DEMONSTRATION OF PRESENCE OF HUMAN HELMINTHES AND PROTOZOA IN FAECAL MATTER FROM PIT LATRINES OF SELECTED RESIDENTIAL COMPOUNDS IN LUSAKA, ZAMBIA

By

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DECLARATIONS

I Zamiwe Mbewe declare that:

(i) The research reported in this thesis is my own original work except where indicated;

(ii) This thesis does not contain other persons writing, graphs, data, pictures or other information unless specifically acknowledged as being sourced from other researchers and persons; and

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As the candidate's supervisor I have approved/refuted this dissertation for approval

Dr. Mohamed Abdel-Rahman Shehata

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ABSTRACT

The peri-urban on-site sanitation has unique challenges such as: lack of space and resources to construct new pit latrines. To ensure sustainability, the emptying of filled pit latrines is necessary. However, handling faecal matter especially if contaminated is risky. An assessment for helminthes and Protozoa was undertaken on faecal matter from four peri-urban areas of Lusaka, Zambia namely: Kanyama, Madimba, George and Chaisa compounds. The study aimed at finding out whether pit latrine faecal matter is contaminated with helminth ova, larvae and protozoan oocysts. Laboratory examinations through the Ammonium bicarbonate (AMBIC) method; Crystal violet stain and *Cryptosporidia species* recovery technique detected the presence, type, and viability of ova, larva and oocysts. Data was analysed by excel sheets and the use of statistical paired sample t-tests. It was established that 96% of Kanyama Compound pit latrines had *Cryptosporidia species* oocysts; 60% had *Ascaris species* ova which were viable in 4% of the pit latrines. Whereas 20% had *Enterobius species* ova and 16% had *Strongyloides species* larva. It was observed, 96% of George Compound pit latrines had *Cryptosporidia species* oocysts and 68% had *Ascaris species* ova which were viable in 48% of the pit latrines. Whereas 4% had *Enterobius species* ova and 4% had *Strongyloides species* larva. It was noted that 92% of Madimba Compound pit latrines had *Cryptosporidia species* oocysts and 40% had *Ascaris species* ova which were viable in 20% of the pit latrines. Whereas 8% had *Enterobius species* ova; 40% had *Cyclospora species* and 16% had *Strongyloides species* larva. Further, it was observed that 96% of Chaisa Compound pit latrines had *Cryptosporidia species* oocysts and 56% had *Ascaris species* ova which were viable in 36% of the pit latrines. Whereas 4% had *Enterobius species* ova and 8% had *Strongyloides species* larva. Using *Ascaris species* as indicator parasite, George Compound was the most affected by the parasites as it indicated a highest number of pit latrines with *Ascaris species* ova with high viability although it showed only 4 types followed by Kanyama Compound despite registering 11 types of parasites. Madimba compound indicated 9 types and Chaisa compounds had 4 types. The four compounds had a substantial enumeration of ova and oocysts with top sludge showing large numbers that reduced with depth. The t-test for paired samples showed that for Chaisa and George compounds, only *Ascaris species* varied in the mean concentration of top and bottom layer samples; George and Madimba compounds samples only varied in *Cryptosporidia species;* whereas Kanyama Compound samples showed no variation at all. Faecal matter was found to be contaminated with helminth and protozoa especially in the top layer sand hence not safe to public health, groundwater and the general environment.

Key words: Helminth, Protozoa, Faecal matter, Public health and Groundwater.

DEDICATION

For

Victor, Evelyn, King and Majesty

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LIST OF ACRONYMS

AIC	Akaike Information Criteria
AMBIC	Ammonium bicarbonate
AIDS	Acquired Immune Deficiency Syndrome
BOD	Biochemical Oxygen Demand
CBD	Central Business District
CI	*Cryptosporidia* Incidence
CLTS	Community Led Total Sanitation
COD	Chemical Oxygen Demand
CSO	Central Statistics Office
DALY	Disability Adjusted Life Years
DRA	Demand Response Approach
EED	Environmental Enteric Dysfunction
ELISA	Enzyme-Linked Immune Sorbent Assay
EPA	Environmental Protection Authority
ESA	East and Southern Africa
FC	Faecal Coliforms
GH-GWTU	Greenhouse Greywater Treatment Unit
GPS	Global Positioning System
GRZ	Government Republic of Zambia
IB-GWTU	Ice-Block Greywater Treatment Unit
IWRM	Integrated Water Resources Management
KAP	Koga-Agar Plate
LWSC	Lusaka Water and Sewerage Company
MDA	Mass Drug Administration
NBLH	Negative Binomial logit hurdle

NECOS	Network for Environmental Concerns and Solutions
NGO	Non Governmental Organisation
OD	Open Defecation
OSS	On Site Sanitation
PCR-RFLP	Polymerase Chain Reaction-Restriction Fragment Length Polymorphism
pH	Potential Hydrogen
QMRA	Quantitative Microbial Risk Assessment
RPM	Revolutions Per Minute
SARDC	Southern Africa Research Development Centre
SCGHCU	Semi-Centralised Greenhouse Composting Unit
SC-WCU	Semi-Centralised Winter Composting Unit
SME	Small and Medium Enterprise
STH	Soil Transmitted Helminthes
TC	Total Coliforms
TSS	Total Suspended Solids
UNZA	University of Zambia
VIP	Ventilated Improved Pit latrines
WASH	Water Sanitation and Hygiene
WDC	Ward Development Committee
WHO	World Health Organisation
WRC	Water Research Commission
WWTP	Waste Water Treatment Plant
ZINB	Zero Inflated Negative Binomial

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CHAPTER ONE: BACKGROUND INFORMATION

The chapter outlines the background information about the study. It introduces the study and spells out clearly the general objective as well as the specific objectives of the study.

1.1 Introduction

In several regions of the world, faecal matter is being used for agricultural purposes and production of biogas (Strauss at el, 1992). This clearly indicates that faecal matter is no longer regarded as a waste product, but a valuable resource. Verhagen and Carrasco (2013) noted in Ghana that any sanitation service model needed to address the full sanitation chain, which included safe and hygienic collection, storage, and safe and final disposal or the productive uses of faecal sludge.

There is a growing demand for the treated and untreated sewage faecal matter for land application in Lusaka (Phiri et al, 2014). This is also true for exhumed faecal matter from pit latrines in Lusaka peri-urban areas. The generated faecal matter at household level in Kanyama Compound for instance is normally harvested into a pit latrine from where it is exhumed and transported to a faecal matter digester where it gets stabilised while the extraction of biogas which is a source of energy is done. The stabilised residual faecal sludge is periodically removed from the digesters, sun dried and transported to crop fields for soil conditioning and improvement of crop yields thereby ensuring good food security for households. As observed recently by WaterAid (2013) biogas is a by-product of anaerobic digestion, the breaking down of organic material in the absence of air. The gas is rich in methane and can be used as a fuel for cooking, lighting and generating electricity. Anaerobic digesting takes place in what is known as a digester. Traditionally, digesters have been directly linked to the latrine so the fresh faeces are subjected to digestion immediately (WaterAid, 2013). In addition, the practice has the potential to improve agronomic productivity, soil fertility and add to cost effectiveness as well as sustainability in agriculture and of wastewater as well as pit latrine faecal matter management (Seidu et al, 2008).

Faecal matter from on-site sanitation systems is rich in nutrients, organic matter and constituents which contribute to replenishing the humus layer and soil nutrient reservoir and to improving soil structure and water-holding capacity. The consequences of not understanding the helminth content of pit latrine faecal matter is that the players in the value chain might be exposing themselves during pit latrine emptying and use to helminthes and protozoa. According to Phiri et al (2014), in biosolids, among the most important and prevalent intestinal parasites in Zambia, are the helminthes [*Ascaris species*; *Ancylostoma duodenale* (hookworms); *Taenia species* (Tape worms) and protozoa (*Cryptosporidium species; Entamoeba histolytica*)]. The evidence of human helminthes and protozoa in faecal matter from pit latrine of selected residential compounds in Lusaka Zambia was needed so that a suitable approach could be developed on how to handle the faecal matter. Although not all faecal matter is contaminated, the presence and concentration of pathogens in pit latrine faecal matter are the main factors that can affect the health of the community and infect the sources of domestic water (Australia and Victoria Environmental Protection Authority-EPA 2004). This research aimed at generating the scientific information to support the suitable options for the handling of faecal matter that has accumulated around the per-urban areas over the years especially the exhumed faecal matter which was already being used for biogas production and as fertiliser in Kanyama Compound, Lusaka.

1.1.1 General Objective of the Study

The main objective of the study was to assess the presence, types, viability and mean concentration of helminth pathogens in pit latrine faecal matter of selected peri-urban areas of Lusaka, Zambia.

1.1.1.1 Specific Objectives

(i) To assess whether pit latrine faecal matter is contaminated with helminthes and protozoa;

(ii) To find out the types of helminth and protozoa in the pit latrines of

the study areas;

(iii) To assess the helminth ova viability with faecal matter depth; and

(iv) To estimate the mean concentration of the observed helminth pathogens and protozoa

CHAPTER TWO: LITERATURE REVIEW

This chapter delves to consider what other researchers have done and even the extent to which they studied in fields related to this study as well as identification of gaps. It should also be appreciated that very few studies have been done on the evidence of helminthes and protozoa in faecal matter from pit latrines of selected residential compounds in Lusaka, Zambia with regard to pit latrine pollution of groundwater, environment and public health.

2.1.1 Helminth and Protozoa Studies Done Outside Africa

This section of the chapter considers some studies related to helminth and protozoa studies done outside Africa

Finland

Mattila (2005) did a PhD study on appropriate management of onsite sanitation, Tampere, Finland. He observed that the World was facing an enormous sanitation crisis: about 2.6 billion people lack appropriate sanitation. Mattila (2005) noted that in Finland, the lack of proper sanitation was not jeopardizing people's lives, but caused deterioration of the environment, especially eutrophication of surface water and in some cases also pollution of groundwater. The research experiences of Mattila (2005) and product development of on-site sanitation could help in solving the world's sanitation crisis if only given enough resources.

Mongolia

Uddin (2015) did a study on sustainable sanitation technologies for vulnerable peri-urban communities in Ulaanbaatar, Mongolia. During the study he noted that many of the world's undeveloped or partially-developed countries, and even in some developed ones, frequent outbreaks of various water, sanitation and hygiene (WASH)- and greywater-borne diseases were still prevalent due to lack/absence/failure of WASH system. As a result, poor sanitation accounts for the death of a child every 20 seconds which led to 1.5 million preventable death each year. Therefore development of

alternative sanitation technologies which were sustainable could be a problem-solver in the field of WASH, could be one of the options to tackle the WASH- and greywater-borne challenges around the world including Mongolia. The study was carried out for the first time in the coldest capital in the world, namely Ulaanbaatar, Mongolia during the period of 2012 and 2014 to assess technical feasibility, replicability and social acceptability of various sustainable sanitation technologies (e.g. eco-toilets, greywater and human feaces treatment technologies) for the vulnerable communities.

The results from Ice-Block Greywater Treatment Unit (IB-GWTU) showed that the maximum removal rates of chemical oxygen demand (COD), NH_4^+, NO_2^-, total suspended solid (TSS), PO_4^-, and Escherichia coli were up to 98%, 99%, 97%, 97%, 87%, and 98% respectively. As for Greenhouse Greywater Treatment Unit (GH-GWTU), the maximum removal rate of over 90% of those parameters was achieved. Both technologies showed a high potential to significantly reduce chemical and biological contaminants.

Results from composting human feaces in Semi-Centralized Winter Composting Unit (SC-WCU) showed that the temperatures above 55 and 65ºC were maintained for 2 weeks and more than a week respectively, which satisfies all the sanitation requirements including World Health Organization and Germany standard. In the Semi-Centralized Greenhouse Composting Unit (SCGHCU), over 70ºC of pile temperature was achieved which met the international standards in terms of temperature and pathogen die-off. Biological test results indicated that there was not a single Salmonella and E.coli found in the compost and the compost meet the standard of heavy metal content. The study confirmed that the greywater and human feaces treatment units were technically feasible towards meeting international standards/guidelines for greywater reuse and compost products, and able to significantly contribute to develop sustainable sanitation system and to close the study area's sanitation loop in a manner that could be replicable in other parts of the world. Alternative and re-invented financial sources such as micro-finance, social capital, corporate WASH responsibility could be considered as potential sources

of funding for replicating technologies and services in the study area and these could be applied in other parts of the low and middle-income regions of the world. An integration of Safe Water Supply and Sustainable Sanitation System was highly suggested to decrease the prevalence of WASH-borne diseases and to reduce the mortality rate at global level.

United States of America

Graham and Polizzotto (2013) did a study on pit latrines and their impacts on groundwater quality: a systematic review, United States of America (U.S.A). During the research, it was observed that pit latrines were one of the most common human excreta disposal systems in low-income countries, and their use was on the rise as countries aimed to meet the sanitation-related target of the Millennium Development Goals. There was concern, however, that discharges of chemical and microbial contaminants from pit latrines to groundwater might negatively affect human health.

