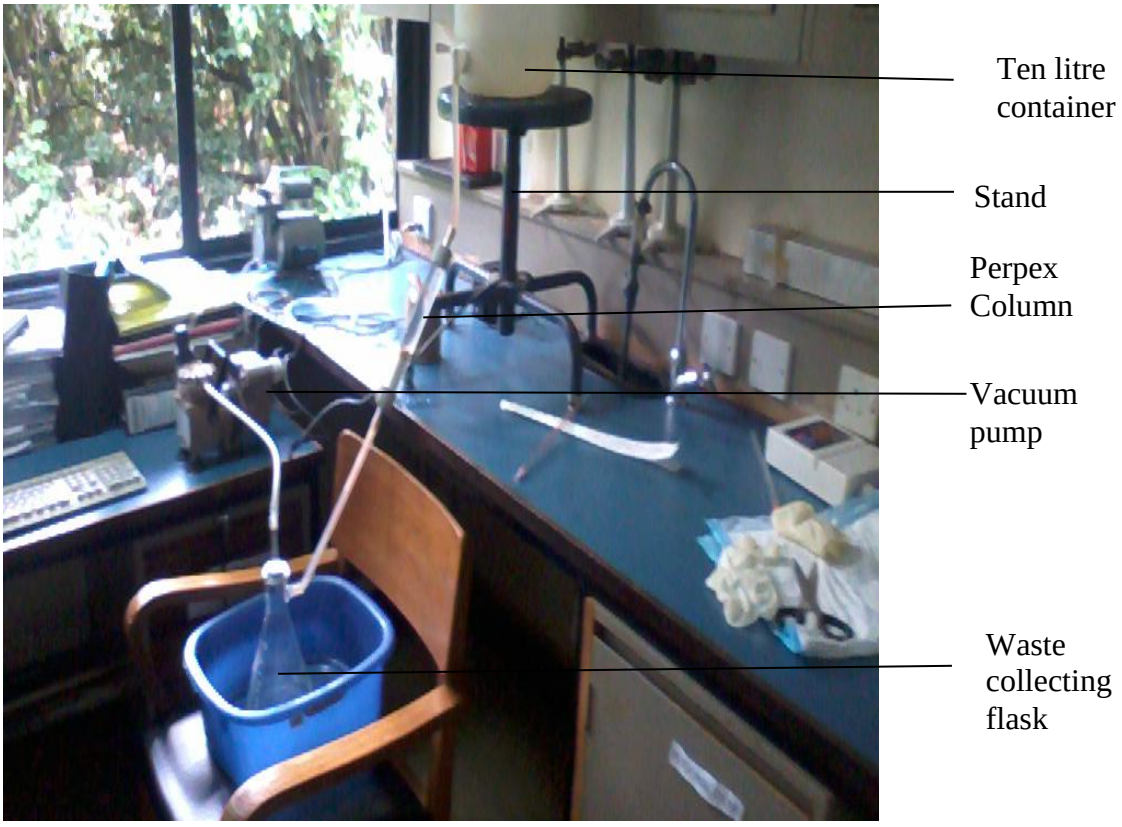

Plate 3.10: Arrangement of the apparatus for the filtration process.

3.5.3 Filtration of the samples to recover the viruses

Once the filtration apparatus had been assembled, water samples were drained into the perpex column by application of negative pressure resulting into filtration through the positively charged glass wool columns at a flow rate of 10 l/h. The volumes filtered varied depending on the turbidity levels which were affected by the source of the sample. For example samples collected from the site close to the sewage effluent were a little turbid than those collected directly from the beach. Waters with low turbidity were filtered up to 200 ml. Samples that were found to have a pH >7.5 as measured before

filtration were subjected to adjustment by use of 0.05 N HCl to <7 .5. A vacuum pump was used to drain the water through the column. Being negatively charged, the viruses got adsorbed on the glass wool filters which are positively charged.

3.6 Secondary concentration of the viruses

The viruses recovered from the filtration process were subjected to secondary concentrated by washing using PEG/NaCl in a glycine beef extract buffer (GBEB) based on the methods described by Millen *et al.* (2012).

3.6.1 Preparation of Glycine beef extract buffer

Glycine beef extract buffer (GBEG) was prepared by adding about 4 g and 5 g of glycine and beef extract respectively in a litre of water. To adjust the pH to 9.5 either 1 M NaOH or 1 M HCl was added to the mixture depending on the value. The mixture was autoclaved at a temperature of 121 ^{0}C for 35 minutes. The buffer was stored at -4 ^{0}C until use.

3.6.2 Filter elution and flocculation

The columns were removed from the container attached after the test samples had drained through the perspex columns and assembled on a G-Clump with a collecting container put in place to receive the elements. The adsorbed viruses were eluted by saturating the filters with 50 ml of 9.5 pH GBEB and left for 15 minutes. After 15 minutes contact, the excess mixture volume was sucked out through the filter using a syringe before evacuation with air. The pH of the resultant eluent was adjusted to 7.5 or 7.0 with 1 N

HCl. The eluent was then flocculated using polyethylene glycol 8000 (8 % [wt/vol]) and NaCl (final concentration, 0.2 M). This mixture was then stirred for 1 hr at a temperature of 4 °C before being subjected to incubation overnight at 4 °C. It was then centrifuged at 4,200 rpm for 45 min at 4 °C and the pellets resuspended in a 2 ml of sterile phosphate-buffered saline solution [0.15 M Na_2HPO_4 solution (pH 7.0)]. It was stored in a separate sterile 20 ml tube at a temperature of -70 °C until use.

3.7 Nucleic acid extraction

The process of nucleic acid extraction from the virus concentrates involved the use of an automated nucleic acid extraction system. The method of choice was based on whether the virus in focus was a DNA or an RNA type. A higher concentration and better purity of the isolated nucleic acids was further enhanced by precipitation after quantification. Virus concentrates that were thick probably because of fecal debris were refined by adding chloroform. Suspensions from sites L5 and site L6 were treated using 200 µl of chloroform (10 % Chloroform-Merck) as they had higher debris concentration than the rest of sites. The mixture was rigorously vortexed for 30 seconds followed by centrifugation for 30 seconds at 3000 rpm (Eppendorf 5402 microcentrifuge, Hamburg Germany). The resulting supernatant was used for nucleic acid extraction in which 1 ml was used. The supernatant was centrifuged at 300 rpm for 30 seconds to separate the phases (Kiulia *et al.*, 2010).

3.7.1 Automated nucleic acid extraction for a DNA virus

The DNA was extracted using automated commercially available MagNA Pure total nucleic acid extraction kit (Roche Diagnostics, Indianapolis, IN, USA) according to the manufacturer's instructions. MagNA Pure has the advantage of a standardised procedure for extracting nucleic acid from up to 32 samples in parallel. One millilitre of the virus concentrate was used for isolation using the kit. The nucleic acid extracted was eluted and the elute stored in Tris-EDTA (pH 8.0) at a temperature of -20 ^{0}C until use. The principle of this isolation procedure is based on magnetic-bead technology, which is as follows: samples are lysed with incubation with a buffer that contains chemotropic salts and proteinase K. Free nucleic acid from lysates is bound to the surface of added magnetic glass particles and unbound substance are removed by several washing steps.

3.7.2 Automated nucleic acid extraction for a RNA virus

The process of RNA extraction involved the use of commercially available RNeasy mini kit (QIAGEN category No. 74106) and QIAvac 24 vacuum manifold (QIAGEN category No. 19403) following manufacturer's instruction. One ml of the recovered virus concentrate was added to the RNeasy spin columns. Filtration was done using QIAvac at 500 mm Hg vacuum approximately. The extracted nucleic acid was eluted and then aliquoted into well labelled tubes. To minimise chances of degradation the extracted RNA was added to the RNase free water before storage. Storage was done by freezing the RNA at a temperature of −70 ^{0}C until time of use.

3.7.3 **DNA purification, quantification and quality assessment**

Diluted DNA samples were purified to ensure that PCR inhibitors are removed. Briefly 100 µl of the DNA was mixed with 40 µl of silica particles. To this mixture, 900 ml of guanidinium thiocyanate lysis buffer (GuSCN) was added followed by incubation at room temperature for about 10 min. Centrifugation was then carried out for one minute on the resultant mixture at 2000 rpm. The supernatant was discarded; the pellet washed using double distilled water and then dried briefly for five minutes. Elution buffer (50 µl of 1x Tris-EDTA) was used to elute the purified DNA from the beads at a temperature of 4 °C.

The concentration and the purity assessment of the DNA were determined using a spectrophotometer (Beuret, 2003). The purified DNA samples were diluted to the ratio of 1:50 through addition of 50 µl of the DNA to 1000 µl of sterile double distilled water before being vortexed to give a reading between 0.1-1.0. A Beckman spectrophotometer was then used for reading the absorbance at 260 nm. The concentration of the dsDNA was then calculated in µg/ml using the following formula (Barbas *et al.*, 2007):

dsDNA concentration (µg/ml) = OD_{260} reading X Dilution factor X 50 µg/ml

An optical density (OD) reading of 1.0 at 260 nm was considered to be equal to 50 µg/ml of DNA.

To verify the purity of the extracted DNA, the ratio OD_{260}/OD_{280} was calculated using the following formula:

dsDNA purity (A_{260}/A_{280}) = A_{260} reading ÷ A_{280} reading

Nucleic acids samples with absorbance ratios ranging from 1.8 to 2.0 were considered to be of good purity. Samples that had ratios outside this range were normalised using 10 % ethanol.

3.7.4 RNA purification, quantification and quality assessment

The extracted RNA samples were subjected to purification to remove possible organic compound contaminants such as phenols and proteins. The samples were mixed with 2 µl of RQ1 RNase-free DNase, 2 µl of DNase buffer (Promega, Madison, WI) and 1 µl of RNase inhibitor (Promega) in a 20 µl volume followed by centrifugation for 10 minutes. To inactivate the residual DNase, a DNase stopping solution (Promega) was added to the mixture. Elution was then carried out to a collecting tube to remove the purified RNA from the columns. Fifty microliters of RNase-free water was added to prevent degradation and left to settle for about 1 min before being centrifuged at 10,000 rpm for 2 min (Eppendorf).

UV spectroscopy was used in verification of the concentration and purity of the RNA from the A260:A280. For the determination of the concentration, the absorbance of RNA sample at 260 nm was measured. The RNA was first diluted 40 fold in which 2 µl of the RNA sample was dissolved in 78 µl of sterile double distilled water. The concentration of the RNA was then calculated in µg/ml using the following formula (Barbas *et al.,* 2007):

RNA concentration (µg/ml) = OD_{260} reading X Dilution factor X 40 µg/ml

An optical density (OD) reading of 1.0 at 260 nm was considered to be equal to 40 µg/ml

of RNA. The purity of the RNA was evaluated using the A260/A280 ratio which was calculated as follows: RNA purity (A_{260}/A_{280}) = A_{260} reading ÷ A_{280} reading

Purified RNA samples that had a ratio ranging from 1.8 to 2.0 were considered to be of good quality. The ratio of 1.90 for an average A260:A280 was recorded with 86 % of the RNA samples falling within 1.8-2.0 which is the range accepted for good quality nucleic acids. For DNA, the average A260:A280 ratio recorded with 82 % of the samples falling within 1.8–2.0 (Appendix III).