Existing survey and population data was used in the research to calculate global pit latrine coverage. The scientific literature on the occurrence of contaminants originating from pit latrines was reviewed and the factors affecting transport of these contaminants were considered. Data were extracted from peer-reviewed articles, books, and reports identified using Web of Science, Google, and document reference lists. Graham and Polizzotto (2013), estimated that approximately 1.77 billion people use pit latrines as their primary means of sanitation. Studies of pit latrines and groundwater were limited and had generally focused on only a few indicator contaminants. Although groundwater contamination was frequently observed downstream of latrines, contaminant transport distances, recommendations based on empirical studies, and siting guidelines were variable and not well aligned with one another.

The study concluded that in order to improve environmental and human health, future research should examine a larger set of contextual variables, improve measurement approaches, and develop better criteria for siting pit latrines. The results obtained by

Graham and Polizzotto (2013), could be utilized by local and international experts seeking a carrier in the planning and design of sustainable sanitation facilities in developing countries or for those who have newly filled a post in governmental, non-governmental or academic institutions.

USAID

Ngure et al (2017) did a review on water, sanitation and hygiene partners and learning for sustainability. Towards a hygienic environment for infants and young children: A review of the literature. Under nutrition and stunting are related to poor overall health, increased susceptibility to infections, lower economic productivity, cognitive deficits and learning disabilities, and increased mortality. Concurrent exposures of inadequate diet and poor water, sanitation, and hygiene (WASH) conditions predispose infants and young children (infant and young children) to a debilitating cycle of infections and under nutrition in early life.

Additionally, over the past decade, environmental enteric dysfunction (EED), a condition characterized by inflammation of the small intestinal lining that inhibits permeability and nutrient absorption, has been identified as a potential major mediating pathway linking poor WASH conditions and chronic under nutrition. Though the etiology of EED is not fully understood, it has been linked with ingestion by infant and young children of high loads of human and animal feacal microbes. The EED hypothesis proposes chronic exposure to feacal microbes, independent of the effects of diarrhea, as a significant underlying cause of child growth faltering. EED was thought to explain why even the most rigorous dietary interventions have only a modest effect on reducing child stunting.

For nearly six decades, routes of pathogen transmission have been identified and summarized in a seminal “F-diagram” via fluids, fingers, flies, fields (floors, earth, dirt), fomites (surfaces), and food. The traditional F-diagram focuses exclusively on human excreta, tracing the transmission of pathogens through different exposure routes into

water and food and onto hands that were then ingested by a host. Based on existing evidence, it was suggested that two refinements be made to the F-diagram: (1) inclusion of domestic animal excreta as a distinct and potentially important source of risk, and (2) in the case of infant and young children, framing ingestion of pathogens via eating dirt (geophagy) and/or human and animal excreta and through mouthing behaviors as an additional *direct* transmission pathway not disrupted by the traditional suite of WASH measures targeted at adults and older children.

WASH interventions to disrupt transmission pathways in the F-diagram had traditionally focused on increasing access to an improved water supply, improving drinking water quality, and refining hand hygiene and sanitation measures (through the reduction of open defecation (OD) and the adoption of improved toilets). These strategies paid less attention to behaviors and technologies separating infant and young children from human and animal feaces through interventions such as clean play spaces, infant and young children mouthing behaviors, and safe disposal of infant and young children feaces.

The USAID Water, Sanitation, and Hygiene Partnerships and Learning for Sustainability project conducted a review of the scientific and grey literature, complemented by dozens of key informant interviews with researchers and field implementers, to synthesize the latest understanding of key pathways of feacal microbe ingestion by infant and young children and their links to diarrhea, EED, and poor nutrition and development outcomes. Specifically, the review sought to: (i) consider both human and animal sources of feacal contamination and the pathways presenting major exposure risks to infant and young children, including sources and pathways previously underemphasized (and to the extent possible, sought to understand the relative potential importance of the various pathways in terms of magnitude of pathogen transmission); (ii) examine technological and behavioral interventions to disrupt the transmission pathways (including established WASH measures) as well as interventions that address the underemphasized pathways such as clean play spaces, animal

husbandry, infant and young children feeding practices, and infant and young children-focused WASH behaviors; and (iii) identify areas of priority for implementation research to address the issue of clean play spaces for infants and young children.

The findings of the literature review informed the design of research to measure the additive effects of specific measures to reduce infant and young children exposure to feacal pathogens and other feacal microbes in their home environments when coupled with independent or integrated WASH and nutrition interventions.

The review begun by documenting the traditional transmission pathways outlined in the F-diagram and the effectiveness of traditional rural WASH interventions to disrupt them. Key findings were as follows: (i) The evidence of WASH interventions reducing the risk of diarrhea among under five-year old children was mixed, and was stronger for particular categories of WASH interventions, such as water supply and point-of-use water treatment, than for others; (ii) An emerging body of evidence from high-quality (experimental) studies also showed mixed effects of improved sanitation on health outcomes such as diarrhea, EED, soil-transmitted helminths (STH), and child growth outcomes. However, a recent community-led total sanitation (CLTS) trial provides evidence suggesting that promoting intensive behavior changes over a 12-month period can improve child linear growth; (iii) Evidence also suggested that, unless threshold levels of community sanitation coverage were reached to achieve herd effects, household sanitation improvements might be insufficient to mitigate feacal-oral pathogen exposure and achieve desired health benefits; (iv) Substantial evidence had been found on the effectiveness of hand washing with soap in preventing diarrhea and STH infections. However, the magnitude of the effect differed between studies and across contexts; (v) The additive or synergistic effects of different WASH interventions on child health were complex and not well understood, and evidence on the effect of focused and combined WASH interventions on child health outcomes, such as diarrhea and growth, was far from definitive; and (vi) Food hygiene was largely a neglected

aspect of WASH and the few existing intervention studies were weak in quality and not easily generalized.

While evidence of the impact of traditional WASH interventions on improving health outcomes was mixed, the review suggested other underemphasized sources and pathways for feacal pathogens that might have forced some re-evaluation of the types of interventions needed to limit pathogen exposure in infant and young children. Risks from human excreta have been well-documented, but uncontained animal feaces also were abundant in rural areas in developing countries, constituting an important reservoir of zoonotic pathogens and non-commensal feacal microbes to the domestic environment and water supply sources. While the relative magnitudes of pathogen exposure from these sources remain largely unknown, substantial data exist to demonstrate that animal feaces contribute significant contamination to the home environment.

Direct ingestion of feacally contaminated soil and/or animal feaces was observed to be a critical pathway for exposure of feacal pathogens by infant and young children. infant and young children ingest dirt and faeces through mouthing soiled fingers, play objects, and household items as well as through exploratory ingestion of contaminated soil and/or poultry feaces.

In addition to the interventions intended to disrupt the pathways of the traditional F-diagram (e.g., increasing use of improved latrines, improving access to safe water, handwashing with soap at critical junctures and, to a lesser extent, emphasizing food hygiene), efforts have been made to construct barriers that prevent feacal contamination of the domestic environment and associated ingestion of soil and animal feaces by infant and young children. These barriers include finished flooring; improved animal husbandry practices, such as corralling and other means to separate infant and young children and animals; play mats for immobile infants; and a playmat/playpen combination for crawling and mobile infants.

While the introduction of barriers was gaining attention in infant and young children and nutrition communities of practice, to date there have been no published studies finding that protective play spaces prevent feacal ingestion or improve child health. Likewise, a few implementing organizations have conducted formative research and executed interventions to address poultry feaces through modified animal husbandry practices; however, data were not generally available on the effectiveness of these approaches.

Reducing or eliminating animal feaces in household compounds was challenging. Both ruminant and fowl feaces were used productively as fuel, farm fertilizer, and in building materials. Because of its utility, households were reluctant to get rid of what they consider to be a valuable resource. Storage and handling of fresh animal feaces for such purposes exposes household members including children to contamination, suggesting that a focus on safer management of animal feaces and handwashing after handling might have been more successful than focusing on eliminating animal feaces from household compounds altogether.

Researchers have measured the impact of uncontained feaces on the health and growth of the infant and young children cohort, but until recently, little attention has been focused on feaces management *of* the infant and young children cohort. This review highlighted findings from recent analyses indicating that infant and young children feaces disposal is sub-optimal in many LMICs. Field practitioners responded to these findings with interventions that promote the safe disposal of infant and young children feaces out of the belief that promoting age-cohort specific behaviors (supported by enabling technologies such as potties, diaper cloths, and use of hoes) facilitate improved infant and young children feaces disposal practices. Data supporting this recommendation, however, have come from program evaluations with low rigor. In some deployments, CLTS has begun to include a focus on safe disposal of infant and young children feaces as part of triggering, follow-up, and verification of open defecation-free status. Nutrition programs also have begun to include safe disposal of

infant and young children feaces and hand washing after handling child feaces (often not regarded as “dirty” by their caregivers) as part of comprehensive infant and young children growth initiatives.

The review noted that relative magnitudes of transmission pathways of enteric microbes in infant and young children were not well-defined and might have been highly context-specific, making it difficult to conclude which pathways represent the highest risk. Still, the growing attention to risks from direct and indirect consumption of animal waste and direct consumption of contaminated soil point to the potential benefits of separating infant and young children from feacal pathogen sources in the home environment.

Play mat and play space interventions might be promising, but assessing their true potential requires a better understanding of the biological plausibility of their protective effects against feacal contaminant risk; extended periods of protection on a mat or within a play yard may not be sufficient to prevent risk posed by even short periods of infant and young children time spent in areas of increased hazard (e.g. on bare soil with excreta present, or eating contaminated food from contaminated hands). The exposure-response relationship for ingestion of animal excreta or contaminated soil remains a source of considerable uncertainty. Due to the heterogeneous nature of the formative and observational studies of feacal microbe transmission pathways, developing findings on the relative magnitudes of feacal microbe exposures across different pathways was not possible in this review; further research was recommended to make better and more informed programmatic decisions.

Analysis of the available literature, as well as feedback from key informant interviews, underscored how little was understood about the etiology of EED. Given that EED might facilitate linear growth failure *independently of diarrhea,* the historic research focused on diarrhea as the primary outcome variable may have missed key relationships among various WASH interventions, EED and child growth. Measuring EED, however, was far more complex and expensive than measuring diarrhea (including specific

diarrhea-causing pathogens). There was a need to develop less costly proxy measures predictive of EED and child stunting.

The review underscored a significant evidence gap on various technologies and social behaviour change interventions promoted through current programmatic efforts. Additional research was recommended to investigate the effectiveness, adoption, constraints, and scale potential of measures to reduce infant and young children exposure to feacal pathogens, including safe play spaces, improved animal husbandry practices, and flooring, among others. The research explored the additive benefits of these interventions when coupled with traditional WASH measures such as expanded water supply, improved water quality, utilization of improved toilets, hand washing with soap, and promotion of other hygienic behaviours.

Africa, Asia and Latin America

Strauss and Montangero (1992) did a study which indicated that in many areas of Africa, Asia and Latin America, helminth, notably nematode infections (*Ascaris, Trichuris, Ancylostoma, Strongyloides, etc.*) were highly prevalent. *Ascaris* ova were reported to be particularly persistent in the environment. The bulk of helminth ova contained in faecal or in wastewater treatment plant sludges end up in the biosolids generated during treatment. Hence, in many places, nematode ova were the indicators-of-choice to determine hygienic quality and safety where biosolids were to be used as a soil conditioner and fertilizer. The concentration of helminth ova in the biosolids was largely dependent on the prevalence and intensity of infection in the population from which faecal sludge or wastewater was collected.

Strauss and Montangero (1992) strongly recommended that where biosolids use in agriculture was a practice or being aimed at, treatment should aim at reducing helminth egg counts and viability, or solids storage must be long enough to achieve the desired reduction they observed.

Cambodia

Khieu (2014) carried out a research on epidemiology and clinical aspects of *Strongyloides stercoralis* infection in Cambodia. The threadworm *Strongyloides stercoralis* is endemic in settings where sanitary conditions are poor and where the climate is warm and humid. More than 70 tropical countries in Southeast Asia, Sub-Saharan Africa, West Indies and Latin America are considered as high endemic settings. However, *S. stercoralis* is also prevalent in subtropical and temperate regions including Australia, Japan, Canada, United States and Europe. The global prevalence of *S. stercoralis* is heterogeneous. It is believed that about 30 - 100million people worldwide are infected with *S. stercoralis*. But the true number and the global burden of infection remain unknown and most probably are today underestimating in many areas of the tropical resource poor countries. The low sensitivity of the currently available diagnostic tools and a scarcity of specialized survey are most important factors for that.

Moreover, many epidemiological aspects of *S. stercoralis* infection are poorly understood or unknown. It is not known in detail where *S. stercoralis* is endemic, which infection rates and intensities can typically be expected in different settings and populations, and when an individual was infected at first-time and how quickly the re-infection can occur after successful treatment. Epidemiological information on *S. stercoralis* such as large-scale prevalence, re-infection, risk factors, clinical features and treatment efficacy are unknown in Cambodia and many parts of Southeast Asia.

The study aimed to understand the importance of *S. stercoralis* infection in Cambodia by pursuing four main objectives: (i) assess *S. stercoralis* infection and risk factors, validate diagnostic methods and determine treatment efficacy among schoolchildren, (ii) determine large-scale prevalence and risk factors in two socioeconomic and ecological distinctly different settings, (iii) determine re-infection rates among schoolchildren, and (iv) document clinical aspects of patients with high intensity of *S. Stercoralis* infection in rural communities.

School- and community-based studies were carried out in four primary schools and 120 villages of three provinces (Kandal, Preah Vihear and Takeo) in Cambodia, from 2009 to 2011. After obtaining the written informed consent from participants, an individual questionnaire was administrated to obtain demographic, risk-perception and behavioral data. The head of household was interviewed with a household questionnaire on socioeconomic indicators of the household such as house type, household assets, latrine and livestock. After the interview, each participant was given a pre-labeled plastic container (ID code, name, sex, age and date) for stool sample collection. In case a multiple stool samples analysis, another stool container was distributed upon collection of the first or second sample.

The feacal materials were analyzed by Baermann method and Koga-agar plate (KAP) culture for diagnosing *S. stercoralis* and Kato-Katz method for helminth co-infections. Two school-based studies were performed in four primary schools in Kandal Province. In 2009, a cross-sectional study was carried out among 458 children, examining three feacal samples per child, to assess risk factors, diagnostic methods and treatment efficacy after three weeks of ivermectin treatment (100μg/kg/day for two days).

A two-year cohort study was conducted among 302 schoolchildren from 2009 to 2011, analyzing two stool samples per child, to determine re-infection and risk factors of *S. stercoralis*. Two large-scale cross-sectional community-based studies were conducted in 2010 and 2011 to assess infection prevalence and risk factors in two provinces (2396 participants from 60 villages of Preah Vihear province, analyzed two stool samples per participant; and 2861 participants from 60 villages of Takeo province, examined one stool sample per participant). Bayesian kriging was used to predict risk at non-surveyed locations in Preah Vihear province. A caseseries study, nested in the survey in Preah Vihear province in 2010, was carried out to document the clinical features of 21 *S. stercoralis* cases, with high numbers of *S. stercoralis* larvae in their feacal specimen detected by Baermann technique.

A cross-sectional school-based survey in 2009 found that 24.4% of 458 schoolchildren were infected with *S. stercoralis*. The prevalence of *S. stercoralis* infection increased considerably (from 18.6% to 24.4%) when three stool samples were examined. The sensitivity of KAP culture and Baermann technique was 88.4% and 75.0%, respectively. Clinical features such as itchy skin and diarrheal episodes were significantly associated with *S. stercoralis* infection. Children who reported defecating in latrines were significantly less infected with *S. stercoralis* than those who did not use latrines (OR: 0.4; 95% CI: 0.2 – 0.6; $P<0.001$). Almost three-quarters of the infections could have been reduced by proper sanitation (PAR: 0.7; 95% CI: 0.5– 0.9). Ivermectin (200 μg/kg BW, PO, over 2 days) was highly efficacious against *S. stercoralis* infection, with a cure rate of 98.3% three weeks after treatment. In Preah Vihear and Takeo provinces, *S. stercoralis* infection prevalence among general population was 44.7% and 21.0%, respectively. In both areas found that the male participants were significantly more infected than females ($P<0.001$) in all age classes. In Preah Vihear province, northern Cambodia, *S. Stercoralis* infection statistically increased with age, starting at 31.4% in children less than 6 years to a peak of at 51.2% in participants older than 50 years. Participants defecating in latrines were significantly less infected with *S. stercoralis* than those who did not use latrine (OR: 0.5; 95% CI: 0.4 - 0.8; $P<0.001$). *S. stercoralis* infection exhibited almost no tendency to spatial clustering in this province. Infection risk significantly decreased with increasing rainfall and soil organic carbon content and to increase in lands occupied by rice fields.

In Takeo province, Southern Cambodia, *S. stercoralis* infection prevalence reached 14.5% in children under or equal to 5 years and 28.0% in participants aged between 56 and 60 years. Participants who reported having a latrine were statistically less infected with *S. stercoralis* infection than those who did not possess latrine at home (OR: 0.7; 95% CI: 04 - 0.8; *P*: 0.003). Muscle pain and urticaria were significantly associated with *S. stercoralis* infection. A two-year cohort study among 302 schoolchildren revealed a prevalence rate of 24.2% and 22.5% at baseline (2009) and follow-up (2011), respectively. Almost one-third (31.5%) of 73 treated *S. stercoralis* cases at baseline

were re-infected at follow-up. But, almost 70% of children infected at baseline and treated remained free of re-infection for the period of two years. Children reported having shoes and defecating in toilet were statistically less infected with *S. stercoralis* than those who did not possess shoes (OR: 0.3; 95% CI: 0.1 – 0.5; *P*: 0.031) and use latrine (OR: 0.4; 95% CI: 0.2 – 0.9; *P*<0.001) at follow-up. None of the reported clinical symptom was significantly associated with *S. stercoralis* infection at follow-up. Clinical symptoms of 21 *S. stercoralis* patients with high intensity infection (more than 250 larvae in Baermann test) from Preah Vihear province were documented in 2010.

The median age of the patients was 11 years (range: 5 - 67); 23.8% were females. Eleven patients (52.4%) were younger than 16 years. Out of 21 patients, 20 (95.2%), 18 (85.7%) and 14 (66.7%) reported frequent abdominal pain, diarrhea and periods of sensation of itching, respectively, during the previous six months. Five patients (23.8%) reported having experienced urticaria the week preceding the examination. One patient suffered from extended urticaria. Three weeks after ivermectin treatment (200µg/kg BW, single oral dose), most symptoms (diarrhea, abdominal pain and urticaria) almost entirely resolved.

S. stercoralis infection was observed to be highly prevalent in rural communities of Cambodia where appropriate diagnosis and treatment do not exist. The reinfection rate of *S. stercoralis* among schoolchildren after two years of ivermectin treatment was considerable, but more than two-third remains free of infection for at least 2 years. Preschool- and school-aged children were highly affected. Persona hygiene and sanitation including wearing shoes, possession and use of latrines, were significant predictors of *S. stercoralis* infection. Gastrointestinal and cutaneous symptoms were associated with *S. stercoralis* infection and resolve almost entirely after ivermectin treatment. Thus, *S. stercoralis* infection should no longer be neglected in Cambodia and elsewhere in tropical resource poor countries. Access to adequate diagnosis and treatment of *S. stercoralis* infection was an urgent need in Cambodia.

Palestine

Al-Zain (2009) carried out a reaserachon the impact of socioeconomic conditions and parasitic infection on hemoglobin level among children in Um-Unnasser Village, Gaza Strip. A cross-sectional study was conducted to examine the impact of socioeconomic conditions and intestinal parasitic infection (IPI) on hemoglobin level among children aged 2-15 years in Um-Unnasser village, North Gaza, Palestine. The data were collected using structured questionnaire and laboratory analysis of blood and feacal samples. Of 256 children, 25% were anemic and prevalence was higher in children aged below six years. Overall prevalence of IPI was 46.9%. *Ascaris lumbricoides* (11.3%), *Giardia lamblia* (8.2%), *Hymenolepis nana* (6.2%), *Entamoeba histolytica* (5.1%), *Strongyloides stercoralis* (2.0%), *Enterobius vermicularis* (2.7%), and Trichuris trichiura (0.3%) were the most frequently found, whereas 10.9% of children had multiple parasitic infection. An association was determined between some socioeconomic conditions and parasitic infection and anemia. These socioeconomic factors included age group of the studied children, father's educational level and work status. It was found that children with double parasitic infection had lower hemoglobin level than those who had single parasitic infections except in cases of A. lumbricoides and G. lamblia.

Panama

Kaiser (2006) did a study on an analysis of the use of desiccant as a method of pathogen removal in compost latrines in rural Panama. It was observed that in Panama only 51% of the rural population had access to sanitation facilities. The author of this report experienced these statistics first hand as a Peace Corps Volunteer while implementing and studying compost latrines in the rural indigenous region of Panama, La Comarca Ngäbe Bugle. Compost latrines were a viable solution to the sanitation problems of the developing world. They provided a method of safely destroying harmful pathogens and produce a free organic fertilizer. Traditionally, compost latrines had been built with the intention of pathogen removal through aerobic decomposition at high thermophilic temperatures. However, in the developing world studies showed that the actual method

of pathogen destruction was desiccation. The addition of dry organic materials with high pH such as wood ash increase the pH in the latrine and reduce moisture levels, accelerating pathogen removal. Therefore, the quantity and type of desiccant added to the compost latrine becomes critical to the operation of the latrine for pathogen removal. By conducting a survey of compost latrines in six communities in rural indigenous Panama, the author assessed the use of desiccant in the latrines to determine if the latrines were being operated to destroy pathogens. Through observations and interviews with the latrine caretakers the author collected data including; the amount and type of desiccant used, the method of applying the desiccant and the final use of the compost.

The results of the study show that 98.6% of the households interviewed reported using desiccant in their latrines. However, the results of the latrine inspections showed that only 71% were using the correct amount of desiccant. The average reported amount of desiccant added to the latrine was 2.8 handfuls (1.5 – 3 cups) per use. This fell within the desired range of 1 – 2 cups. In terms of the types of desiccant being used, 76.8% of the households reported using wood ash, which was important for raising the pH of the pile. However 20% of the households reported only using sawdust in their latrines. This report also analyzed the educational component of Peace Corps compost latrine projects in Panama to determine if the proper mechanism for pathogen destruction was emphasized. The results showed that although adding desiccant to the latrines was stressed throughout the trainings, there were still indicators that pathogen removal through high temperature decomposition was assumed.

Mehl (2008) did a research on pathogen destruction and aerobic decomposition in composting latrines: A study from rural Panama. Composting latrines were a common sanitation solution in rural Panama. These latrines were assumed to effectively destroy pathogens in human excrement through aerobic decomposition at high temperature—the composting process. However, the majority of composting latrines in developing countries never reach high enough temperatures for pathogen removal. Instead,

desiccation at high pH may be the responsible means of pathogen removal; yet, the breakdown of organic matter is hindered at high pH and low moisture levels.

To assess the relationship between temperature, high pH, desiccation, decomposition, and pathogen destruction, a survey to observe the use of desiccant and obtain temperature and pH measurements was conducted on 63 composting latrines in five indigenous communities. Furthermore, compost samples were taken to a laboratory for chemical and microbiological analysis to test for pH, % moisture, carbon-to-nitrogen (C/N) ratio, and presence of pathogens.

The temperature results supported previous findings that compost latrines do not get hot enough to kill pathogens; rather, the latrines remained close to ambient temperatures. The pH results showed that many latrines were operating within the range for ideal decomposition, pH of 7.5-8.5 but only 17% of latrines measured pH 9 or above, the recommended pH for pathogen destruction. Most composting latrine users added desiccant materials, sawdust and wood ash, to lower the moisture level and provide the necessary carbon for decomposition. However, it seemed not enough desiccant materials were added because moisture levels remained above the suggested maximum of 25% for pathogen destruction and C/N ratios were in the range of the ratio of raw human faeces. More importantly, the results of the microbiological analysis showed various pathogens, mainly helminthes, still present in the compost samples that had been stored for the recommended 6-month storage time. From these results, it followed that pathogens were not being removed in composting latrines nor was aerobic decomposition taking place.

Mehl (2008) concluded that as a means of sanitation, composting latrines must be operated to destroy pathogens. Storage time should be increased to a minimum of 1-year, and users should be instructed to add more desiccant materials of both the high pH type (e.g., wood ash) and bulky type (e.g., sawdust). It was recommended that the

current composting latrine design used in Panama will need to be adjusted for the longer storage time.

Sweden

Fidjeland (2015) did a research on sanitisation of faecal sludge by Ammonia, Uppsala, Sweden. Faecal sludge contains valuable plant nutrients and can be used as a fertiliser in agriculture, instead of being emitted as a pollutant. As this involves a risk of pathogen transmission, it is crucial to inactivate the pathogens in faecal sludge. One treatment alternative is ammonia sanitisation, as uncharged ammonia (NH_3) inactivates pathogens. The aim of the thesis was to study how the pathogen inactivation depends on treatment factors, mainly NH_3 concentration, temperature and storage time, and based on this to make treatment recommendations that ensure pathogen inactivation.

Salmonella inactivation was rapid and could be eliminated within a few days. Reovirus and adenovirus were inactivated more slowly than that, but more rapidly than bacteriophages PhiX174, 28B and MS2. *Ascaris* eggs were generally inactivated more slowly than the other studied organisms, especially at low temperatures (<20 °C). *Ascaris* egg inactivation was modelled as a function of NH3 concentration and temperature, which enabled the prediction of required treatment time. An assessment of health risk associated with consumption of crops eaten raw indicated that a 4.5 log10 reduction of *Ascaris* eggs and a 7.5 log10 reduction of rotavirus were required for unrestricted use of ammonia-treated faecal sludge as a fertiliser.

It was noted that faecal sludge contains some ammonia mainly due to the ammonia in urine, but the concentrations can be low due to dilution with flush water and losses to air. Mixing source-separated urine and faeces from urine-diverting dry toilets would give a high enough NH_3 concentration for pathogen inactivation. Estimations of NH_3 concentrations in faecal sludge from vacuum, pour-flush and low-flush toilets indicated that the ammonia concentrations required for stable pH might not be reached without the addition of ammonia. The addition could be urea, which was a common mineral

fertiliser that hydrolyses to ammonia and carbonate through the enzyme urease found in faeces.

The study concluded that Ammonia sanitisation of faecal sludge was a simple and robust technology enabling a high degree of pathogen inactivation. It could considerably reduce the health risk for farmers, food consumers and downstream populations. Minimising flush water volumes in order to reduce the treatment costs was recommended.