3.8 PCR amplification of the purified nucleic acid samples

Reverse Transcription (RT)-nested PCR was used for amplification of the RNA samples, while nested PCR was used for amplification of DNA samples. The choice of use of the Nested PCR for amplification was based on the need to enhance the levels of specificity of the amplifications and minimise chances of getting false-positive outcome through possible non-specific amplifications. In this study the PCR analysis was only used for determination of the virus presence or absence as the virus nucleic acids are always in small amounts in environmental samples such as the lake. Sequence of primers and probes for the PCR process are provided in Table 3.2.

3.8.1 Polymerase chain reaction for enterovirus

The PCR procedure for enteroviruses was modified from the procedure according to Puig *et al.* (1994). The specific primers (Ent1/Ent2) used originated from the enterovirus genome at the 5' non translated region, aligned with the sequences that are already published (Puig *et al.,* 1994). The specific primers and probes target the EVs 5’ end non

coding region of the genome enabling amplification and identification of a wide range of species of enterovirus (Puig *et al.,* 1994).

Table 3.2: Primers that were used in this study

Virus	Function	Sequence(5'-3')	Product length	Reference
Human Adeno Virus-F40/41	hexAA1885(F)	GCCGCAGTGGTCTTACATGCACATC	300	Santos *et al.,* (2004)
	hexAA1913(R)	CAGCACGCCGCGGATGTCAAAGT		
	HAdV-F4041-hex214probe	6-FAM-CGACKGGCACGAAKCGCAGCGT-BHQ-1		
Human Adenovirus	nehexAA1893(F)	GCCACCGAGACGTACTTCAGCCTG	143	Santos *et al.,* (2004)
	nehexAA1905(R)	TTGTACGAGTACGCGGTATCCTCGCGG TC		
Human Enterovirus	Ent-1(F)	CGGTACCTTTGTACGCCTGT	534	Puig *et al.,* (1994)
	Ent-2(R)	ATTGTCACCATAAGCAGCCA		
	EntProbe	6-FAM-TCCGGCCCCTGAATGCGGCTAAT-TAMRA		
Human Enterovirus	neEnt-1(F)	TCCGGCCCCTGAATGCGGCTA	138	Puig *et al.,* (1994)
	neEnt-2(R)	GAAACACGGACACCCAAAGTA		

* F-Forward primer, R-Reverse primer

The specific primers and probes target the EVs 5' end non coding region of the genome enabling amplification and identification of a wide range of species of enterovirus (Puig *et al.,* 1994). Complementary DNA (cDNA) was first synthesised from the enterovirus RNA by reverse transcription process. Reverse transcription was carried out using a First-Strand Synthesis Superscript III reverse transcription kit (Invitrogen, Carlsbad, CA) (Puig *et al.,* 1994). Briefly, 5 µl of the extracted nucleic acid was added to a mixture of 4 µl of 5x transcriptor reaction buffer (Roche), 200 µM each deoxynucleoside triphosphates, 1 µl of Moloney murine leukemia virus reverse transcriptase (Promega), 20 U of Protector

RNase inhibitor (Roche) and 5 µl of 2.5 µM enterovirus reverse primer (Table 3.2) to a total volume of 8 µl. The conditions were set at temperature at 55 °C for 30 min and the enzyme was inactivated at 85 °C for 5 min. Precaution was taken to minimise contamination of the samples by the amplified enteroviral DNA molecules by use of separate labs for the amplified samples and the reagents.

The one-step PCR reaction involved addition of 10 µl of the cDNA to a PCR mixture containing 50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25 °C), 1.5 mM $MgCl_2$, 0.01 % gelatin, 0.1 % Triton X-100, 200 µM of each (dNTP), 0.5 µM each of the enterovirus primers (Ent-1 (F) and Ent-2 (R)) and 2 U of thermostable Taq DNA polymerase (Promega) to a 50 µl reaction volume. To prevent evaporation, the samples were overlaid with 75 µl of mineral oil. A programmable thermal cycler (Progene, Techne, Cambridge, UK) was used for the thermal cycling process in which the temperature for initial denaturation was set at 94 ºC for 4 min. Thirty amplification cycles were carried out which included denaturation at 94 ºC for 90 s, annealing at 55 ºC for 90 s and extension at 72 ºC for 120 s. A set of forward and reverse primers were used in first round of PCR to amplify a fragment of 534 bp.

For the second round of PCR, nested primers which amplified a 138 bp fragment of enterovirus, were used. The nested PCR mixture consisted of 1x PCR buffer, 2.9 mM $MgCl_2$, 200 µM of each dNTP, 0.4 µM of each of the nested primers (neEnt-1(F) and neEnt-2 (R)), 2 U of thermostable Taq DNA polymerase (Promega) and 2 µl of the one-step RT-PCR products, for a final reaction mixture volume of 50 µl. Thirty five cycles

were carried out (denaturation set at 94 ºC for 25 s, annealing at 55 ºC for 25 s and elongation at 72 ºC for 45 s). This was followed by a final extension for 7 min at 72 ºC and an observation of a fragment of 138 bp was expected. To prevent samples from spilling from one lane into another, the samples were located in the gel on alternate lanes. The PCR products were further confirmed by gel electrophoresis.

3.8.2 Polymerase chain reaction of adenovirus

The PCR procedure for adenovirus was adopted from that described by Allard *et al.* (1990) and modified by Santos *et al.* (2004).The specific primers used for the first step of PCR to amplify adenovirus hexon gene (380 pb) were a published set collected from the open reading frame of the DNA sequence of the hexon genes of Ad40 and Ad4l. All the PCR products from the first PCR step were amplified for a second time using a different set of primers (nested-PCR) that is nehexAA1893 and nehexAA1905 targeting a product length of 143 bp. For the one step PCR, 10 µl of the extracted adenovirus nucleic acid samples were mixed with 40 µl of the PCR mixture.

The PCR mixture consisted of 1x PCR buffer, 2.5 mM $MgCl_2$, 200 µM of each dNTP, 1 µM concentration of each primer, 1 U of thermostable Taq DNA polymerase (Promega), 2 µl enzyme mix (LightCycler FastStart DNA master kit; Roche) and the PCR grade water forming a total volume of 50 µl. The amplification was carried out in a thermocycler that was set at the following conditions: Initial denaturation at 95 ºC for 15 min. There were 35 cycles that consisted of 95 ºC for 0.5 min each for denaturation, 57 ºC for 0.5 min annealing and 72 ºC for 0.5 min elongation followed by a final elongation step at 72 ºC for 5 min. For nested PCR, 1 µl of the first round PCR amplification was

subjected to further amplification. The conditions of amplification were the same as those of the first PCR with a new set of primers (nested primer pair nehexAA1893 and nehexAA1905 which amplified a 143 bp fragment) (Allard *et Al.*, 1992). The concentrations of the primers were changed to 0.5 µM while that of $MgCl_2$ was changed to 3.5 mM. PCR mixtures without DNA were used as negative controls for the analysis, respectively. The PCR products were further confirmed by gel electrophoresis.

3.9 Gel preparation and electrophoresis

Separation of the PCR products was done by gel electrophoresis using a 2 % SeaKem ME agarose gel (FMC Bioproducts, Rockland, Maine) in a 0.5x Tris-borate-EDTA buffers. The gel was stained with 1 mg/ml of ethidium bromide. Two grams of agarose powder was dissolved in 100 ml of TBE [(Tris borate EDTA), 54 g Tris base, 27.5 g boric acid, 200 ml EDTA] buffer. A microwave was used to heat the solution for 2 min while covered to avoid evaporation. One µg/ml ethidum bromide was added to the mixture after it was allowed to settle and cool for 5 min following the heating. The agarose was then poured in to the tray and the combs were inserted with the bottom half of the comb immersed and left to solidify for 30 minutes. The tray was placed on a dark surface to help see the wells in the agar before addition of the loading buffer and the samples. The solidified agarose gel was transferred to the electrophoresis chamber and the loading dye (50 ml EDTA, 5 ml NaCl solution, 5 ml glycerol and 0.01 g bromophenal blue) was prepared and loaded to the chamber covering the gel. The combs were removed and the molecular weight marker was carefully loaded to the first and the last lanes of the

gel. The positive and the negative controls were added to the second last and the third last lanes respectively from both ends.

Twelve microliters of the amplified DNA samples were carefully loaded into the other wells of the gel with great care being taken during the loading process to avoid puncturing of the bottoms of the wells in the gel. The voltage was adjusted to 100 for about for 10 minutes. Visualization of the gel was carried out using a UV transilluminator (Alpha Innotech Corporation, SanLeandro, CA). The bands on the gel were observed and photographed using a digital camera. The amplicons size was compared to 100 bp molecular weight (MW) ladder (Promega) and the positive control.

3.10 Data analysis

Paired *t* test was used to analyse comparison between the variations in seasons (wet and dry seasons) and the variables (the presence or absence of the viruses) while ANOVA test was used to analyse comparison between the variations between the sites and the variables. The Odds ratios were calculated to determine the relationship between virus presence and the orientation of the sampling sites to the sewage treatment plants and pit latrines. Descriptive analysis was done to determine the frequency distribution of the water quality physical characteristics as well as the prevalence of the two virus species across the sites. Correlation and Regression analysis were also used to analyse the factors with significant influence on the outcome variable, contamination. SAS ver. 9.1 (SAS Institute, Cary, NC, USA) was used to carry out all the analyses in which the P- values were considered statistically significant at P-values <0.05.

CHAPTER FOUR

RESULTS

4.1 Physical characteristics of water samples from the sampling sites

There were varying results as far as the physical quality these parameters are concerned (Table 4.1). The results show that the samples had pH values ranging from 7.00 to 7.06 that were within the WHO acceptable levels of 6.50 – 8.00 (Table 4.1). A few samples had pH greater than 7.00 and 1 N HCl was used to adjust the pH to 7.00 at the filtration stage to enhance adsorption of the viruses.

Analysis indicate that there was significant difference in pH ($p = 0.0090$) during the dry and wet season as well as between the sites ($p=0.0077$) (Table 4.1). The highest pH was observed in site L5 (7.06) while the lowest pH was observed in sites L1, L2, L3 and L4 (Table 4.1). There was significant interaction ($p=0.0077$) between site and season in influencing the pH ($p=0.0077$). Paired comparison using *t* test shows that there was significant difference ($p=0.014$, $t = 2.51$) in pH between the wet and dry season (Appendix II). Highest pH was recorded during the wet season.

Mean temperature varied between 25.47 ^{o}C to 25.92 ^{o}C for all the sampling periods as measured on site (Table 4.1). The results indicate that the water temperature was within the acceptable limits of ± 2 ^{o}C from 25.00 ^{o}C. Statistical analysis showed that there was significant difference in temperature ($p < 0.0001$) during the dry and wet season. There was also significant difference in temperature ($p=0.0002$) between the sites. The highest temperature was recorded from site L6 (25.92 °C) as compared to the rest of the sites L1,

Table 4.1: Two way analysis of variance with Post hoc analysis using Tukey's HSD on physical parameters

Treatment	pH	Temp (°C)	EC (µS/cm)	TDS (mg/l)	DO (mg/l)	Turbidity (NTU)
Season						
Dry	7.00± 0.00a*	25.90± 0.03a	70.73± 3.89a	50.14± 3.24a	8.81± 0.12a	9.98± 0.25a
Wet	7.03± 0.01b	25.56± 0.07a	0.28± 0.05b	47.56± 2.08b	8.58± 0.11b	19.73± 0.85b
P values						
Season	0.0090	<0.0001	<0.0001	0.0028	0.0447	<.0001
Site						
L1	7.00± 0.00b	25.47± 0.13c	56.97± 11.40a	87.17± 6.13a	9.55± 0.20a	6.06± 0.25e
L2	7.00± 0.00b	25.78± 0.07abc	60.02± 10.14a	30.58± 1.66c	9.46± 0.24a	16.00± 1.05c
L3	7.00± 0.00b	25.83± 0.06ba	34.63± 5.90b	50.72± 1.58b	8.46± 0.17bc	11.00± 0.40d
L4	7.00± 0.00b	25.53± 0.13bc	20.62± 3.38c	25.47± 0.78d	8.63± 0.15b	15.50± 1.11c
L5	7.06± 0.03a	25.83± 0.06ab	3.91± 0.00c	50.00± 3.66b	8.08± 0.11bc	21.50± 1.56a
L6	7.03± 0.02ab	25.92± 0.05a	4.25± 0.00c	49.17± 3.52b	7.98± 0.10c	19.08± 1.34b
WHO Standards	6.8-8.5	25 ºC ±2	500-5000	500-1000	8-9	< 5
P values						
Site	0.0077	0.0002	<0.0001	<0.0001	0.0447	<.0001
Site*Season	0.0077	0.0002	<0.0001	<0.0001	<.0001	<.0001

*values followed by the same letter along the column are not significantly different based on Tukey's HSD at $p \leq 0.05$.

L2, L3, L4 and L5. There was significant interaction (p=0.0077) between site and season in influencing the temperature (p=0.0002). There was significant difference (p=0.0001, t = -4.52) in temperature between the wet and dry season (Appendix II) from the paired comparison using *t* test. Electrical conductivity (EC) values ranged from 70.73 μS/cm to 0.28 μS/cm with two way ANOVA test showing that there was significant difference in EC (P<0.0001) during the dry and wet season (Table 4.1). The highest mean EC was observed during the dry season (70.73 μS/cm) while the lowest was observed during the wet season (0.28 μS/cm) (Table 4.1). There was also significant difference in EC (p<0.0001) between the sites with water samples from site L2 having the highest EC (60.02 μS/cm) while the lowest EC was recorded from the water samples from site L5 (3.91 μS/cm) (Table 4.1). There was significant interaction between site and season in influencing the EC (<0.0001). There was significant difference (p=0.0001, t = -11.49) in Electrical Conductivity between the wet and dry season (Appendix II).

Differences between the sites show that Total Dissolved Solids (TDS) was highest at site L1 at 87.17 mg/l while site L4 had the lowest level of TDS at 25.47 (Table 4.1). The TDS values were significantly different (p =0.0028) during the dry and wet season. Similarly there was significant difference in the TDS values between the sites (p<0.0001) (Table 4.1). Highest mean for TDS was recorded during the dry season (50.1389 mg/l) compared to the wet season (47.56 mg/l). There was significant interaction (p<0.0001) between site and season in influencing the TDS (p<0.0001). In terms of seasonal variations, the two way ANOVA test indicate that there was significant difference in Dissolved Oxygen (DO) with a p value of 0.0447 during the dry and wet season (Table 4.1). The highest

mean DO was observed during the dry season (8.81 mg/l) while the lowest was observed during the wet season (8.58 mg/l) as shown in Table 4.2. There was also significant difference in DO ($p=0.0447$ between the sites. In regards to sites, the highest DO levels were recorded at site L1 (9.55 mg/l) in comparison to the rest of the sites (Table 4.1). There was significant interaction between site and season in influencing the DO ($p<.0001$). There was no significant difference ($p=0.578$, $t = -0.56$) in Dissolved Oxygen between the wet and dry season (Appendix II).

Both conductivity and TDS were below the thresholds recommended by WHO at 500-5000 mS/cm and 1000 mg/l respectively for fresh water bodies. Majority of the samples for the DO levels were within the recommended range by WHO OF 8-9 except samples from sites L1 and L2. There was a significant difference ($p=0.498$, $t = -0.68$) in Total dissolved solids between the wet and dry season (Appendix II).

Turbidity values were significantly different ($p<0.0001$) during the dry and wet season, the highest mean being observed during the wet season (19.73 NTU). Similarly, there was significant difference in turbidity ($p<0.0001$) between the sites with the highest turbidity being observed in site L5 (21.50 NTU) and closely followed by site L6 (19.08 NTU). The lowest turbidity was recorded at site L1 (6.06) NTU (Table 4.1). There was a significant interaction ($p<0.0001$) between site and season in influencing the turbidity (Table 4.1). Most samples had turbidity levels that were above the recommended values according to WHO for environmental waters of 5 NTU (Table 4.1). There was a

significant difference (p=0.047, t = 1.99) in turbidity between the wet and dry season (Appendix II).

Based on Pearson correlation analysis, there was a positive association between the physical parameters and the two enteric viruses ranging from r=0.045 (p=0.508) between Total Dissolved Solids and the adenoviruses to r=0.128 (p=0.060) between pH and enteroviruses. For the pairs with negative correlation, the range was from as low as r= -0.027 (p=0.693) between electrical conductivity and enteroviruses to a high of r=-0.083 (p=0.222) between DO and the adenoviruses (Table 4.2). There were no significant correlation between any of the physical parameters and the number of enteroviruses or adenovirus found (p>0.05).

Table 4.2: Pearson correlation of physical parameters and viruses present

	pH	**Temp (^{0}C)**	**EC**	**TDS**	**DO**	**Turbidity**
HAdV	0.089	0.114	-0.062	0.045	-0.083	0.108
	0.193	0.096	0.366	0.508	0.222	0.113
EV	0.128	0.090	-0.027	0.119	-0.068	0.087
	0.060	0.188	0.693	0.082	0.317	0.202

Cell Contents: Pearson correlation; P-Value at 0.05 probability level
Temp-Temperature, EC-Electrical Conductivity, TDS-Total Dissolved Solids, DO-Dissolved oxygen, HAdV-Human adenovirus detected, EV-Enterovirus detected

4.3 Viruses detected from the samples from the six sampling sites

Out of the 216 water samples that were processed for the identification of the enteric viruses, only 7 (3.24 %) were enterovirus positive based on the nested PCR results. Eleven out of the 216 water samples assessed (5.09 %) showed positive results for

adenoviruses based on the nested PCR results. In total 18 (8.33 %) of the samples out of 216 tested positive for the enteric viruses.

No bands were visualised in any of the samples with one step PCR products; whereas the nested PCR products of the samples displayed 138 bp fragment for RNA and 143 bp fragment for adenovirus respectively (Plate 4.1 and 4.2). It was therefore clear that viruses were detected in the agarose electrophoresis only after two PCR amplifications that is the one-step PCR amplification followed by the nested PCR amplification.

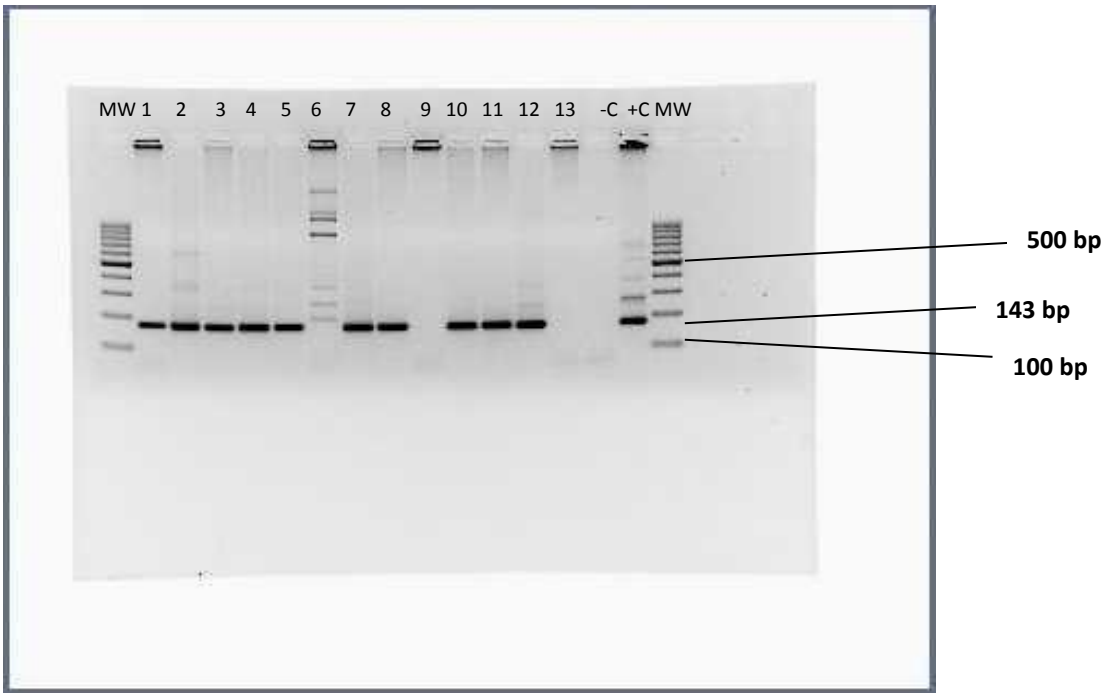

Plate 4.1. Agarose 1 % gel electrophoresis showing PCR amplicons generated by each of the adenovirus specific primers. MW, 100 bp molecular weight marker (Promega); Lane 1, sample from L1; Lanes 6 & 13, samples from sites L2 & L4; Lanes 2-5, samples from site L5; Lanes 7 & 8, samples from site L3; Lanes 10 to12, samples from site L6; +C, adenovirus positive control and –C, negative control.

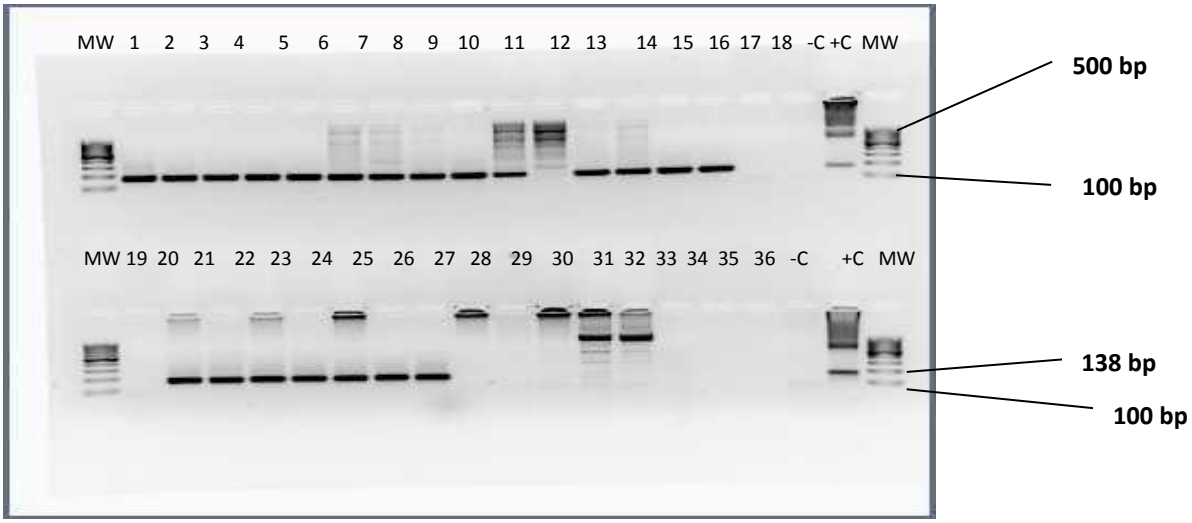

Plate 4.2. Agarose 1 % gel electrophoresis showing PCR amplicons generated by each of the EV specific primers**.** MW, 100 bp Molecular weight marker (Promega), Lanes 1-6, samples from site L5; Lanes 7-10, samples from site L2; Lanes 11-15, samples from site L4; Lanes 16-19, samples from site L1; Lane 20-26, samples from site L6; Lanes 27-36, samples from from site L3; +C-enterovirus positive control and –C-negative control.