Höglund (2001), did a research on evaluation of microbial health risks associated with the reuse of source-separated human urine. Human excreta contain plant nutrients and have the potential to be used as a fertiliser in agriculture. Urine contributes the major proportion of the nutrients (N, P and K) in domestic wastewater whereas faeces contribute a smaller amount and involves greater health risks if reused due to the possible presence of enteric pathogens. Human urine does not generally contain pathogens that can be transmitted through the environment. Source-separation of urine and faeces was possible by using urine-separating (or urine diverting) toilets, available as simple dry toilets or porcelain flush toilets with divided bowls. The risk for transmission of disease when handling and reusing the urine was largely dependent on the cross-contamination by faeces.

In this research, the presence of human faeces in urine samples was successfully determined by analysing for faecal sterols. Cross-contamination was evident in 22% of the samples from urine collection tanks, and in these quantified to a mean (± SD) of 9.1 ± 5.6 mg faeces per litre urine mixture. Testing for indicator bacteria was shown to be an unsuitable method for determining faecal contamination in human urine since *E. Coli* had a rapid inactivation in the urine and faecal streptococci were found to grow within the system. The fate of any enteric pathogens present in urine is crucial for the risk for transmission of infectious diseases. Gram-negative bacteria (e.g. *Salmonella* and *E. coli*) were rapidly inactivated (time for 90% reduction, T90 <5 days) in source-separated

urine at its natural pH value of 9. Gram-positive faecal streptococci were more persistent with a T90 of approximately 30 days at 4°C. Clostridia spore numbers were not reduced at all during 80 days.

Similarly, *rhesus* rotavirus and *Salmonella typhimurium* phage 28B were not inactivated in urine at low temperature (5°C), whereas at 20°C their T90-values were 35 and 71 days, respectively. *Cryptosporidium* oocysts were less persistent with a T90 of 29 days at 4°C. Factors that affect the persistence of microorganisms in source-separated human urine include temperature, pH, dilution and presence of ammonia. By using Quantitative Microbial Risk Assessment (QMRA), the risks for bacterial and protozoan infections related to handling and reuse of urine were calculated to be <10-3 for all exposure routes independent of the urine storage time and temperature evaluated. The risk for viral infection was higher, calculated at 0.56 for accidental ingestion of 1 ml of unstored urine. If the urine mixture was stored at 20°C for six months the risk for viral infection was reduced to 5.4 × 10-4.

The research concluded that by following recommendations for storage and reuse, which were dependent on the type of crop to be fertilised, it was possible to significantly decrease the risk for infections. So far, the level of risk that was acceptable was unknown. The acceptable risk would be one of the main factors determining the future utilisation of source-separated human urine in agriculture.

Thailand

Luong (2002) did a study on prevention of intestinal worm infections through improved sanitation and hygiene. Helminth infections refer to worms that live as parasites in the human body. Worm infection occurs when infective eggs, or larvae, enter the body, mature, lay eggs and feed off the person. People get infected with worms when living in an unclean environment of poor sanitation and unhygienic habits. The three main types of common intestinal worms that infect humans are large intestinal roundworm (*Ascaris*

lumbricoides), hookworm *(Ancylostoma duodenale and Necator americanus*) and whipworm (*Trichuris trichuira*).

Analysis of infection prevalence by age group indicated that all age groups have infections. But the incidence of hookworm infection tends to grow with increasing age. Therefore, adolescent girls and women of childbearing age were generally infected with hookworm. This document focused on hookworm and roundworm infections and their control through drug treatment as an entry point to promote improved sanitation and hygiene behaviour. These were the key preventive measures leading to a clean living environment, which was necessary for the proper development and well being of our children.

Worm infections were one of the major health problems confronting millions of school-age children. These parasites consumed nutrients from the children they infected, thus aggravating malnutrition and retarding physical development. They also destroyed the tissues and organs in which they lived. They caused abdominal pain, diarrhoea, intestinal obstruction, anaemia, ulcers and various other health problems. These ailments did impair learning and slow cognitive development, ultimately resulting in poor school performances of a child. It was not uncommon for heavy or long-term infection to result in death, if treatment was not given in time. It was especially important to note that the stunting of children's growth due to worm infections was not easily recognized because it occurred almost imperceptibly over time. Thus, the full impact of intestinal infections was often greatly under-reported or overlooked. Intestinal worm infections destroyed the well being and learning potential of millions of children in many developing countries.

Pre-school and school-age children and women of childbearing age, including adolescent girls, had the higher proportion of worm infections. Although intestinal worms could infect all members of a population, these specific groups were observed to be at greater risk of heavy infections than others and were more vulnerable to the

harmful effects of chronic infections. These vulnerable groups would benefit most from preventive interventions.

Intestinal worms, known by their species name as helminth, were different in several unique ways from other infectious organisms, such as bacteria and viruses. Understanding their differences enables planners and implementers to effectively formulate a sustainable integrated de-worming programme for improvement of children's health and development.

Helminths do not multiply in number within an infected person. Each adult worm is the result of the host being exposed to an infective egg, or larva, which enters the body either by penetrating through skin or by being ingested, depending on the species. Helminths accumulate gradually inside the host over time, so the onset of disease tends to be slow and may go unrecognized; when moderate to heavy worm infections have been acquired, onset of chronic disease occurs. Severity of disease caused by helminths would depend on the number of worms inside the infected person as well as the age, health and nutritional well being of that person and the duration of infection.

There are a number of drugs that kill several species of helminths at the same time with a single dose. Re-infection, however, was quite frequent. Hence, drug treatment alone without the improvement of sanitation and hygiene practices would not break the routes of worm transmission. It was typical to find that helminths were not evenly or randomly distributed among people in a community; worms tend to clump or aggregate in their distribution. For example, 70 per cent of all worms may be found in as few as 30 per cent of all people in a community.

The study pointed out that anti-helminth drug treatment using Albendazole or Mebendazole that kill several species of intestinal worms at the same time would only result in a short-term reduction of infection in a target population. Re-infection was quite frequent within a relatively short period of time. The long-term key preventive

interventions mentioned below were the basic requirements to break the intestinal worm transmission routes and life cycles: (i) Provision and use of safe and adequate water supply; (ii) Improvement of environmental sanitation; and (iii) Practicing good sanitation and hygiene habits.

It was noted that for both roundworm and hookworm infections, the important preventive interventions involving people's behaviour change are: (i) Building and using sanitary latrine for safe disposal of human excreta; (ii) Washing hands with soap before eating or feeding children and after defecation; and (iii) Washing and cooking vegetables thoroughly. While the implementation of a water supply and sanitation programme at the household and community level may take time, personal precaution against hookworm infection was to wear suitable footwear to protect the skin of feet, ankles and legs from coming into contact with infective larvae.

It was recommended in the study that in areas where human excreta were used as organic fertilizer, people should be educated and motivated to use only the fully digested or properly composted human excreta and not to apply the raw or partially digested excreta to the field. Fully digested excreta were taken from a latrine pit where it has been sufficiently stored for one to two years. During the period, disease pathogens and worms' eggs have died out completely. It will not pollute the soil or the environment as well as the vegetables grown in the field. An integrated de-worming programme should also educate people about the proper method of composting the human excreta for use as organic fertilizer.

Vietnam

Forl (2007) did a study on Helminths and Allergic Disease in Vietnam. Allergic disease is uncommon in developing countries, especially in rural areas. A protective effect of helminth infection has been implicated as a potential explanation. The objective of the study was to determine whether reduced exposure to helminth infection is associated with a higher risk of allergen skin sensitisation and allergic disease, and whether such

an association could be explained by a helminth-induced up-regulation of certain cytokines, in particular anti-inflammatory IL-10.

About 1,742 rural Vietnamese schoolchildren were invited to take part in a cross-sectional baseline survey followed by a randomised, double blind, placebo-controlled trial of anti-helminthic therapy at 0, 3, 6, and 9 months to compare the change in exercise-induced bronchospasm (primary outcome), wheeze, rhinitis, eczema, and allergen skin sensitisation (secondary outcomes) at 12 months. 244 secondary schoolchildren also had venous blood taken to measure helminth induced IL-10, IFN- , IL-5, and IL-13. Out of these 244 children, 144 were infected with hookworm and had bloods taken again at 12 months.

During baseline survey, 1,601 schoolchildren (92% of those eligible) in grades 1-9 aged 6-18 participated in the baseline survey. 0.4% (6/1601) of children had a fall in peak flow after exercise of at least 15%. Doctor-diagnosed asthma was equally rare (0.4%, 6/1601), while 5.0% (80/1601) of children had experienced wheezing over the past 12 months. 6.9% (110/1601) of parents reported that their children had suffered of hay fever in the past 12 months, and in 2.6% (41/1601) of cases, the diagnosis was confirmed by a doctor. 5.6% of children (89/1601) reported an itchy rash over the past 12 months. 0.9% (14/1601) had a history of flexural involvement and on examination 0.5% (8/1601) proved to have flexural eczema on the day of the survey.

Skin prick test positivity was commoner than allergic disease. 33.5% (537/1601) of children had at least one positive skin prick test (dust mites 14.4%, cockroach 27.6%). The cross-sectional analysis yielded only significant results for allergen skin sensitisation. In univariate analysis, sensitisation was less frequent in children with hookworm or *Ascaris* infection, and increased in those with better sanitation, including flush toilets and piped drinking water. In multivariate analysis, the risk of allergen skin sensitisation to house dust mite was reduced in those with *Ascaris lumbricoides* infection (adjusted OR=0.28, 95% CI 0.10-0.78) and in children with higher hookworm

burden (adjusted OR for 350+ versus no eggs per gram faeces=0.61, 0.39-0.96), and increased in those using flush toilets (adjusted OR for flush toilet versus none/bush/pit=2.51, 1.00-6.28). In contrast, sensitisation to cockroach was not independently related to helminth infection but was increased in those regularly drinking piped or well water rather than from a stream (adjusted OR=1.33, 1.02-1.75).

During the intervention study 1,566 children in grades 1-8 completed the baseline survey and all consented to be randomised to either anti-helminthic treatment or placebo. 1487 children (95%) completed the intervention study. There was no effect of therapy on the primary outcome, exercise-induced ronchoconstriction (within-participant mean % fall in peak flow from baseline after anti-helminthic treatment 2.25 (SD 7.3) vs placebo 2.19 (SD 7.8, mean difference 0.06 (95% CI -0.71-0.83), p=0.9), or on the prevalence of the secondary clinical outcomes questionnaire-reported wheeze (adjusted OR=1.16, 0.35-3.82), rhinitis (adjusted OR= 1.39, 0.89-2.15), or flexural eczema (adjusted OR=1.17, 0.39-3.49). However, anti-helmithic therapy was associated with a significant allergen skin sensitisation risk increase in the treatment compared to the placebo group (adjusted OR=1.31, 1.02-1.67). In post-hoc analysis the effect was particularly strong for children infected with *Ascaris lumbricoides* at baseline (adjusted OR=4.90, 1.48-16.19), the majority of whom were co-infected with hookworm.

Cytokine profiles showed that Hookworm-induced IL-10 was inversely related to allergen skin sensitisation (any positive skin prick test) at baseline, but this result missed conventional statistical significance (univariate OR=0.70, 0.48- 1.03; adjusted OR=0.72, 0.44-1.18). No other cytokine response was associated with skin prick test positivity at baseline (univariate OR IFN- =1.15, 0.71-1.85; univariate OR IL-5=0.84, 0.53-1.33). Similary, no significant changes in any of the cytokine profiles were observed following anti-helminthic therapy in the treatment compared to the placebo group (p=0.3 for all three cytokines).

Forl (2007) concluded that the baseline study suggested that hookworm and *Ascaris* infection, sanitation and water supply independently reduced the risk of allergic sensitisation. The intervention study confirmed that helminth infection and allergic sensitisation were inversely related and that the effect of *Ascaris* and hookworm infections on skin prick test responses was additive. However, little evidence was found to suggest that the effect was mediated by IL-10. There was also insufficient evidence to suggest that loss of exposure to gut worms for 12 months resulted in an increase in clinical allergic disease. The effect of more prolonged deworming warranted further research.

2.1.2 Helminth and Protozoa Studies Done Within Africa Excluding Zambia

This section of the chapter considers some studies related to helminth and protozoa studies done within Africa

East and Southern Africa

Phiri et al (2003) did a study on the emergence of *Taenia solium* cysticercosis in Eastern and Southern Africa as a serious agricultural problem and public health risk. It was noted during the study that pig production had increased significantly in the Eastern and Southern Africa (ESA) region during the past decade, especially in rural, resource-poor, smallholder communities. Concurrent with the increase in smallholder pig keeping and pork consumption, there had been increasing reports of porcine cysticercosis in the ESA region. This article reviewed the findings concerning the presence and impact of porcine cysticercosis in seven of the ESA countries. Most of the reported findings were based on surveys utilising lingual palpation and post-mortem examination, however, some also used serological assays. In Tanzania, community-based studies on porcine cysticercosis indicated a prevalence of 17.4% in the northern highlands district of Mbulu and a prevalence range of 5.1–16.9% in the southern highlands. In Kenya existing surveys in the southwestern part of the country where smallholder pig keeping was observed to be popular indicated that 10–14% of pigs were positive for cysticercosis by lingual examination. Whereas, Uganda had the most pigs in Eastern

Africa, which were kept under smallholder conditions. Preliminary surveys in 1998 and 1999 at slaughterhouses in Kampala indicated a prevalence of porcine cysticercosis between 0.12 and 1.2%, however, a rural survey in northern Uganda in 1999 indicated 34–45% of pigs slaughtered in selected villages were infected. Additionally, a new survey of 297 pigs slaughtered in Kampala in 2002 indicated that pigs from the central region of the country were negative for cysticercosis while 33.7% of the pigs coming from the rural Lira District in the north were positive. Interestingly 8 piglet foetuses removed from an infected slaughtered sow coming from Lira District were all found to harbour cysts of *T. solium* hence providing evidence of congenital transmission of porcine cysticercosis. In Mozambique, abattoir records indicated that porcine cysticercosis was present in all provinces of the country. A serological survey on pigs in rural Tete Province found 15% of pigs positive.