All the six different sampling sites tested positive for either adenoviruses or enteroviruses at some point. Adenovirus DNA was isolated in 4 out of the 6 sites same as enterovirus. When the samples were tested for the first time in the first month of sampling which was October 2011, adenovirus was detected in 1 out of the 36 water samples (2.78 %) while enterovirus was not detected from any of the 36 samples. The adenovirus DNA that was detected at this first batch of the samples was from one of the samples from site L5. Seven more samples out of the rest 30 water samples from the subsequent collections in this site tested positive for viral contamination (both enterovirus and adenovirus). The positive samples came from the same source (site L5) as the first positive identification sampled in five different occasions and were taken in five subsequent. From site L5, adenovirus DNA was detected four times at different sampling periods and in different 10 litre container samples, whereas enteroviruses were detected three times in two different

samples at different times after the initial finding of adenoviruses. In total 8 out of 36 samples from site L5 tested positive with the enteric viruses in question. The other five different sites tested negative during the first sampling in October 2011. Three of these sites (60 %) tested positive five times during the observation period with adenoviruses. Similarly four of these sites also tested positive for enterovirus seven times. These sites included L2, L4, L5 and L6. All the samples from the other two sites L1 and L3 tested negative for enteroviral contamination. However, these sites tested positive for adenovirus. The sites that tested negative for adenovirus were L2 and L4. In general, 100 % (6 out of 6) of the sites tested positive at least once for either enterovirus or adenovirus. Overall eleven samples tested positive for adenovirus (Table 4.7) from four different sites, whereas enterovirus was only detected in seven samples also from four different sites.

Table 4.3: Detection of the two enteric viruses from all the sampling points

Area (Number of samples (n=36)	Vol of water filtered (l)	Total No. of samples	Positive samples (%)	Adenovirus (%)	Enterovirus (%)
L1 (n=6)	360	36	1(2.78)	1 (9.1)	0 (0)
L2 (n=6)	360	36	1(2.78)	0 (0)	1 (14.3)
L3 (n=6)	360	36	2(5.56)	2 (18.2)	0 (0)
L4 (n=6)	360	36	1(2.78)	0 (0)	1 (14.3)
L5 (n=6)	360	36	8(22.22)	5 (45.5)	3 (42.9)
L6 (n=6)	360	36	5(13.89)	3 (27.3)	2 (28.6)
Total	2160	216	18(8.3)	11(61)	7(39)

The values in the brackets represent the proportion of the total number of the samples and each type of virus detected by site.

The proportion of the viruses detected was higher in L5 and L6 for both viruses as compared to other sites with adenoviruses being the most prevalent virus type during this study period. The site whose samples were highly contaminated with the viruses was site L5 where 22.22% of the samples tested positive for enteric viruses, while the least contaminated sites were sites LI, L2 and L3 with 2.27% of the samples testing positive.

There was a significant difference in the level of contamination of the sites with adenoviruses (p=0.0373), on the contrary the contamination of the sites with enteroviruses was not significantly different (p=0.3046) (Table 4.4). The highest mean adenovirus was observed in site L5 (0.14) while the lowest mean was observed in site L1 and L4 (0.03). The highest mean enterovirus was observed in site L5 (0.08) while the lowest mean was observed in sites L2 and L4 (0.03). Adenoviruses were never recorded from sites L2 and L4 while enteroviruses were not observed from sites L1 and L3 (Table 4.4).

Table 4.4: Mean number of water samples from the six sites detected with adenovirus and enterovirus.

Treatment	**Adenovirus**	**Enterovirus**
Site		
L1	0.03±0.03a	<LOD
L2	<LOD	0.03±0.03a
L3	0.06±0.04a	<LOD
L4	<LOD	0.03±0.03a
L5	0.14±0.06a	0.08±0.05a
L6	0.08±0.05a	0.06±0.04a
P values		
Site	0.0373	0.3046

*values followed by the same letter along the column are not significantly different based on Tukey's HSD at p≤0.05; LOD-Limit of detection

4.5 Influence of seasonality on the contamination of lake waters along Homa Bay town by the enteric viruses

Data from Livestock Development and Fisheries Development Kenya from 2011 to 2012 indicates the highest recording of rainfall was in April 2012 during the entire six months sampling period. The maxim recording was about 165 mm (Figure 4.1).

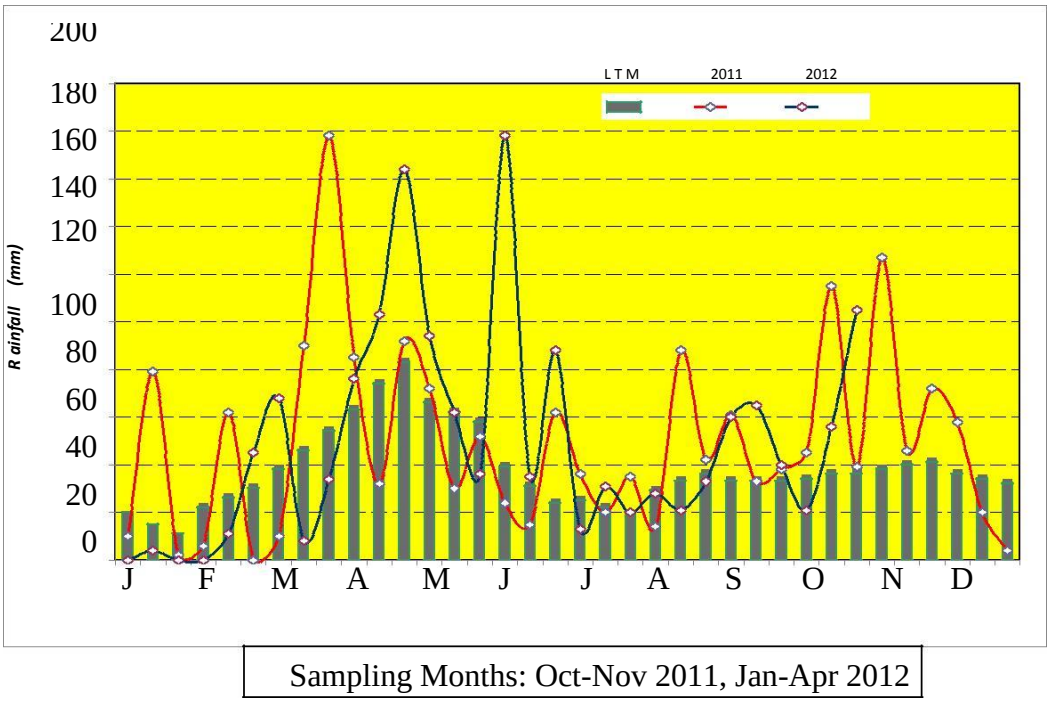

Figure 4.1: 2012 Rainfall distributions, Homa Bay, Nyanza.
Source: FAO, WFP, FEWS NET and KFSSG (2012).

January 2012 recorded the lowest rainfall over the sampling period with the highest recorded amount being less than 10 mm. Viruses were detected at least once in each of the six sites sampled during the two seasons, but no site was virus-positive in every sampling month (Table 4.5). Out of 108 samples collected between October and November and in April which were rainy months, 10 (9.26 %) tested positive for viral

contamination. Similarly out of the 108 samples collected from January to March during the dry season, 8 (7.41 %) tested positive for adenovirus and enterovirus contamination.

Table 4.5: Summary of viruses detection from each site during the wet and the dry seasons

Month	Season	Enteric virus	L1	L2	L3	L4	L5	L6
Oct 2011	Wet	Enterovirus	0	0	0	0	0	0
		Adenovirus	0	0	0	0	1/11	0
Nov 2011	Wet	Enterovirus	0	0	0	0	2/7	0
		Adenovirus	1/11	0	0	0	2/11	0
Jan 2012	Dry	Enterovirus	0	0	0	0	1/7	0
		Adenovirus	0	0	2/11	0	0	0
Feb 2012	Dry	Enterovirus	0	0	0	1/7	0	0
		Adenovirus	0	0	0	0	0	2/11
Mar 2012	Dry	Enterovirus	0	1/7	0	0	0	0
		Adenovirus	0	0	0	0	0	1/11
April 2012	Wet	Enterovirus	0	0	0	0	0	2/7
		Adenovirus	0	0	0	0	2/11	0

The numerator represents the number of viruses detected in a given month, while the denominator represents the total number of the specific virus detected during the whole period. Zeros represent non detection.

There was no significant difference in the number of adenovirus detected ($p = 0.7440$) during the dry and wet season (Table 4.6). The highest mean adenovirus detected was observed during the wet season (0.06) while the lowest was observed during the dry season (0.05) as shown in Table 4.6. There was a significant interaction ($p=0.0003$) between site and season in influencing the number of adenovirus detected (Table 4.6). There was no significant difference in the number of samples positive for enteroviruses ($p = 0.7010$) during the dry and wet season (Table 4.6). The highest mean for enterovirus

was recorded during the wet season (0.04) in comparison to the dry season (0.03) with a very small margin (Table 4.6). There was a significant interaction (p=0.0077) between site and season in influencing the detection of enteroviruses (p=0.3046).

Table 4.6: Mean number of the samples detected with viruses during the dry and wet seasons

Treatment	Adenovirus	Enterovirus
Season		
Dry	0.05±0.02a	0.03±0.02a
Wet	0.06±0.02a	0.04±0.02a
Site		
L1	0.03±0.03a	<LOD
L2	<LOD	0.03±0.03a
L3	0.06±0.04a	<LOD
L4	<LOD	0.03±0.03a
L5	0.14±0.06a	0.08±0.05a
L6	0.08±0.05a	0.06±0.04a
P values		
Season	0.7440	0.7010
Site	0.0373	0.3046
Site*Season	0.0003	0.3046

Values followed by the same letter along the column are not significantly different based on Tukey's HSD at p≤0.05; LOD-Limit of detection

4.6 Influence of proximity of the defunct sewage treatment plant and pit latrines to the shore on the contamination of the lake waters by the enteric viruses

A total of sixteen pit latrines were identified from the study area mostly owned by individual homesteads while others owned communally especially the people from the nearby market where the remaining fish products commonly known as "*Mgongo wazi*" from the nearby fish processing factory (Capital Fish) are sold. Two of them were located at an estimated distance of about 50m from the shoreline, 5 between 51m-100m while 9

beyond 100m. Numerous other pit latrines were however located outside the capture zones of the study area. Two pit latrines were identified close (about 50 m) to site L3 from which some samples tested positive for the viral contamination. Positive samples were also recorded from sites that were about 70 and 100 m away from the pit latrines. From the sites that were closer to sources of contaminations like sewage effluents such as L5 and L6 viruses were detected in higher frequency than the other sites with adenovirus being detected the most.

Odds Ratio analysis (Table 4.14) indicates that pit latrines that were 50 and 70 metres away had a significant influence on the contamination of Lake Victoria waters with Odds Ratio (OR) of 20.28 (P = 0.005) and OR of 11.45 (P = 0.029) respectively.

Table 4.7: Multivariate analysis proximity to the sewage plant and pit latrines as factors influencing contamination of Lake Victoria waters along Homa Bay town

Factors	Odds Ratio	P-value	95 % Confidence Interval	
Distance from the nearest latrine				
50	20.28	0.005	2.42	169.74
70	11.45	0.029	1.28	102.13
80	4.18	0.250	0.37	47.68
100	4.18	0.250	0.37	47.68
300	(Reference)			
Distance from the sewage plant				
20	4.86	0.014	0.95	24.75
70	2.74	0.039	0.49	15.17
700	1.00	0.044	0.13	7.514
1500	1.00	0.044	0.13	7.514
2000	0.49	0.563	0.04	5.608
3000	(Reference)			

The variable contamination was derived from the adenovirus and enterovirus as indicators.

Similarly sites that are 20, 70,700 and 1500 metres away from the defunct sewage plant have significant likelihood of being contaminated with OR of 4.86 (P = 0.014), OR of 2.74 (P = 0.039), OR of 1.00 (P = 0.044) and OR of 1.00 (P = 0.044) respectively.

There was a significant negative correlation between the estimated distance from the latrines and the number of samples testing for adenovirus (p=0.007, r=-0.183). Similarly there was a slight significant negative correlation between the distance from the sewage treatment plant and the number of adenovirus found (p=0.042, r=-0.139). There was no significant correlation between the detection of enterovirus and the distance from the latrines and the estimated distance from the sewage treatment plant (p>0.05) (Table 4.8).

Table 4.8: Pearson correlation of estimated distance from the sewage treatment plant and the latrines and viruses present

	Estimated distance from nearby latrines	Adenovirus detected	Enteroviruses detected
Adenovirus detected	-0.183 0.007		
Enteroviruses detected	-0.088 0.198	-0.042 0.535	
Distance from sewage treatment plant	0.207 0.002	-0.139 0.042	-0.081 0.238

*Cell Contents: Pearson correlation value; P-Value at 0.05 probability level

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Characteristics of the study sites and water samples

Most of the samples from all the sites had a normal range of values from the analysis of the physical quality parameters. The values were within the WHO acceptable standards (WHO, 2004) except for turbidity. Most of the samples had a neutral pH most of 7.0, qualifying the water source to be fit for domestic consumption (Onda *et al.,* 2012). The average temperature of the samples was 25^{o}C with a range of 24^{o}C to 26^{o}C which falls within the recommended temperature ranges for inland waters and suitable for aquatic life which does not thrive well when the temperature changes by a ±2 ^{o}C. The turbidity range of 5-30 was not within the WHO recommended levels which is below 5 NTU. These higher levels of turbidity recorded during the study can be attributed to agricultural activities from the surrounding community especially during the rainy seasons properly as a result of runoff carrying silt (Alkarkhi *et al.,* 2009). Higher turbidity makes it difficult to execute various water purification processes such as flocculation and filtration thereby increasing the cost of water treatment and consequently leading to increased chances of feco-oral contaminations (Alkarkhi *et al.,* 2009).

Electrical Conductivity (EC) and Total dissolved solids (TDS) varied from wet to dry season with slightly higher recordings during dry seasons. The results of conductivity and TDS fell within the thresholds recommended by WHO for fresh water which can be used for domestic purposes. WHO recommends a range of 500-5000 mS/cm for conductivity and less than 1000 mg/l for Total dissolved solids (TDS). There were high concentrations

of Dissolved oxygen recorded although a larger percentage fell within the WHO recommended range of 8-9 mg/l for surface water. A DO below 5 mg/l is highly polluted and not suitable for aquatic life and may act as an indicator for poor water quality (DFID, 1999). There was a variation of results of DO with a range of 7.8-10.40 recorded across the two seasons symbolizing availably of limited organic waste in the lake. Water from most of the sites however is suitable for domestic use except for site L5 and L6. Based on the observation, these sites are identified as not suitable for domestic use since their pollution intensity from the nearby dilapidated sewage treatment system is higher.

5.1.2 Adenoviruses and enteroviruses detected from samples for the six sites

Studies have revealed that different types of viruses are present in surface waters worldwide (Haramoto *et al.*, 2018). In the present study, the results generally suggest that the lake water is contaminated with enteric viruses, even though the study never included analysis of the viral concentration levels in the lake waters. The level of contamination varied according to the location of a sampling site. The six sampling sites were not the same in their levels of contaminations as indicated by the varied percentage of the number of samples testing positive for contamination at every site. Eight per cent of all the samples (18 out of 216) tested positive for the enteric viruses thus confirming that the LV waters along Homa Bay town is contaminated with Human enteric viruses. The results may still be an underestimation of the actual levels of contamination in that certain limiting factors such as incomprehensive procedures could have affected the overall outcome. Findings from the present study show that the sites from which the samples were obtained are contaminated with human pathogenic enteric viruses. This suggests

that there is poor viral quality of the lake water, and therefore it is not safe for domestic usage without proper treatment. It is however important to take cognisant of a probability of the fact that not all the viruses detected are infectious. There is a probability of existent of non-infectious particles in the lake (Hot *et al.,* 2003). Reverse Transcription-PCR and nested PCR methods used in the present study can detect both the infectious and non-infectious virions without discrimination thus the possibility non-infectious or inactive particles from the positive samples.

All the six selected sites tested positive for viral contamination suggesting that this lake is contaminated with the viruses and there is a possibility of other natural water courses getting contaminated as well. The viral contamination of the lake could be attributable to increase in population density which has resulted into massive discharge of human wastes into the lake. The poor viral quality of the lake may also be due to contact with animal faeces. Little research has been documented concerning presence of viruses in environmental samples in Kenya, despite success in related research in different various sites around the globe (Shah *et al.,* 2017). From the results of this study, there is a clear indication that water may play a very important role as a source of infection in the surrounding community in Kenya and Africa in general.

Presence of viruses in the lake water suggests that viruses can possibly end up in the lake through different ways. One of the main pathways is through sanitary facilities such as sewers, pit latrines and septic tanks. Location of such facilities within the vicinity of the water ways as well as poor maintenance is likely to enhance rapid bypass mechanism

exacerbated by the flow of water in the soil and this forms localized pathway system. Viruses can also end up in the lake through the aquifer pathway which involves movement of viruses through the subsoil to the water table. The viruses are small enough to go through the porous aquifer rocks or sediments during seepage of contaminated sources (Eftim *et al.,* 2017). Virus persistence in the environment is an interesting and an important consideration and this can be confirmed by the fact that viruses were detected from the lake water samples in this study. Research activities have shown that viruses can survive in different environments for a fairly long time even though they never replicate outside the host cell (Ettayebi *et al.*, 2016).

The presence and persistence of viruses in environmental samples as observed from this study are likely to cause serious public health related issues to the surrounding community (Wong *et al.,* 2009). Precautions should therefore be taken especially when it comes to the detection of the specific viruses circulating in the community as well as in the treatment of the water sources for domestic use. As part of the detection procedure, glass wool filtration method for viral concentration process was used. This method was chosen because of it is less costly, easy to operate and can be applied to samples with low and high levels of fecal contamination. Besides, it also has the additional advantage of avoiding inhibition of the reverse transcription and PCR amplification (Grabow *et al.*, 2007), which is a common problem in many environmental samples (Vilaginès *et al*., 1993). The procedure for viral recovery and nucleic acid extraction from the environmental samples was relatively simple and can be applied indiscriminately to DNA and RNA viruses.

The two human specific viruses considered under this study were evaluated as possible indicators for contamination of environmental waters by viruses. Positive samples were recorded form all the six sampling points. Slightly higher number of samples tested positive for adenoviruses indicating that adenoviruses were more prevalent than enteroviruses. Adenovirus has been shown to be a perfect indicator for viral contamination of the environmental samples due to its ability to resist common environmental treatments (Akihara *et al.,* 2005). There is potential risk of adenovirus infection to the surrounding community that depend on the lake for various economic activities due to the fact that adenovirus was detected in higher numbers. Human adenovirus related infection outbreaks have been documented in previous studies especially in recreational waters (van Heerden *et al.,* 2005a). Adenoviruses are known to be persistent in environmental waters and are less susceptible to the effects of solar radiation. This could be one of the reasons as to why more adenoviruses were detected compared to enteroviruses (D´Ugo *et al.,* 2016). Adenoviruses have specifically been found to always outnumber enteroviruses in occurrence when both are isolated in environmental samples (Carter, 2005).

Prior studies have shown that adenoviruses are resistant to various chemical inactivation such as chlorination (Katayama *et al.,* 2008). It has been reported that adenoviruses also infect and can be spread in the environment by adults besides the children with diarrhoea. This could be one of the factors contributing to its dominance in the detection. This dominance and persistence of adenovirus in the environment makes it ideal as potential indicator for viral contamination of the lake (Chaudhry *et al.,* 2015). Adenovirus isolation

from the environmental samples has been proposed as intimation for the contamination by human enteric viruses (Pina *et al.,* 1998). In comparison to other enteric viruses, adenoviruses can be found in abundance in sewage contaminated environments and are also rather stable in wastewaters according to previous documented studies (Pina *et al.,* 1998). Certainly this study gives a clue to the fact that there could be high amount of waterborne enteric viruses in LV waters along Homa Bay town than we may imagine and exactly affirms that LV waters are actually contaminated with human fecal matter. The viruses have been isolated from similar other open environmental waters all over the world such as rivers water, lakes, oceans and pools (Chapron *et al.,* 2000).

Most of the samples (92 %) tested negative for viral contamination. The higher percentage of negative results could be ascribed to the fact that viruses concentration in environmental samples is always low (D´Ugo *et al.,* 2016). This means that there could be viruses that were undetected during the study from the negative samples. This poses inherent public health risks to the consumers of the lake water for various domestic and recreational uses especially due to the fact that there is a constant human activity along the study area. The viruses may also end up contaminating drinking water within the region due to the fact that they have the ability to percolate. Depending on the type of the virus, estimates suggest that there is increased risk of viral infection to the consumers if the concentration of the viruses in drinking water is higher than 1.9×10^{-3} $litre^{-1}$ to 2.22×10^{-7} $litre^{-1}$, depending on the virus type (Regli *et al.,* 1991).

Detection of these viruses from the LV waters means the surrounding community is at the risk of enteric virus infection. The surrounding community actually draws water from nearby beaches or areas used even for recreational purposes. Examples of recreational activities that were noted in the sampled areas include swimming, boat rowing sport, tourism and fishing. Presence of viruses in such recreational areas may pose serious public health risks. These viruses are important pathogens and have been isolated repeatedly from various waters which have been impacted directly or indirectly by fecal contamination just like in Homa Bay region case (van Heerden *et al.*, 2005b).