In Zimbabwe, a retrospective study in official abattoirs around the country from 1994 to 2001 reported a mean prevalence of 0.34% which was in contrast to a post-mortem survey in 1999, which showed that the prevalence of porcine cysticercosis in rural west Zimbabwe where smallholder pig keeping is popular was 28.6%. In Zambia, abattoir records reported porcine cysticercosis in six of the nine provinces. Routine meat inspection of 1316 pigs at a slaughter slab in Lusaka showed that 20.6% of the pigs had cysticercosis whereas serological testing of 874 pigs at the same abattoir indicated that 56.6% were found to have circulating antigens of *Taenia solium*. Field surveys based on lingual palpation in Southern and Eastern Provinces of Zambia revealed prevalence of 8.2–28.4 and 5.2%, respectively.

South Africa has the largest number of pigs in Southern Africa and cysticercosis has been recognised as a problem in the country for many decades. There was strong evidence supporting the high prevalence of neurocysticercosis infecting humans from resource-poor areas of the country where pigs are being raised under smallholder conditions. In spite of this community-based surveys on porcine cysticercosis have never been conducted in South Africa and the last slaughterhouse survey was conducted

nearly 40 years ago. The prevalence of porcine cysticercosis found in these ESA countries rank among the highest in the world and the disease was emerging as an important constraint for the nutritional and economic well-being of resource-poor smallholder farming communities. The current findings suggested the widespread presence of human tapeworm carriers and thus a high risk of human cysticercosis in both rural areas and urban centers in the ESA region. More research was recommended in the region to assess the extent and public health and economic impact of *T. solium* infection in order to determine whether and what prevention and control efforts could be needed.

Ethiopia

Ganta (2015) did a research on Soil Transmitted Helminth Parasitic Infections and their Association with Haemoglobin Concentration and Vitamin A Deficiency among School Children in Mello Koza Woreda, Southern Ethiopia. Soil-transmitted helminth (STH) infections are the major public health problems in many developing countries including Ethiopia. The objective of this study was to determine the prevalence and intensity of Soil Transmitted Helminth parasitic infections in and primary school children. And also to examine their association with haemoglobin concentration, vitamin-A deficiency, anthropometric measurements and risk factors of the infections.

A cross-sectional survey study was carried out and a total of 400 school children were selected using stratified random sampling technique in Salayish Mender-3 pre- school and primary school from March to April, 2014. A structured and pre-tested questionnaire was administered to respondents to generate information on risk factors of STH infections. Stool samples were collected and processed for microscopic examination using the Direct-Wet Mount, Formol-ether concentration and Kato-Katz methods. Blood samples were collected and processed in the laboratory for determination of haemoglobin and serum retinol concentration.

Data were analyzed using SPSS statistical software version 20.0. The overall prevalence of STH infections in the studied school was 48.75%, of which 52.8% were males and 47.2% were females. The prevalence of infection with *Ascaris lumbricoides*, hookworm species, *Trichuris trichiura*, *Schistosoma mansoni* and Strongyloides stercoralis were 34.90%, 29.90%, 17.00%, 10.80% and 6.70%, respectively. The prevalence of low haemoglobin concentration (Hb<110g/L) and vitamin-A deficiency (SRC<0.70μM/L) was 28.75% and 23.25%, respectively. On the other hand, the prevalence of stunting, wasting and underweight was 32.70%, 25.00% and 18.27%, respectively. The STH parasitic infection was associated with low haemoglobine level and low serum retinol concentration in the study area. Risk factors like low level of maternal education, unprotected drinking water source, latrine availability at home, level of knowledge about parasitic infection and shoe wearing habits were significantly associated with STH infections.

Finally, the study revealed that STH infection, anaemea, vitamin-A deficiency and under nutrition, are serious health problems in the studied school (Salayish Mender-3 Pre-School and Primary School). Hence, community-based intervention, particularly school-based deworming program and water, sanitation and hygiene programs are recommended to students of the selected study area.

Getachew (2014) did a study on prevalence and intensity of intestinal parasitic infections and associated risk factors among households around Akaki River and Aba Samuel Dam, Addis Ababa, Ethiopia. Intestinal parasites are widely prevalent in developing countries, probably due to poor sanitation and inadequate personal hygiene. The objective of the study was to determine the prevalence and intensity of intestinal parasitic infections among households in the selected study areas around Akaki River and Aba Samuel dam, Addis Ababa city.

In the cross-sectional descriptive study, the formol-ether concentration technique, Kato-Katz technique and macroscopic examination were employed to identify parasites from

425 stool samples; structured questionnaires were also used. Data was entered and analyzed by SPSS version 16 statistical package software. The generalized estimation equation method was used to determine correlated risk factors and infections at the household level. Out of 425 households who had stool examination, 171 of them had at least one parasite.

Therefore, the overall prevalence of intestinal parasitic infection was 171(40.2%). Of those, the dominant parasite was *A.lumbricoides* (11.53%), followed by *T. trichiura* (8.7%), the prevalence of *Entamoeba histolytica*/*dispar* was 33 (7.8%) and *Giardia lamblia* was 28(6.59%). The presence of double infection was only 37(8.7%) and triple infections were only 5(1.2%). The intensity of intestinal helminth parasitic infections as measured by geometric mean of egg per gram of stool was generally low. High prevalence of intestinal parasitic infection was not in general significantly associated with drinking water, education status, Presence/absence of toilet and level of knowledge on parasitic infection,($P>0.05$). Factors like consumption of raw meat and uncooked vegetables, not washing hands before meal and after toilet use and family size were significantly associated with intestinal parasitic infections ($P<0.05$).

Based on these results, it can be concluded that intestinal parasitic infections were one of the major problems of households in the study areas, Akaki River and Aba Samuel dam. Thus, local health sector should collaborate with community health program for providing health education to increase the knowledge, attitude and practice (KAP) of households about intestinal parasitic infections, their transmission mechanisms and prevention and control methods.

Ghana

Amoah (2008) carried out a research on wastewater irrigated vegetable production: contamination pathway for health risk reduction in Accra, Kumasi and Tamale – Ghana. The study was conducted in three Ghanaian cities (Accra, Kumasi, and Tamale) selected from different agro-ecological zones (Coastal Savanna for Accra, Moist Semi

Deciduous Forest for Kumasi, and Guinea Savanna for Tamale) in two phases. The main research methods used included surveys, crop and water analysis, and field trials. In phase I, questionnaire interviews were used to gather background information from a total of 1058 subjects (farmers, sellers, and consumers) from the three cities on wastewater use, distribution, and handling of wastewater irrigated vegetables. In addition, direct observation and focus group discussions were carried out.

Samples of wastewater irrigated lettuce, cabbage, and spring onions were also collected from selected markets in Accra, Kumasi, and Tamale to determine the current level of exposure of the Ghanaian urban population to hazardous pesticide and faecal bacteria contamination through the consumption of fresh vegetables produced with wastewater.

All three vegetables were analysed for total coliform (TC) and faecal coliform (FC) as well as helminth egg populations on all three vegetables using standard methods. Lettuce samples were also analysed for pesticide residue. In view of the qualitative nature of most of the results from phase I, scientific quantitative data was provided to complement the qualitative results, identify intervention points, and provide the baseline for assessing the effectiveness of interventions.

The requisite quantitative scientific data and implications were covered in the second phase of the study. Lettuce was used as a test crop because of the higher levels of FC from markets (phase 1) compared to cabbage and spring onions. Tamale was also dropped because farmers used similar irrigation water sources as in Accra. At this stage, the microbiological and physicochemical quality of irrigation water from different urban sources was assessed. From two vegetable production sites each in Accra and Kumasi, lettuce samples irrigated with water from drain, stream, well, and piped water, were collected at designated points along the "farm to fork" pathway and analysed for TC, FC, and helminth egg populations.

Attempts were also made to isolate and characterize representative types of faecal coliforms present on farm samples collected during the pathway study to assess the potential health risk to consumers. Methods used for washing vegetables before consumption at households and street food kitchens were surveyed and modifications to improve on their efficacies in removal of FC and helminth eggs carried out in the laboratory. Some of the agronomic practices employed by farmers who used wastewater for vegetable production could be sources of both microbiological and chemical contamination and potentially put farmers, sellers and consumers at risk. For example, 52 to 65% of farmers in Accra, Kumasi, and Tamale irrigated their crops on the day of harvesting, affirming the need to develop measures to minimize the risk associated with wastewater use in vegetable production.

Majority of the irrigated vegetable sellers in Accra, Kumasi and Tamale condemned the use of polluted surface water for growing vegetables but could not indicate which input poses the highest risk. All respondents interviewed washed their vegetable before consumption. This was an indication that the last stage before consumption could be one of the best entry points where health risk reduction strategies could be put in place. Market vegetables from all three cities carried FC and helminth egg populations ranging between 4.0 x 103 to 9.3 x 108 g-1 for FC and 1.1 to 2.7 g-1 for helminth and exceeded ICMSF recommended standards.

A number of different types of helminth eggs, including that of *Ascaris lumbricoides*, *Ancylostoma duodenale*, *Schistosoma heamatobium and Trichuris trichiura*, were also identified on lettuce, cabbage, and spring onions from the markets. Most of pesticide residues on lettuce exceeded the Maximum Residue Limit (MRL) for consumption. Faecal coliform and helminth egg populations in irrigation water from different sources exceeded WHO recommended standards for unrestricted irrigation. From all the irrigation water sources in both Kumasi and Accra, *Ascaris lumbricoides* was the most predominant species recorded; population density ranged between 2 to 4 eggs l-1.

However, heavy metal concentrations were mostly within limits of international standard. Despite poor sanitary conditions in markets, postharvest handling, and distribution of lettuce did not significantly increase the farm-gate contamination levels. High FC levels exceeding common guidelines for food quality were also recorded on lettuce irrespective of the irrigation water source. The results also showed that apart from wastewater, contaminated soil, and poultry manure also contribute to crop contamination. Identified bacterial isolates from lettuce sampled from the farms belonged to genera (*Cedecea, Enterobacter, Erwina, Escherichia, Klebsiella, Kluyvera, Aeromonas, Chryseomonas, and Serratia*). Presumptive test for *E. coli* 0157:H7 was positive for about 13% of isolates from Accra and 20% from Kumasi.

The food vendor/consumer surveys revealed that the large majority of the households and street kitchens used various methods to wash vegetables before consumption. Washing vegetables, irrespective of the method used reduced FC and helminth populations in lettuce, but at varying degrees. Attempts made to improve on selected existing washing methods showed that there was promise for vinegar (> 4 log reductions possible at increased concentration and contact time) but with high financial implications for poor households; and this could reduce its adoption potential. The WHO has set a health protection level of 10-6 DALY (Disability Adjusted Life Years) which is achievable by 6 -7 log reduction. However, none of the methods tested achieved the level of reduction alone.

The study concluded that wastewater irrigated vegetable production threatens public health from the perspective of microbiological and pesticide contaminations. The need to reduce the potential health risks resulting from FC and helminth contamination of wastewater irrigated vegetables needs a more holistic approach than a simple focus on the polluted irrigation water sources. The adoption of the multiple barrier approach, where complementary risk reduction strategies are applied at various entry points before the vegetables reach the kitchen, is likely to make more significant impact.

Yaw (2012) did a study on prevalence of soil-transmitted helminthes among pupils in Gia and Kajelo primary schools in the Kassena-Nankana east and West districts in the upper east region of Ghana. Soil-Transmitted Helminthes was prevalent in many communities in the Kassena-Nankana District of the Upper East Region of Ghana. Gia was one community where the prevalence of soil-transmitted helminthes had been reported in 2007 to be 10% prevalent by the direct wet mount method. This study determined the current prevalence of soil-transmitted helminthes. Among primary school pupils in Gia and Kajelo communities in the Kassena-Nankana district using direct wet mount and the Formol-ether concentration techniques. It was observed that stool samples that are negative for parasites by the direct wet mount method should be re-examined using the Formol-ether concentration technique as the confirmatory test as it was noted that the approach would improve the detection of helminthes from stool specimens for accurate diagnosis of soil-transmitted helminthes infections and ultimately improve the quality of life of individuals in these communities.

Kenya

Owiiti (2008) did a study on prevalence of soil-transmitted helminths infections among school children in Bondo District, Nyanza Province, Kenya**.** A study to determine the prevalence of soil-transmitted helminth (STH) wasconducted among 418 school children in 20 primary schools in five divisions(Maranda, Madiany, Rarieda, Nyang'oma and Usigu) of Bondo district, Nyanza Province, Kenya.