Centres for Disease Control and Prevention (CDC) estimates that most fresh water sources used for drinking water in developing countries is contaminated by human enteric viruses (CDC, 2000). The significance of enteric viruses as waterborne pathogens has just as of late been perceived despite the fact that they have been related with waterborne outbreaks (Calgua *et al.*, 2008). According to the World Health Organisation and the CDC the role of enteric viruses as waterborne pathogens is continuously getting recognition in environmental waters (Albinana-Gimenez *et al.*, 2009).

5.1.3 Influence of seasonality on the contamination of lake waters by the viruses

Recent studies have revealed seasonal profiles of virus concentration in certain environmental waters. For example, human caliciviruses have been found to be more prevalent during the colder months of the year (Haramoto *et al.*, 2018). In the present study, it was observed that there was slightly higher rainfall that resulted in the month of November 2011 and thus the lake received a lot of runoff water 2011 which was the

second month of sampling. However during this high rainfall period, the results show that there was neither a significant increase in the number of viruses detected in the month of November nor in the following months of January or February which were also wet seasons. This result is consistent with findings of some studies in regards to seasonal concentrations of the two enteric viruses. Adenoviruses and Enteroviruses have been reported to show relatively little change in concentration with changes in seasonal trends throughout the year (Katayama *et al.*, 2008).

Based on the results, virus detections varied through the sampling months that included both the wet and the dry weather from individual sites. Rainfall activities may lead to increase in the level of contamination of the surface waters by enteric viruses as a result of occasional discharges from the run-offs. This was never the case with this study as the detection of the two types of enteric viruses never changed significantly during flooding heavy rainfall months of October 2011, November 2011 and April 2012. All the three heavy precipitation months resulted in episodic recharge events, as indicated by rising water levels and storm sewer flows. Virus detections also peaked in January to March 2012, a season when no rainfall was recorded. Despite the detections varying with time at each site, there was no difference in the timing of virus detection among the six sites ($P = 0.622$), suggesting that virus contamination was the result of some other factors common to the sites sampled. Point sources of contamination could be the common factor that leads to contamination of environmental waters (Fong and Lipp, 2005).

Studies have found that virus can persist suspended in environmental water for several days at both very low and very high temperatures (Bitton *et al.,* 1982). It has been reported from past studies that viral persistence in the environmental waters increases with decrease in temperature. This means that viruses are likely to persist more if the lake is cooler than when warm (Brashear *et al.,* 1982). There was no significant difference in variation in temperature recorded during both the wet and the dry seasons and this could be one of the reasons as to why there was no significant deference in the detection of viruses in both dry and rainy seasons according to the results.

Viruses have been isolated throughout the year with slight increase in concentration during rainy seasons though not always (Brashear *et al.,* 1982). As for this study, there seems to be no correlation between contamination of the lake with the viruses to rainy seasons as the presence were somehow evenly distributed across both dry and wet seasons. The number of viruses detected did not change significantly during the wet months of sampling of October, November and April when there were severe flooding, compared to the dry moths of January, February and March. Despite the fact there is no significant change in detection during wet seasons, it can be assumed that viruses may have been transported to the sampling sites later after getting desorbed from subsurface sediments after infiltration of rainfall hence can be detected at a later date (Eftim *et al.,* 2017).

Studies have shown that there are chances of increase in ease of transportation of viruses with increase in water flow and this may increase chances of viral detection (Xagoraraki

et al., 2007). However few viruses were detected during increased water flow period in the wet months and a possible explanation for this could be the dilution effect of the flooding activities (Kota *et al.,* 2014). Some samples collected during the dry months tested positive. During these dry seasons less water flow happens but contamination of the lake water by the viruses can still occur through aquifer pathways. Virus detection during this period is not affected by the dilution effect due to less water flow (Wolfaardt *et al.,* 1995). Contamination during the dry season can be through the ground flow probably exacerbated by the positive surface waters which is in contrast to the wet season when contamination is likely to be through water flow in the upper part of the soil (Tani *et al.,* 1995). Generally there was low count of the positive samples during the dry seasons as only 7.41 % of the samples tested positive. The occurrence of viruses recorded from the sampling sites receiving significant contributions of surface water during the dry seasons when there are no floods may be lower (Lipp *et al.,* 2001).

5.1.4 Influence of proximity of the defunct sewage treatment plant and pit latrines to the shore on the contamination of the lake waters by enteric viruses

The occurrence of viruses in environmental samples can be affected by among other factors, discharge of fecal pollution such as sewage and pit latrines. Fecal contamination of the water was much more in the sites closer to the sewage plant compared to those sites that were far away as indicated by the number of samples that tested positive. However, viral contamination was realised from all the sites indicating the same levels of water quality. At least one type of virus was detected from every site, however sites L5 and L6 which were located much closer to the dilapidated County sewage treatment plant had higher number of samples with viruses recorded. This is a clear indication that the

fecal contamination levels of these two sites were higher than the rest due to the sewage effluent. Other than sites L5 and L6 that had contamination of the two virus types screened, the other sites were virus negative for certain types of viruses on different samples. Enterovirus was not detected in L1 and L3 sites, while adenovirus was not detected in L2 and L4 sites.

The absence of these enteric viruses in certain samples is not surprising because they are generally present in infected humans. Therefore, the dilapidated county sewage treatment plant presumed to be collecting wastewater from healthy humans may not have these enteric viruses. Site 5, with high surface water contamination with sewage effluent, was virus positive for quite a number of samples within four different months of sampling. The positive samples were collected during the months of October, November, January and April. Site 6 which is closer to site 5 also had an intermediate sewage contamination, but had a lower virus occurrence rates compared to site five. Therefore, this gives an impression that the County sewage treatment plant could be one of the fecal contamination sources to the lake. Besides the sewage treatment plant, the study has a close proximity to other possible sources of fecal contamination such as pit latrines, soak pits, septic tanks and the landfills though this is located about 2 km from the study area. Homa bay town has numerous pit latrines and septic tanks as an alternative to the inadequate sewage treatment system. Some of the pit latrines are located far outside the catchment of the study areas, but this town is located on a hilly ground and the possibility of infiltration down to the lake.

It has been demonstrated by previous studies that presence of pit latrines within a short radius of the water sources increases significantly chances of viral contamination (Van Puijenbroek *et al.,* 2015). This narrative is in concordance with the fact that viruses can be transported through several routes including underground water. Past studies have generally hypothesized that viruses can travel longer distances beyond 1 km under optimum conditions (Kiulia *et al*., 2009). The risks associated with closeness of pit latrines and chances of microbial contamination to the water sources have been given due attention in developed counties. This is however not the case in most developing countries (Menor, 2007).

In general some sites which were located within the vicinity of the sewage outlets and the beach may have created the potential for fecal pollution especially during wet conditions; HAdVs were detected in mostly site L5 as well as in site L6. Enterovirus was also detected majorly in both sites. The two sites were the closest to the sewage treatment plant outlets emptying directly into the lake. It is therefore very possible that the sewage treatment plant was one of the sources of fecal termination that may have contributed to the occurrence of HAdV and enteroviruses in the LV waters along Homa Bay town. There is likelihood of other many sites around the study area, towns or other human activities along the commonly used sites along the lake discharging untreated or under-treated sewage into this lake thereby increasing chances of viral contamination (Haramoto *et al.,* 2018).

From the Odds Ratio analysis there is an indication that the pit latrines that were 50 metres from the lake shore were 20.28 times more likely to cause contamination to the Lake Victoria as compared to those that are 300 metres away while those that were 70 metres away were 11.45 times more likely to cause contamination than those that are 300 metres away (Table 4.14). Similarly sites that are 20 metres away from the sewage plant can easily get contaminated with the viruses 4.8570 times than those that are 3000 metres away while those that are 70 metres away are 2.7419 times more likely to get contaminated compared to those that are 3000 metres away and those that are 700 and 1500 metres away are equally likely to be contaminated as compared to those that are 3000 metres away (Table 4.14).

The results largely provides evidence on the extent of viral contamination of the lake waters in Homa Bay town and suggests the most probable sources of this contamination which is majorly from the spillage of the raw sewage from the dilapidated municipal sewage treatment plant. The distance between the shoreline and the pit latrines was also estimated to be generally short and this may as well increase the chances of viruses being transited to the lake from the pit latrines.

Other than the dilapidated County sewage treatment plant and the pit latrines around the capture zones of the study area, other potential sources of viral contamination to the lake were identified in the area. They include: Septic tanks and soak pits which are a common method of fecal disposal in the area besides the pit latrines. Other potential sources of fecal pollution were the Landfills, farming activities which include waste water irrigation

system, use of manure as well as livestock. One of the landfills was located in a hilly area about 2 km from the study area. Another landfill was identified about 3 km from the study area but along the banks of one of the rivers that drains into to the lake. Some of the common wastes disposed here are the remains of the fish products commonly known as "*Mgongo wazi*" from the nearby fish processing factory. Several livestock were observed grazing next to site L3 and there is a possibility of them contributing to the fecal load of the lake thereby leading to viral contamination in case of zoonotic pathogens (Millen *et al.*, 2012). However there are no results concerning this assumption as this study was limited to human specific viruses only.

The surrounding of the study sites is a residential area and has a system of sewer lines. Some of the sewer pipes are broken and leaks and given the downhill nature on which the town is located, the viruses can be ferried easily to the catchment area of the study site. One of the sites (L3) was closer to one of the pit latrines observed in the study area compared to other sites tested positive for viral contamination. All other sites including the ones far away from pit latrines and also far away from the dilapidated municipal sewage treatment plant were however found to be contaminated as well. Many of the people residing around the study area heavily rely on pit latrines which may never be sufficient in number to handle the large population considering that this population is always on a steady rise. This is besides the apparent lack of proper infrastructure when it comes to sewerage system and these factors are likely to contribute to the fecal contamination of the lake. The ever increasing population and the socioeconomic activities in the town will automatically lead to an increase in the number of fecal

disposal facilities such as pit latrines. This increase is likely to lower the risk of human infections that would occur through direct contact with fecal matter; however the increase in the number of such facilities could also lead to the fecal contamination of the surrounding lake water (Lipp *et al.*, 2001).

5.2 Conclusions

i. Most of the samples had an average of the physical water quality parameters that were within the range accepted by the WHO. The water is therefore suitable for domestic use as far as the physical water quality parameters are concerned. The physical water parameters did not affect the occurrence of enteric viruses in Lake Victoria waters along Homa Bay town.

ii. Positive virus detection confirms that there is sufficient evidence to conclude that Lake Victoria waters along Homa Bay town are contaminated with human pathogenic enteric viruses and that the lake waters are constantly getting impacted by human fecal pollution which is a public health issue. The evidence that HAdV and enterovirus strains have been detected in these waters also supports the evidence that the lake water may be an important reservoir of enteric viruses in this community and therefore proper treatment is advisable before use.

iii. Adenoviruses and enteroviruses occurrence in LV waters are relatively constant throughout the year without significant variations in their profile with changes in season. Changes in season are therefore an unreliable factor for prediction of viral contamination peaks in the lake.

iv. Being that most of the positive samples were from sites closer to the dilapidated sewage treatment plant, we can conclude that there is significant correlation between the levels of viral contamination of the lake and its proximity to the dilapidated sewage treatment plant as well as the pit latrines as the main sources of fecal pollution.

5.3 Recommendations

i. With regards to physical water quality parameters being found to be within the WHO acceptable levels for domestic use, continuous sampling and analysis of the LV waters for the same are recommended. This would be important in continuous understanding the hydrological characteristics changes of the lake for proper management of its quality in reference to the WHO set standards.

ii. The lake waters have been found to be contaminated with enteric viruses, some of which may be resistant to different environmental treatments with different genetic variability. Further studies are required to determine the diversity and the extent of contamination of the LV waters with enteric viruses for a relatively longer period.

iii. Having identified the dilapidated sewage treatment plant and the pit latrines as point sources of fecal pollution to the lake and consequently viral contamination, it is recommended that such elements be incorporated as part of comprehensive approach to the prediction of viral contamination in efforts to control fecal pollution.

iv. There was no significant influence of season in detection of the viruses as per the present study, however, more sampling in different regions and during different seasons is recommended to establish their geographical distribution and relatedness to seasonal distribution patterns.

5.4 Areas of future research

i. Although this study has established the occurrence of enteric viruses in LV waters, there are no documented reports concerning their implications as far as public health is concerned in the region. Further research and analysis is therefore recommended to ascertain the true position concerning the public heath implication.

ii. Other potential sources of fecal pollution of the lake were identified within the catchment of the study area including landfills, manure, septic tanks, and irrigation activities. Further research is therefore recommended to determine their contribute to the fecal load of the lake vis a vis the enteric virus occurrence.

iii. Livestock were observed grazing next to one of the sampling sites and this could suggest that livestock may also be contributing to the fecal load in the lake and possibility of presence of potential zoonotic pathogens. Further research is therefore recommended to determine the extent to which livestock contributes to the fecal load of the lake and the potential for zoonotic pathogens.

iv. With this study limited to the determination of the presence or absence of the viruses only, Hexon sequencing approach, gene typing and other molecular methods is recommended so as to assist in the determination of the genetic relatedness of the viruses circulating in that community.

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APPENDICES

Appendix I: Buffers and solutions

a) Buffers for Nucleic Acid extraction (Glycine beef extract buffer-GBEB)
   - Glycine 3.754 g
   - Beef extract 5 g
   - Water
   - 1 M NaOH
   - 1 M HCl
   - TBE- 100 ml
   - Tris base- 4 g
   - Boric acid-2.75 g

b) PCR buffer
   - 10-50 mM Tris-HCl pH 8.3,
   - Up to 50 mM KCl, 1.5 mM or higher $MgCl_2$,
   - Primers 0.2 – 1 µM each primer,
   - 50 – 200 µM each dNTP,
   - Gelatin or BSA to 100 µg/ml, and/or non-ionic detergents such as Tween-20 or Nonidet P-40 or Triton X-100 (0.05 - 0.10 % v/v).

c) Reagent preparation

   Preparation of 1 M HCl was carried out by dissolving 82.5 ml of HCl stock solution to water to make a solution. Water was then added to make a total volume of 1000 cm^3 of solution. Preparation of 1 M NaOH involved dissolving of 40 g of Sodium hydroxide pellets in water and then topped up to make 1000 cm^3 of solution.

Appendix II: Data analyses

A). Frequency distribution of the pH and the temperature of the samples from all the months

TEMP (ºC)	Frequency	Percentage	pH	Frequency	Percentage
24	12	5.56	7	210	97.22
25	35	16.2	7.5	6	2.78
26	169	78.24			
Total	216	100	Total	216	100

B). Physical parameters seasonal comparison using *t* test

a) pH

Paired T-Test and CI: Wet, Dry
Paired T for Wet - Dry

	N	Mean	StDev	SE Mean
Wet	108	7.0278	0.1151	0.0111
Dry	108	7.0000	0.0000	0.0000
Difference	108	0.0278	0.1151	0.0111

95% CI for mean difference: (0.0058, 0.0497)
T-Test of mean difference = 0 (vs ≠ 0): T-Value = 2.51 P-Value = 0.014

b) Temperature

Paired T-Test and CI: Wet, Dry
Paired T for Wet - Dry

	N	Mean	StDev	SE Mean
Wet	108	25.5556	0.6881	0.0662
Dry	108	25.8981	0.3039	0.0292
Difference	108	-0.3426	0.7873	0.0758

95% CI for mean difference: (-0.4928, -0.1924)
T-Test of mean difference = 0 (vs ≠ 0): T-Value = -4.52 P-Value = 0.000

c) Electrical Conductivity

Paired T-Test and CI: Wet, Dry
Paired T for Wet - Dry

	N	Mean	StDev	SE Mean
Wet	215	12.86	12.69	0.87
Dry	215	48.42	36.37	2.48
Difference	215	-35.56	45.39	3.10

95% CI for mean difference: (-41.66, -29.46)
T-Test of mean difference = 0 (vs ≠ 0): T-Value = -11.49 P-Value = 0.000

d) Total dissolved solids

Paired T-Test and CI: Wet, Dry
Paired T for Wet - Dry

	N	Mean	StDev	SE Mean
Wet	108	47.56	21.59	2.08
Dry	108	50.14	33.72	3.24
Difference	108	-2.57	39.30	3.78

95% CI for mean difference: (-10.07, 4.92)
T-Test of mean difference = 0 (vs ≠ 0): T-Value = -0.68 P-Value = 0.498

e) Dissolved Oxygen

Paired T-Test and CI: Wet, Dry
Paired T for Wet - Dry

	N	Mean	StDev	SE Mean
Wet	215	27.97	24.79	1.69
Dry	215	29.00	30.87	2.11
Difference	215	-1.04	27.32	1.86

95% CI for mean difference: (-4.71, 2.64)
T-Test of mean difference = 0 (vs ≠ 0): T-Value = -0.56 P-Value = 0.578

f) Turbidity

Paired T-Test and CI: Wet, Dry
Paired T for Wet - Dry

	N	Mean	StDev	SE Mean
Wet	322	25.26	21.21	1.18
Dry	322	22.68	26.80	1.49
Difference	322	2.58	23.26	1.30

95% CI for mean difference: (0.03, 5.13)
T-Test of mean difference = 0 (vs ≠ 0): T-Value = 1.99 P-Value = 0.047

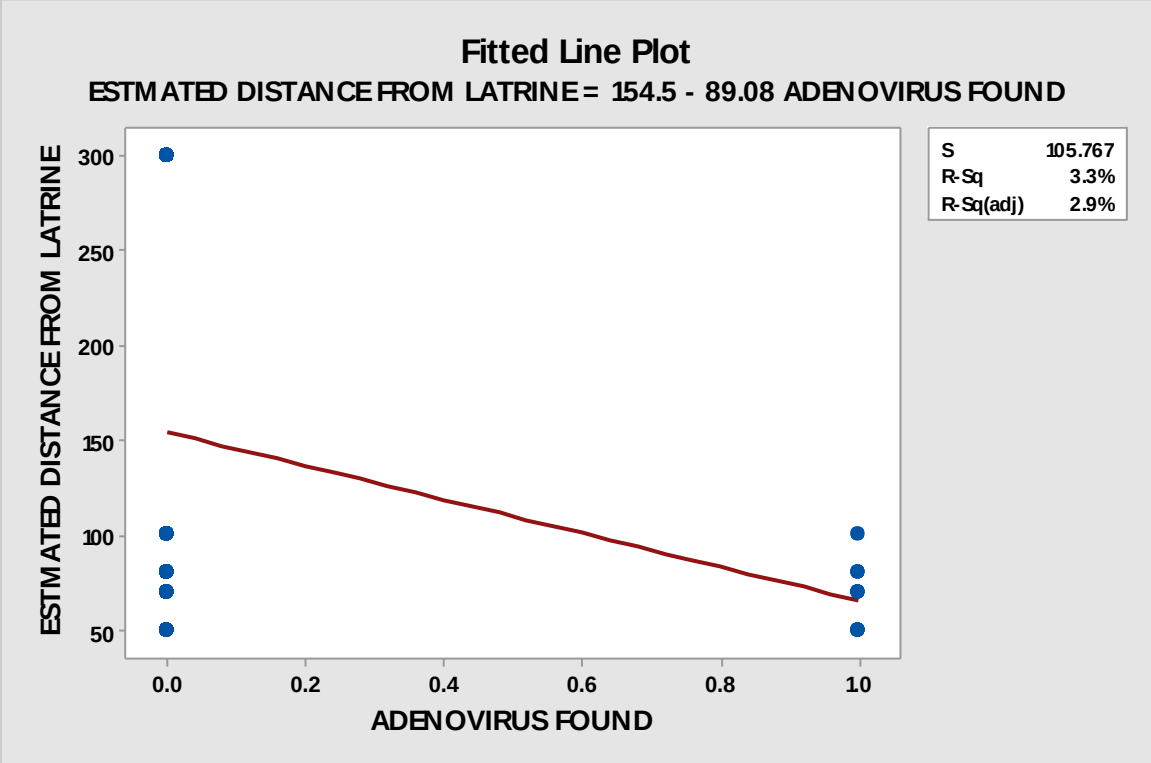
c). Regression analysis

1. **REGRESSION ANALYSIS: ESTMATED DISTANCE FROM LATRINE VERSUS ADENOVIRUS FOUND**

The regression equation is
ESTMATED DISTANCE FROM LATRINE = 154.5 - 89.08 ADENOVIRUS FOUND
S = 105.767 R-Sq = 3.3% R-Sq(adj) = 2.9%
Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	82846	82846.3	7.41	0.007
Error	214	2393954	11186.7		
Total	215	2476800			

FITTED LINE: ESTMATED DISTANCE FROM LATRINE VERSUS ADENOVIRUS FOUND



2. REGRESSION ANALYSIS: ESTMATED DISTANCE FROM LATRINE VERSUS ENTEROVIRUS FOUND

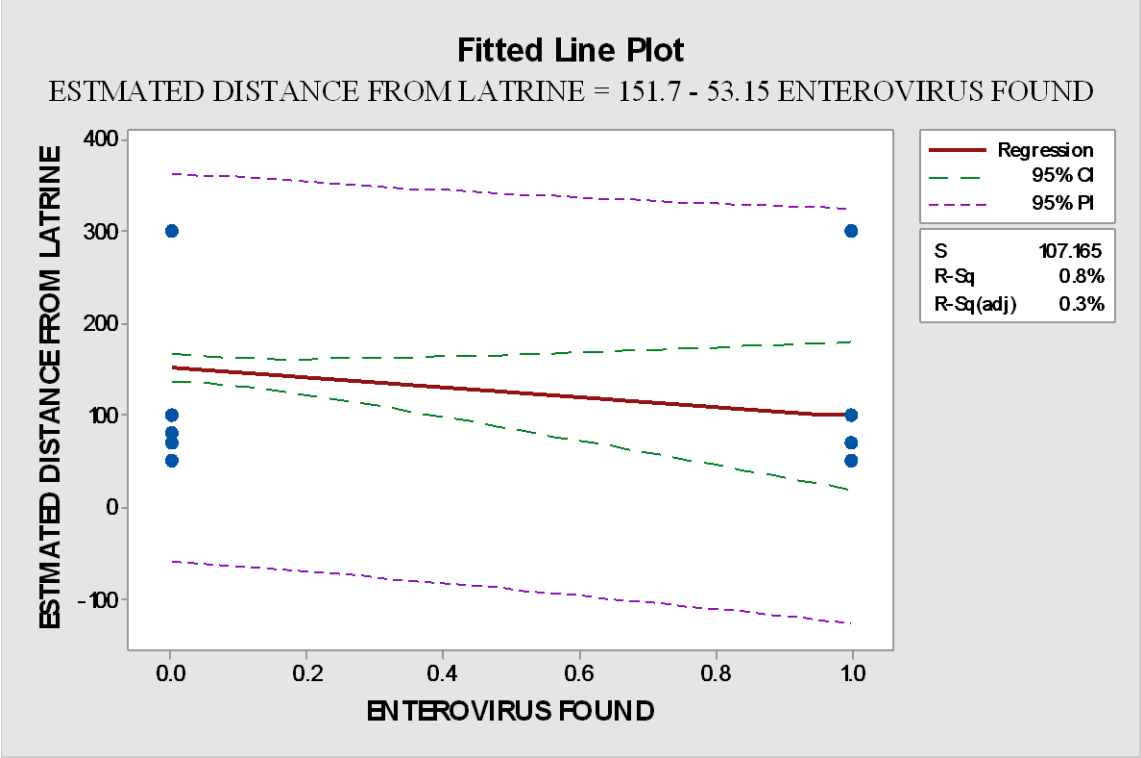
The regression equation is estmated distance from latrine = 151.7 - 53.15 ENTEROVIRUS FOUND