In a cross-sectional survey stool samples were obtained from children aged 5-15 years and examined for the presence of eggs of helminthes, from September to November 2007. The stool samples were examined using the direct method and Formol-Ether concentration. The overall prevalence of STH in the district was found to be 18.4%. *Ascaris lumbricoides* had the highest prevalence at 8.6% followed by Hookworms 5.7%, *Trichuris trichuria* 3.7% and *Strongyloides stercoralis* 0.2% respectively.

The study revealed that Maranda division had the highest prevalence of the helminthes infections. The high prevalence of worm infection in Maranda seems to be associated with the division's dependency on water pan as a source of water for domestic use. Children belonging to the age group of 5-7 years were more infected with STHIs than those of older age groups. It was recommended that further study be done to find out why dependency on water pan as a source of water for domestic use is a risk factor in Maranda.

Robinson (2005) did a study on household adoption of ecological sanitation: An assessment of agricultural value and user perspectives in Nyanza Province, Kenya. Ecological sanitation, or ecosan, refers to a range of sanitation technologies in which human excreta is recovered and retained on-site, and eventually reused. However, when a culture does not have a tradition of reusing or handling human waste, what would motivate a household within that culture to recycle and reuse their waste? More specifically, how do the agricultural value of the material from an ecosan toilet and user perspectives on ecosan systems influence households' adoption of ecosan toilets?

On average, households in the study area produced 4 kilograms of nitrogen and 0.6 kilograms of phosphorous per year from urine collected in the skyloo toilet, the type of urine-diverting ecosan toilet available in the study area in the Nyanza Province, Kenya at the time. These nutrients were the equivalent of a cost savings of about US $12 per year (the GDP per capita in Kenya in 2004 was US $1100).

It was noted that about two-thirds of the households reused the processed feaces and urine in household gardens. Users reported additional major benefits such as the absence of foul odors, inexpensive construction costs (partly due to a materials subsidy by the promoting NGO), and the aesthetic value/social status that the facility brings to the owners' homes. The major negative factors included problems with construction and design of the facility, training new users—especially children—how to use the toilet, and handling human excrement.

The findings suggested that ecosan was a viable sanitation option that filled a niche within the region of Kenya. Ecosan's comparative advantages seemed to be significant enough to outweigh negative cultural sentiments regarding the handling of human excrement to some user groups. Such user groups included the very poor who practiced household agriculture (those who had trouble affording commercial fertilizer and also had reason to want it), those who lived in areas with high nutrient loads to natural waters, households with an exceptional environmental conscious, and households in which adverse hydrogeological conditions (such as a high water table or loose soils) made pit latrines an environmental and human health hazard.

It was concluded that in addition to household-level advantages, the niche that ecosan filled had the potential to make headway towards the Millennium Development Goals' provision of sanitation and to be a valuable contribution to integrated water resource management strategies.

South Africa

Babatunde (2014) investigated the accumulation rate of sludge in pit latrine and the role of digestion process on sludge accumulation rate with a community in eThekwini Municipality. He also looked at the chemical and biological characteristics of pit sludge at different depths within a pit latrine. He observed that providing adequate sanitation to all in the form of VIP latrines as proposed by the South African Government Strategic Framework for Water Services did not end with building toilets. An understanding of the processes occurring in pit latrines would facilitate better management during their lifespan and identifying suitable options for dealing with the accumulated sludge when they eventually reach their capacity.

Babatunde (2014) aimed at providing scientific support for decision making in management of accumulated sludge in ventilated improved pit latrines during their life

span and when they reach their capacity under South African conditions. The approach to the research work was divided into two main thrusts: The first was to provide an understanding of the processes in VIP latrines and mechanism of sludge stabilization in pit latrines. The second approach was to provide management and disposal options for pit latrine sludge before and once it has been exhumed in the context of the eThekwini Pit latrine emptying Programme Two options were used as case studies, namely:

- deep row entrenchment of exhumed pit sludge for agroforestry; and
- in situ-treatment of pit sludge using additives.

Direct measurement of sludge accumulation rate from selected pit latrines within a community in eThekwini Municipality was performed and a laboratory investigation into the effect of moisture content and aerobic/anaerobic conditions on sludge accumulation rate was conducted.

The second experiment investigated the chemical and biological characteristics of pit sludge at different depths within a pit latrine. Research into deep row entrenchment of VIP latrine sludge for agroforestry was conducted. The effect of deep row entrenchment on sludge characteristics and surrounding groundwater at the site was investigated by monitoring changes in sludge characteristics and groundwater quality at the entrenchment site over time.

An investigation into the effect of pit latrine additives on pit sludge was conducted. Two sets of trials were conducted; the first was a laboratory trial conducted to investigate the effect of pit latrines additives on collected sludge samples from pit latrine in laboratory scale test units. The rate of mass loss that could arise from the effect of addition of pit additives to sludge in the test unit was determined. The second was a field trial in which pit additives were added to randomly selected pit latrines within a community in Durban and changes in amount of the sludge in the pit was investigated using a laser tape measure and a stereographic imaging technique.

The main findings of the research were:

The sludge volume accumulation rate in pit latrines investigated was between 120 ℓ/year and 550 ℓ/year regardless of the number of pit users. The overall average sludge accumulation rate was 282 ± 46 ℓ/year. The figures related to per-capita sludge accumulation rate of 56 ℓ/person/year for an average of 5 number of pit users obtained in the study. Statistical analysis performed indicated that sludge accumulation rate on a per capita basis did not decrease with an increase in number of pit users;
In the laboratory batch experiments, it was observed that by increasing the moisture content the rate of degradation of sludge samples decreased. Over a period of 230 days, mass loss was inversely proportional to total moisture content, and it was found that the mass of solids were reduced to somewhere between 17 and 64 % of the original sludge mass.

The observed effect was attributed to the exposure of sludge samples in the test units to oxygen, since sludge samples with higher total moisture content in the test units appeared as increased depth of free liquid between sludge sample and air. The calculated mass loss rates observed were expected to be higher than that which was observed in a pit because the laboratory test had continuous air exposure but pit contents were usually covered over by new materials added to the pit;

Natural stabilization of sludge within the pit did occur if the pit was managed and maintained properly thus providing a long service life for the pit. It was found that the volume of materials had been reduced to between 50 and 75 % of the volume of material added over the 3 years since the pits investigated were last emptied, based on the observed per capita sludge accumulation rate and an estimated addition of the material to the pit per person/year. Thus, by comparing the calculated mass reduction in the batch laboratory experiment with the volume reduction in the field investigation of sludge accumulation rate, it was inferred that sludge densification/compaction played an important role on the stabilization processes in a pit;

The nature of sludge in pit latrines varied significantly within the pit and between different pits. It was observed that below the surface layer in a pit, additional stabilization of sludge did occur and the degree of stabilization within a pit increased with increasing depth from the surface down to the bottom layer of the pit. Sludge samples from the bottom of the pit were well stabilized;

It was also observed from the investigation into deep row entrenchment of pit sludge for agroforestry that further stabilization of pit sludge did occur and as a result of that, nutrients (nitrogen, phosphorus and potassium) locked up in the buried sludge were released as fertilizers. Trees planted near buried VIP sludge showed better growth rate compared to those buried only on soil without VIP latrine sludge and no profound effect on groundwater was observed for the duration in which monitoring was carried out;

Neither laboratory trials nor field trials provided any evidence that the use of pit additives have any beneficial effect on VIP latrine sludge. There were no systematic and statistically significant changes in the rate of mass loss on sludge samples in the laboratory test units as well as changes in sludge content of the pit latrines used for the field trials as a result of pit latrine additives;

Although, it was observed that there was significant reduction in sludge height in pit latrines in which only water was added compared to those in which additives were added and those in which nothing was added (control) using the infrared distance measure, the effect could probably not be explained completely to be as a result of increasing biodegradation rate caused by higher moisture content, since the explanation would have been observed in the laboratory trials as well as in measurement taking using the stereographic imaging techniques;

Instead, flattening of the surface of sludge content in the pit by the addition of water onto the highest part of the pile played a part in the apparent reduction of sludge height observed.

Therefore, Babatunde (2014), concluded that disposal options involving biological treatment such as disposal into wastewater treatment plants and anaerobic digestion were not appropriate because the residual biodegradability of VIP latrine sludge obtained was very low (about 30 %) and as such would only result in accumulation of undigested solid. Among the options considered in the research, deep row entrenchment of VIP latrine sludge for agro forestry seemed to be an appropriate option for the disposal of VIP latrine sludge. There was no evidence that suggested that pit latrine additives had any effect in reducing sludge content in pit latrines. Sludge content in pit latrines had naturally undergone significant degradation and that the options for disposal of pit latrine sludge would be limited by the characteristics of the sludge.

Buckley et al (2008), did an analysis of samples of fresh faecal material and samples from a range of pit latrines within a number of locations which were analysed for organic and inorganic characteristics including total COD, moisture, total solids and inorganic solids. Assessment of the results leads to the development of a conceptual model of biological degradation processes that occur in a pit latrine.

During their analysis, the faecal sludge portions of pit contents were divided into four theoretical categories:

The first category was sludge where readily biodegradable components were still present, wherein rapid aerobic degradation occurred. The layer was observed to be negligibly small and was not measurable practically;

The second category was made up of the top aerobic section of the pit where, aerobic degradation of hydrolysable organic material occurred at a rate limited by the aerobic hydrolysis of complex organic molecules to simpler compounds; and

The third layer was anaerobic due to the occlusion of oxygen by covering material. Anaerobic digestion, it was observed, proceeded at a significantly slower rate than in the layer above, and was controlled by the rate of anaerobic hydrolysis of complex organic molecules to simpler molecules.

The study concluded in the study that in the lowest layer, no further stabilisation of organic material occurred within the remaining life of the pit contents.

Still and Nash (2002), undertook a study on groundwater contamination due to pit latrines, Maputaland. The objective of the study was to establish the hazards posed by pit latrines, and the real risk situations. The study described local and international research and examined the justification of the 75 metre rule. The field research was conducted in the Maputaland area of KwaZulu-Natal, where there were large numbers of both pit latrines and shallow wells. Observations of water quality in a range of well types spread throughout the area were done and the water quality was assessed using samples collected from wells found close to pit latrines. The water samples were monitored for faecal coliforms bacteria and nitrates. It was observed that properly designed and maintained wells produce water of good quality.

Tanzania

Chaggu (2003) undertook a study on Sustainable Environmental Protection Using Modified Pit-Latrines, in Tanzania. In the study, it was indicated that, the substructures for the pits were not water-tight. Therefore, the groundwater could freely flow in and out of the pit, especially in high water table areas resulting in groundwater pollution and even surface water pollution in the neighbourhood. With a growing concern of public health, groundwater pollution and the reuse of nutrients from human waste, it was indicated that there was a serious need to study and improve the pit latrines, especially those in high water table areas. The need to improve the pit latrines in general was emphasised.

Kamasho (2012) did a research on effectiveness of intervention with mass chemotherapy for soil-transmitted helminths among primary school children in Bagamoyo District, Tanzania. In most countries where soil-transmitted helminths (geoheminths) were endemic, school-age children had the highest prevalence and bear the greatest intensity of infection. A deworming programme represented one of the most efficient and cost-effective means to improve child health and education. The objective of the study was to assess the effectiveness of intervention with mass chemotherapy for soil-transmitted helminths among primary school children in Bagamoyo District.

A cross-sectional study design, cluster sampling was used to obtain 300 children of standard I-V of Mlingotini and Pande primary schools. Questionnaires were administered to obtain demographic information, knowledge and factors related to soil-transmitted helminths infection. Fresh stool samples (≤ 24 hours) were collected from every participant and examined by the Kato-Katz thick smear technique to determine infection status.

The results of the study indicated that proportion of the school children who received Albendazole during the last distribution was 97.7% and the prevalence of geohelminths was 0% based on microscopic examination. 63% of the children had poor knowledge regarding soil-transmitted helminths, 23% had adequate knowledge and 14% had moderate knowledge. It was statistically significant that the level of knowledge was increasing with age, Chi squire for age group with level of knowledge was 3.26 and p value <0.05. Some risk factors for having soil-transmitted helminths were high, as 89% of the children were drinking untreated water at their home. Also 64% of the children mentioned sand as their toilet floor material and 0.3% reported never wearing shoes when visiting toilets.

The study concluded that health promotion activities to enhance awareness were important in ensuring that the risks for being infected with soil-transmitted helminths were minimal if not eliminated. The study recommended that regular mass treatment

should be maintained so as to ensure that the prevalence of soil-transmitted helminths remained zero.

Thomas et al (2013) did a review of sanitation and hygiene in Tanzania during which it was observed that Tanzania was not on track to meet its Millennium Development Goal of 62 % improved sanitation coverage by 2015. The failure was due to population growth characterised by rapid urbanisation which the Government of Tanzania was unable to service due to limited capacity, resources and lack of coordination of the other implementing stakeholders. Inadequate sanitation and hygiene resulted in morbidity and mortality for Tanzanian's due to endemic infections resulting in diarrhoea and other illnesses. The review summarised all the available literature to provide the status of sanitation and hygiene and an overview of projects and programs in Tanzania. Finally, gaps were identified in the knowledge available at the time and recommendations made on how to improve sanitation and hygiene in Tanzania. The review identified the stakeholders in sanitation and hygiene at that time in Tanzania to include; office of the Prime Minister, three government ministries, local government areas, 12 donor and multilateral agencies, 5 private donors, 13 international non-government organizations (NGO), 18 local NGO, 2 faith based organizations, 2 networks as well as numerous actors from community based organisations and the commercial sector. Stakeholders interacted through the policy process, funding, implementation, research, evaluation, networks and partnerships.