S = 107.165 R-Sq = 0.8% R-Sq(adj) = 0.3%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	19134	19134.4	1.67	0.198
Error	214	2457666	11484.4		
Total	215	2476800			

FITTED LINE: ESTMATED DISTANCE FROM LATRINE VERSUS ENTEROVIRUS FOUND



3. REGRESSION ANALYSIS: ADENOVIRUS FOUND VERSUS DISTANCE FROM THE SEWER

The regression equation is

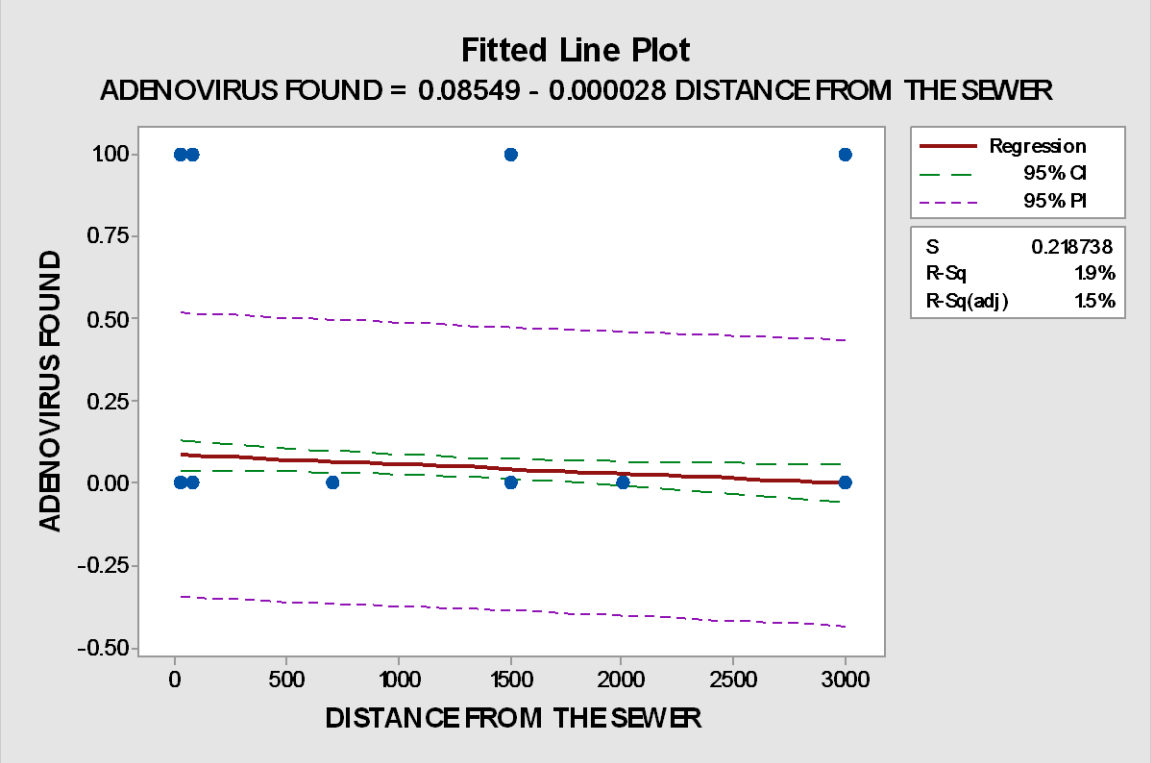
ADENOVIRUS FOUND = 0.08549 - 0.000028 DISTANCE FROM THE SEWER

S = 0.218738 R-Sq = 1.9% R-Sq(adj) = 1.5%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	0.2007	0.200725	4.20	0.042
Error	214	10.2391	0.047846		
Total	215	10.4398			

FITTED LINE: ADENOVIRUS FOUND VERSUS DISTANCE FROM THE SEWER

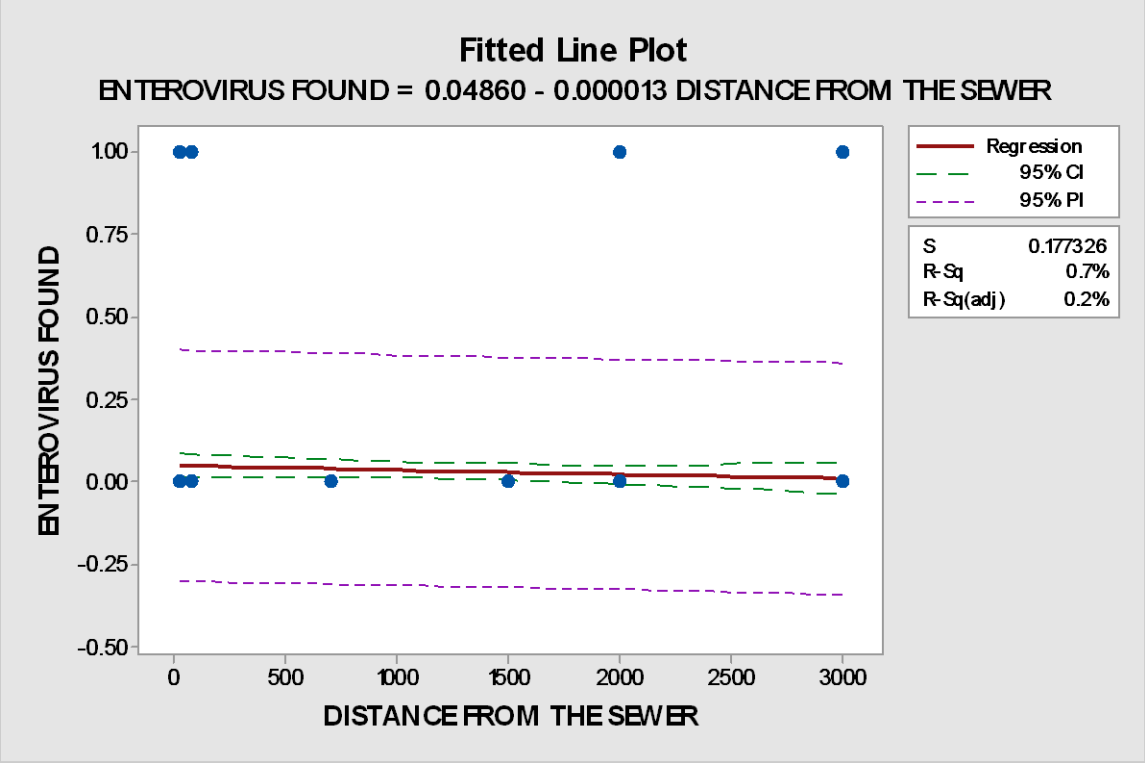


4. REGRESSION ANALYSIS: ENTEROVIRUS FOUND VERSUS DISTANCE FROM THE SEWER

The regression equation is
ENTEROVIRUS FOUND = 0.04860 - 0.000013 DISTANCE FROM THE SEWER
S = 0.177326 R-Sq = 0.7% R-Sq(adj) = 0.2%
Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	0.04405	0.0440505	1.40	0.238
Error	214	6.72910	0.0314444		
Total	215	6.77315			

FITTED LINE: ENTEROVIRUS FOUND VERSUS DISTANCE FROM THE SEWER



D). Pearson Correlation of Physical Parameters and Viruses present

	pH	Temp(^{0}C)	EDFL	Conductivity	TDS	DO	Turbidity	Adenovirus Found	Enterovirus Found
Temp(^{0}c)	*0.032 0.636								
EDFL	-0.147 0.030	-0.120 0.079							
Conductivity	-0.131 0.055	0.267 0.0001	0.108 0.112						
TDS	0.125 0.067	0.147 0.031	-0.465 0.0001	0.331 0.0001					
DO	-0.108 0.115	0.012 0.850	0.263 0.0001	0.245 0.0001	0.214 0.002				
Turbidity	0.247 0.0001	0.011 0.874	-0.005 0.945	-0.542 0.0001	-0.132 0.053	-0.157 0.021			
Adenovirus found	0.089 0.193	0.114 0.096	-0.183 0.007	-0.062 0.366	0.045 0.508	-0.083 0.222	0.108 0.113		
Enterovirus found	0.128 0.060	0.090 0.188	-0.088 0.198	-0.027 0.693	0.119 0.082	-0.068 0.317	0.087 0.202	-0.042 0.535	
EDFS	-0.186 0.006	-0.175 0.010	0.207 0.002	0.345 0.0001	0.358 0.0001	0.485 0.0001	-0.557 0.0001	-0.139 0.042	-0.081 0.238

*Cell Contents: Pearson correlation value. TDS- total dissolved solids, DO –Dissolved Oxygen, EDL-Estimated distance from pit latrines, EDS-Estimated distance from sewage treatment plant

P-Value at 0.05 probability level

E). Frequency distribution of the viruses detected

Frequency distribution of the enterovirus detected

Enterovirus Detected	Frequency	Percentage (%)
Absent	209	96.76
Present	7	3.24
Total	216	100

Frequency distribution of adenovirus detected

Adenovirus Detected	Frequency	Percentage (%)
Absent	205	94.91
Present	11	5.09
Total	216	100

Appendix III: **Absorbance ratios of the extracted nucleic acids**

Month	Season	Nucleic Acid	Sites L1	L2	L3	L4	L5	L6
Oct 2011	Wet	RNA	0	0	0	0	0	0
		DNA	0	0	0	0	1.75	0
Nov 2011	Wet	RNA	0	0	0	0	2(1.9)	0
		DNA	2.2	0	0	0	2(1.9)	0
Jan 2012	Dry	RNA	0	0	0	0	2.1	0
		DNA	0	0	2(1.8)	0	0	0
Feb 2012	Dry	RNA	0	0	0	1.9	0	0
		DNA	0	0	0	0	0	2(1.9)
Mar 2012	Dry	RNA	0	1.91	0	0	0	0
		DNA	0	0	0	0	0	1.89
April 2012	Wet	RNA	0	0	0	0	0	2(1.8)
		DNA	0	0	0	0	2(1.9)	0

Values represent averages of the absorbance ratios of the nucleic acids extracted per site. The values outside the parenthesis represent situations where the number of the nucleic acids recovered from samples from a given site is more than one with the same absorbance ratio. Zero (0) means no nucleic acid was extracted.