The health burden due to poor sanitation and hygiene was significant. Diarrhoea in the preceding two weeks was reported on average in 15 % of children under five years of age and results in 9 % of all mortality for the age group. Cholera and Typhoid was endemic in some areas of Tanzania and outbreaks were common. Then there was the ever present problems of water related parasitic infections such as malaria and schistosomiasis. Prevalence of these infections in Tanzania has been scientifically linked to poor sanitation and hygiene; in particular a access to latrines, poor hand washing behaviour, and inadequate drainage.

Across Tanzania it was estimated that 93 % of the population has access to a latrine. However, when assessing access to improved sanitation that figures drops to 24 %, depending on the definition of improved sanitation used. There are differences between urban and rural areas with urban areas particularly in Dar es Salaam reporting lack of access to affordable sanitation due to costs of construction, high water table and de-sludging expenses.

In urban areas lack of solid waste collection and poor drainage combined with extensive use of pit latrines make for very poor hygiene conditions. Hygiene behaviour in Tanzania varies, although hand washing is widely practiced except not always with soap nor at critical times such as before preparing food or after disposing of children's faeces. Programs and projects implemented across Tanzania vary in their scale. Large government run multi-donor programs such as the Water Sector Development Program (WSDP) operate on a national scale. WSDP has brought together a number of the stakeholders. However, the program has only a fraction of its budget allocated to sanitation and hygiene projects with the majority focused on water supply.

Up-scaling of the World Bank funded Water Sanitation Program (WSP) using a market led approach to hand washing and sanitation adoption has been shown to be successful in reaching large a number of people in the community although quantifying the impacts of the program was not achievable. International and local NGO are conducting programs and projects on smaller scales across most regions of Tanzania. Affordable improved sanitation and safe sustainable pit latrine emptying practices in urban areas are examples of such projects. The projects vary in their approaches and some focus on the needs of specific groups such as pastoral tribes, women and children, refugees, schools and health care clinics. Hygiene education programs through schools have been shown to be effective at changing behaviour. The results from this work varies from ineffective or un-measurable outcomes to sustained up-take and changed behaviour. The

challenge is the replicate the results seen at small project level at scale through national sustainable programs.

Within the literature reviewed there were gaps identified in knowledge of sanitation and hygiene in Tanzania. For sanitation there is a lack of information regarding the markets for sanitation in urban areas. In comparison to sanitation there was less information available about the state of hand washing hardware and behaviour. There was also very little information about oral, anal washing or menstrual hygiene practices.

Recommendations on how to improve sanitation and hygiene in Tanzania are centered around adoption of participatory approaches between all the stakeholders. However, this requires the Tanzanian Government to have clear policies and regulations with respect to sanitation and hygiene. Hence, it is of paramount importance that the draft policy on sanitation is accepted promptly. Further, there needs to be more investment into sanitation and hygiene at all levels of government. NGO and community based organizations have a role to play in both driving this policy but also assisting the government in implementation and efficient use of resources. Tanzania will not reach its 2015 Millennium Development Goal for sanitation. Now that attention has been directed to the importance of sanitation and hygiene a change of direction characterised by a coordinated response between all the stakeholders is needed for real improvements.

Uganda

Dumba et al (2008) carried out a research on intestinal helminths in Luweero District, Uganda. Intestinal helminthiasis is a debilitating parasitic disease found in many parts of Uganda including Luweero District. In the district, the disease causes as high as 9% morbidity in children below five years. There was very scanty district information on the disease based mainly on hospital records despite the figure. The study was carried out to provide data to plan for its effective control. The objective of the research was to investigate risk factors that promote helminth infections among children under five years of age in Luweero District.

During the research, stool samples from 727 children were examined for presence of helminth ova using Kato-Katz technique. The subjects' parents or guardians were interviewed using a semi-structured questionnaire to establish their demographic, social-cultural characteristics; information on water accessibility and usage; child toileting practices and knowledge about helminthiasis.

The results of the research showed that risk factors strongly associated with helminth infections included methods of anal cleaning, how compounds and latrines were maintained, keeping of pigs and age of the subjects, ($P < 0.001$). In addition, methods of hand washing after latrine visits, the respondents' education level, type of house floor and household compound as well as accessibility to water were associated with worm infection.

It was concluded that the hygiene practices of the parents/guardians and environmental surroundings in which the child grew played a big part in determining his or her helminth status. It was recommended that the District Health workers, community leaders and extension staff should educate the community on the importance of personal hygiene and environmental sanitation to minimize the risks of helminth infections.

Malawi and Zambia

Chipeta et al (2014) did a study on Zero adjusted models with applications to analysing helminths count data. It was observed that it is common in public health and epidemiology that the outcome of interest was counts of events occurrence. Analysing these data using classical linear models was mostly inappropriate, even after transformation of outcome variables due to over dispersion. Zero-adjusted mixture count models such as zero-inflated and hurdle count models were applied to count data when over-dispersion and excess zeros exist. Main objective of the current paper was to apply such models to analyse risk factors associated with human helminths (*S. haematobium*) particularly in a case where there's a high proportion of zero counts.

The data were collected during a community-based randomised control trial assessing the impact of mass drug administration (MDA) with praziquantel in Malawi, and a school-based cross sectional epidemiology survey in Zambia.

Count data models including traditional (Poisson and negative binomial) models, zero modified models (zero inflated Poisson and zero inflated negative binomial) and hurdle models (Poisson logit hurdle and negative binomial logit hurdle) were fitted and compared. Using Akaike information criteria (AIC), the negative binomial logit hurdle (NBLH) and zero inflated negative binomial (ZINB) showed best performance in both datasets. With regards to zero count capturing, these models performed better than other models.

In conclusion, this paper showed that zero modified NBLH and ZINB models were more appropriate methods for the analysis of data with excess zeros. The choice between the hurdle and zero-inflated models should be based on the aim and endpoints of the study.

2.1.3 Helminth and Protozoa Studies Done Within Zambia

This section of the chapter considers some studies related to helminth and protozoa studies done within Zambia

Banda (2013) did a study on prevalence and risk factors of cystic Echinococcosis in cattle and humans in Western Province of Zambia. Cystic echinococcosis or hydatidosis is caused by the larval stage of the tapeworm *Echinococcus granulosus*, and is one of the most important parasitic infestations in livestock worldwide and one of the most important parasitic zoonoses.

A cross sectional study was conducted to estimate the prevalence of hydatidosis in cattle and humans of Western Province of Zambia and determine the risk factors associated

with disease occurrence. A retrospective analysis of cattle slaughter data from an eleven year period 1994 to 2007 (except for 1997, 1998 and 2002) was done to determine the presence of hydatid cysts in cattle. A retrospective review of, records of human cystic echinococcosis from Lewanika General Hospital, which is a referral centre for Western Province over four year period (2006 to 2010) was conducted and analyzed to determine the prevalence of the parasite in humans. Disease prevalence in cattle in various districts and camps was done by post mortem examination conducted from October 2007 to November 2008. The viability and fertility tests were done on some of the cysts collected during the study.

A questionnaire survey to identify risk factors of transmission was also carried out. Annual losses due to abattoir condemnation of organs were estimated to determine the direct economic loss due to the disease. A total of 158,456 cattle were slaughtered and inspected out of which, 4689 (3.0%) cases of bovine hydatidosis were recorded. The lung accounted for 93.7 %, followed by liver at 6.26 % and spleen at 0.02 %. Proportion positive in humans was 0.009 % (9 per 100,000 cases attended to). Sixty-seven percent of the human cases diagnosed were in females and 33% in male humans. Hydatidosis was prevalent in 2.1% of the cattle slaughtered in two abattoirs in Western province during the prospective study. District prevalence were at 2.5%, 2.1%, 1.4% and 0.6% for Mongu, Senanga, Kalabo and Lukulu, respectively.

In the questionnaire survey, it was observed that 88% of the households owned at least one dog. Among those that kept dogs (n= 58), the majority (96.6%) of dogs were in the free range system. Most dogs were mostly kept for security (86.2%) and cattle herding (87.9%). Stray dogs in the communities were regularly spotted by 65% of respondents. Most of the HH (94.8%) admitted to feeding offal to dogs and 37.9% of the respondents reported dogs scavenging from abattoirs and local slaughter slabs. It was observed that 98% of the households had dogs that defecated in the immediate surroundings of their dwelling places.

It was found that the liver had 43.7% and the lung had 43% fertile cysts. The overall percentage of fertile cysts was found to be 43.1%. The estimated annual loss associated with organ condemnation was evaluated at K 15, 894, 000 (U$3,311) annually. The study did establish that hydatidosis was prevalent in cattle and humans in Western Province of Zambia and that the risk factors for its transmission existed. The disease also contributed to economic losses due to organ condemnations and was of public health concern.

Mulenga and Fawcett (2002) did a research on the impediments to the implementation of the demand response approach (DRA) methodology in urban sanitation programmes in Zambia and South Africa. The research aimed to identify the issues that may hinder the effective implementation of the DRA methodology in water and sanitation provision to the urban poor and to recommend methods to overcome them. The study therefore explored the barriers at both community and institutional levels that may have had an impact on the implementation of the DRA methodology in sanitation programmes in urban poor communities. In order to identify the barriers to the DRA on the ground, both quantitative and qualitative methods were used to collect data from households in urban informal settlements and sanitation agencies in the two countries.

The study was implemented in two informal settlements in Ndola and two in Lusaka in Zambia and two each in Pretoria and Durban in South Africa. A total of 1,894 households were surveyed and 88 representatives of institutions dealing with water and sanitation issues were also interviewed. The household and institutional surveys took place between August 1999 and July 2000. The research findings and discussion are organised in 5 main sections under the following themes:

- Determinants of demand for improved water and sanitation services;
- Social intermediation issues;
- Technical choices of water and sanitation;
- Institutional issues; and

- Financial and economic issues

These themes provided a basis for determining the key lessons learnt, and recommendations for the effective implementation of the DRA methodology in urban poor sanitation programmes.

The evidence from the study led to the conclusion that household income was a major barrier to the implementation of the DRA in urban poor communities because the majority cannot afford to pay significant tariffs for sanitation services. Poverty in the urban poor communities was therefore the greatest barrier to the implementation of the DRA methodology.

The study also indicated that compared to water, sanitation facilities were not a major priority in all the study areas. This had a negative impact on the DRA because if the households did not consider sanitation to be a major priority it was difficult to expect them to spend their limited resources on it, at the expense of water supply.

Homeowners interviewed, also complained of their failure to be granted land tenure, especially in areas that were still considered illegal by the authorities. In both South Africa and Zambia sanitation agencies and aid agencies did not operate in communities which did not have a legal status. Without the participation of these organisations, the DRA could not be feasible because they were key participants in the implementation of the methodology. Without the community's willingness to pay for improved sanitation services therefore, the DRA was not feasible.

There was clearly a lack of awareness in urban poor communities about the key issues that were vital to the implementation of the DRA methodology such as good community organisation. Likewise, there was a gap in knowledge about the existence of different water and sanitation technologies. Therefore, civic education needed to be undertaken in

order to increase the possibility of providing sustainable sanitation services through the use of the DRA methodology.

The study found that the voluntary nature of community participation in projects had a negative impact on community organisation. This was exacerbated by the unfavourable economic situations in the two countries, which made it difficult for community members to devote more time to non-paying community work at the expense of income generating activities. Experience gained from the field surveys showed that communities could only spend some time for one-off projects and not recurring ones. Readiness to participate in community programmes was found to be higher in cases where households were compensated for their labour contributions, food for work community projects.

Other findings of the research showed that the lack of social integration and coordination in the informal settlements in both Zambia and South Africa in general could be blamed on organisations existing and working in these areas, which often fail to link up with other similar organisations working in the same areas and with similar goals. As a result, efforts in the past to resolve sanitation problems in the informal settlements had often been disjointed.

There were still no clear lines of communication between government agencies and communities despite the existence of community based organisations. Many agencies still ignored the informal settlements as if all of them were still illegal due to the severity of problems faced there and do not make any attempts to change the situation. The study also noted that the inadequate information flow between policy makers and grass root implementers had also worsened the situation. Local Councillors who could be a useful link between communities and local authorities had a very poor record with communities because of their political inclinations and misrepresentation of community priorities. Without the active involvement of these constituencies, it seemed unlikely that the DRA methodology could be successfully implemented in urban poor settlements.

In some cases the study found that there were existing institutional units that were supposed to deal with service provision to the urban poor but such units were normally understaffed and lacked the skills to coordinate with other departments or institutions. An example is the Peri-Urban Section at the Lusaka City Council, which has a clear mandate to address development projects in the informal settlements of the city but, due to understaffing, lack of skilled manpower and financial problems it was unable to fulfil its mission.

Obstacles were also encountered due to the lack of interest, knowledge and commitment to sharing in the implementation of the DRA methodology. In the absence of technical support from local authorities and other agencies, communities have remained poorly organised, making it difficult for them to be engaged in the process of selecting, financing, implementing and managing of sanitation facilities based on expressed demand. The lack of capacity at local authority level further exacerbated the problem.

The survey also revealed that both South Africa and Zambia were undergoing a transformation in their water and sanitation sectors. Transformation was, however, a complex process needing a range of skills which were currently in short supply in both countries. There was very little understanding of the role and function of local government in relation to other levels of government and there were very few interactions between the various line departments. There was also still very little understanding of how to communicate new responsibilities and their implications to senior management and the local council and of how to be accountable to customers within a service delivery framework. These challenges were great even for the most skilled staff and councillors, so several years of training and promotion were required for the necessary capacity to be developed before the legal requirements could be effectively fulfilled. Under such circumstances, it was almost certainly impossible to implement programmes using the DRA methodology due to its complexity. The South African local government structure, had for example, been undergoing changes since 1994; the changes had been so great that many local authorities were still struggling to cope with the new expectations.

Many decisions made at central government level were not implemented due to the lack of resources to implement the decisions, demonstrating the weak linkages between policy and resources. There was no legal framework and no effective strategies to guide the provision of water and sanitation services to the urban poor communities. The lack of capacity, coupled with poor administration and coordination at both community and sanitation agency levels, were major barriers to the implementation of the DRA, because it was interdisciplinary in nature.

For the DRA to work there was need for efficient cost recovery mechanisms in the communities. In both Zambia and South Africa, cost recovery measures were central to the governments' promise to provide household sanitation. Currently, however, cost recovery was very low in the two countries. In South Africa, it was found that the legacy of apartheid had continued to haunt politicians and has made it impossible for them to stick to programmes that promote cost recovery. In the past the white minority had access to some free services or to other services that were heavily subsidised by the state. Consequently, it was difficult for the government to convince the black majority that in order for them to receive services on a sustainable basis they need to pay for them. This hampered many attempts by local authorities to provide services with cost recovery goals, as the DRA advocates.

Significant challenges remain, particularly relating to financing arrangements at both community and institutional levels. A major barrier to the implementation of the DRA was the need to balance financial sustainability and poverty reduction objectives. Financial cost was already a significant barrier preventing many urban poor communities from accessing improved sanitation facilities. A key social issue being faced with the implementation of the DRA therefore was the reconciliation of the demand for improved sanitation services with a limited ability to pay among the urban poor consumers.

The overall findings of the study suggested that implementing the DRA in sanitation programmes in urban poor communities could remain an enigma unless a comprehensive analysis of all the factors that impeded its implementation were given consideration both at community and institutional levels. In as much as all the barriers identified might have impeded the implementation of the DRA in urban poor communities, the main one was poverty.

The research therefore contended that the DRA, as it was formulated and promoted was not a feasible methodology in the implementation of sanitation programmes in urban poor communities. However, it covered some useful principles, which could be utilised in making water and sanitation programmes more sustainable such as community participation, clarification of roles and responsibilities and community contributions to sanitation.

Mwape et el (2012), carried out a research *Taenia solium* Infections in a Rural Area of Eastern Zambia-A Community Based Study. During the study, stool and serum samples were collected from willing participants. Geographical references of the participants' households were determined and household questionnaires administered. Taeniosis was diagnosed in stool samples by coprology and by the polyclonal antibody-based copro-antigen enzyme-linked immune sorbent assay (copro-Ag ELISA), while cysticercosis was diagnosed in serum by the B158/B60 monoclonal antibody-based antigen ELISA (sero-Ag ELISA). Identification of the collected tapeworm after niclosamide treatment and purgation was done using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). A total of 255 households from 20 villages participated in the study, 718 stool and 708 serum samples were collected and examined. Forty-five faecal samples (6.3%) were found positive for taeniosis on copro-Ag ELISA while circulating cysticercus antigen was detected in 5.8% (41/708) individuals. The tapeworm recovered from one of the cases was confirmed to be *T. solium* on PCR-RFLP. Seropositivity (cysticercosis) was significantly positively related to age (p=0.00) and to copro-Ag positivity (taeniosis) (p=0.03) but not to gender.

Change point analysis revealed that the frequency of cysticercus antigens increased significantly in individuals above the age of 30. Copro-Ag positivity was not related to age or gender. The following risk factors were noted to be present in the study community: free-range pig husbandry system and poor sanitation with 47.8% of the households visited lacking latrines. Furthermore, risk factors associated with the transmission and maintenance of the parasite such as free roaming pigs, households without latrines, backyard slaughter of pigs without inspection and consumption of undercooked pork were also present. The findings of this work have identified the need for further research in the transmission dynamics and the burden that this infection has on the resources of poor local people.

Phiri et al (2014) did a study on characterisation of biosolids and evaluating the effectiveness of plastic-covered sun drying beds as a biosolids stabilization method in Lusaka, Zambia. The Lusaka Water and Sewerage Company (LWSC) produced about 800–1,000 kg of treated sewage sludge per day at its Manchinchi wastewater treatment plant (WWTP). The biosolids were used for land application purposes although the contaminant and pathogen composition and quality of the biosolids had not been known. At the time, Zambia did not have legal standards and guidelines for biosolids management or application.

The Manchinchi plant in Lusaka suffers from constant breakdowns such that the effectiveness of the plant to produce quality grade biosolids for land application use was questionable. In peri-urban areas, the problem of poor sanitation was being addressed using different technologies including urine diversion ecosan toilets. The effectiveness of ecosan toilets to stabilize faecal sludge had not been assessed in Zambia. The purpose of the study was to stabilize and characterize the biosolids from Manchinchi plant and ecosan toilets. Stabilization was done by use of drying beds and irradiation. The parameters that were used for characterization were microbiological, parasitological and heavy metals.

The results of the study revealed that b biosolids from the Manchinchi WWTP sun drying bed, ecosan toilets and from an experimental plastic covered drying bed were found to contain different pathogenic microorganisms and contaminant levels. A radiation dose and time-related declining trend in pathogens loads in biosolids were observed. By the third week, no viable *Ascaris* ova were detected. Based on controlled conditions, the biosolids quality was found to be within the internationally acceptable standards for restricted use. The study concluded that both the untreated LWSC biosolids and ecosan sludge contained pathogen levels with the potential to cause environmental and public health hazards if used for agriculture purposes.

Under plastic-covered drying beds, viable Ascaris ova were not detected by the fourth week of treatment and the biosolids were stabilized to levels equivalent to Class C of the Australian standards for restricted land application. Covered drying beds can be considered as cost effective stabilization treatment technology for biosolids in developing countries. The technology has potential benefits for improving public health and reducing environmental pollution in Zambia, especially during the rainy season when biosolids are directly discharged into the environment.

Siwila et al **(**2010) did a study on intestinal helminths and protozoa in children in preschools in Kafue District, Zambia. The aim of the study was to determine the prevalence of the protozoa *Cryptosporidium* and *Giardia*, as well as prevalence and intensity of intestinal helminths in children attending pre-school or day-care centres in Kafue District, Zambia. Prevalence, incidence and seasonal variation of *Cryptosporidium* and *Giardia duodenalis* were studied over a 12-month period in 100 children from four pre-schools in Kafue, Zambia. Questionnaire data and a single stool sample were collected monthly from each child. Samples were processed using a commercial kit (Meridian Diagnostics Inc., USA) and oo(cysts) visualised by immunofluorescence microscopy. *Cryptosporidium* was detected in 30.7% (241/786; 95% CI = 27.5-33.9) while *G. duodenalis* was detected in 29.0% (228/786; 95% CI =

25.8-32.2). A total of 86% experienced one or more episodes of cryptosporidiosis while 75% had giardiasis.

Cumulative incidence per 100 children was 75.4 for *Cryptosporidium* and 49.0 for *G. duodenalis*. Both infections were significantly more common in the wet compared to the dry season (34.8%, 162/466 vs. 24.7%, 79/320, P = 0.003 and 35.2%, 164/466 vs. 20.0%, 64/320, P < 0.001, respectively). Thus, risk ratios (RR) were 1.41 (95% CI = 1.13-1.77) and 1.76 (95% CI = 1.38-2.27) for *Cryptosporidium* and *Giardia,* respectively. Diarrhoea was significantly associated with cryptosporidiosis (RR = 1.23, 95% CI = 1.03-1.47; P = 0.029) but not with giardiasis (RR = 1.12, 95% CI = 0.91-1.53; P = 0.26).

The study concluded that gastro-intestinal protozoa infections are highly prevalent among children attending pre-school in peri-urban Zambia highlighting the need for further studies of risk factors. Single stool samples were collected from 403 children from 10 pre-schools and were subjected to duplicate Kato–Katz thick smears to identify and quantify helminths. A commercial immune-fluorescence kit was used to identify *Cryptosporidium*- and *Giardia*-positive samples. The overall prevalence of helminth infection was 17.9%. *Ascaris species* was found in 12.0%, hookworm in 8.3%, *Taenia* species. in 0.9%, *Hymenolepis species* in 0.6% and *Schistosoma mansoni* in 0.3%.

The overall prevalence of *Cryptosporidium* and *Giardia* was 28.0 and 29.0%, respectively, with more girls infected with *Giardia* (33.8%) than boys (22.7%) (*P* = 0.02). Significant differences in infections with *A. species* and *Cryptosporidium* were observed between the various pre-schools (*P* < 0.001). These findings indicated that intestinal parasites were prevalent in children enrolled in pre-schools in Zambia.

It was recommended that future studies should explore local factors associated with transmission of these infections, and consequently provide the necessary health education to parents and teachers.

Thys et al (2015) carried out a research on why latrines are not used: Communities' perceptions and practices regarding latrines in a *Taenia solium* Endemic rural area in Eastern Zambia. The study objective was to assess the communities' perceptions, practices and knowledge regarding latrines in a *T. solium* endemic rural area in Eastern Zambia inhabited by the Nsenga ethno-linguistic group, and to identify possible barriers to their construction and use.

It was noted during the study that *Taenia solium* cysticercosis is a neglected parasitic zoonosis occurring in many developing countries. Socio-cultural determinants related to its control remain unclear. Studies in Africa have shown that the underuse of sanitary facilities and the widespread occurrence of free-roaming pigs are the major risk factors for porcine cysticercosis. A total of 21 focus group discussions on latrine use were organized separately with men, women and children, in seven villages of the Petauke District.

The themes covered were related to perceived latrine availability (absence-presence, building obstacles) and perceived latrine use (defecation practices, latrine management, socio-cultural constraints). The findings revealed that latrines were not constructed in every household because of the convenient use of existing latrines in the neighborhood. Latrines were perceived to contribute to good hygiene mainly because they prevent pigs from eating human feaces. Men expressed reluctance to abandon the open-air defecation practice mainly because of toilet-associated taboos with in-laws and grown-up children of the opposite gender.

When reviewing conceptual frameworks of people's approach to sanitation, it was found that seeking privacy and taboos hindering latrine use and construction were mainly explained in our study area by the fact that the Nsenga observe a traditionally

matrilineal descent. These findings indicate that in this local context latrine promotion messages should not only focus on health benefits in general. Since only men were responsible for building latrines and mostly men preferred open defecation, sanitation programs should also be directed to men and address related sanitary taboos in order to be effective. It was further concluded that in order to contribute to breaking the vicious cycle between poverty and poor health among livestock owners in developing countries, disease control strategies should always consider the socio-cultural context.

Nkhuwa (2001) undertook a study on relevance of groundwater quality assessment in the Lusaka aquifer. It was observed that Lusaka's geology comprised mainly of karstified marbles interbedded with thin sub-horizones of schists and quartzites. It was noted that differential dissolution of the marbles, had developed a system of conduits and solution channels. With such a formation, pollutants and pathogens could easily access groundwater. Nkhuwa (2001), was concerned about the industrial effluents, domestic and industrial wastes, and sewage together with the use of pit latrines and septic tanks in relation to groundwater quality.

This confirmed a statement in a report from Lusaka City Council (1997), which stated that "the common excremental sanitary disposal systems in peri-urban areas, were on-site traditional pit-latrines, which were characterised among other things by short life span, no standard design, permeability, breeding ground for vermin and pathogenic (bacteria and parasites) making a pit-latrine a source of pollution (air and groundwater), pathogens as well as source of infectious diseases and environmental hazard."

Combining this with population statistics for Lusaka City (CSO, 2011) literature shows that 70% of the more than 400,000 households in Lusaka are in peri-urban areas and use pit latrines for excreta disposal making faecal matter to be the highest type and quantity of pollutant for Lusaka groundwater.

2.2 The Helminthes and Protozoa

2.2.1 (a) *Ascaris species*

These parasites are large. Populations of both species of *Ascaris* can co-exist without any gene flow between them. Males are 31cm long and 4mm wide whereas females are 49cm long and 6mm wide. The females lay upto 200,000 ova a day. (Roberts and Janovy, 2005). The ova are oval to round measuring 75 micrometers long and 45 micrometers wide with a thick lumpy outer shell. When passed through faeces the mammiliated layer is bile stained golden brown (Nnaemeka et al, 2010). Infections occur when unhatched juveniles are swallowed with contaminated food or water (Brusca and Brusca, 2003). They hatch in the duodenum where they penetrate the submucosa and into the blood stream (Figure 2.1), to the lungs though the heart (Nnaemeka et al, 2010). In the lungs they get into the airspaces. From there they are swallowed back into the intestines where they begin to produces ova after undergoing stages of molting (Nnaemeka et al, 2010).

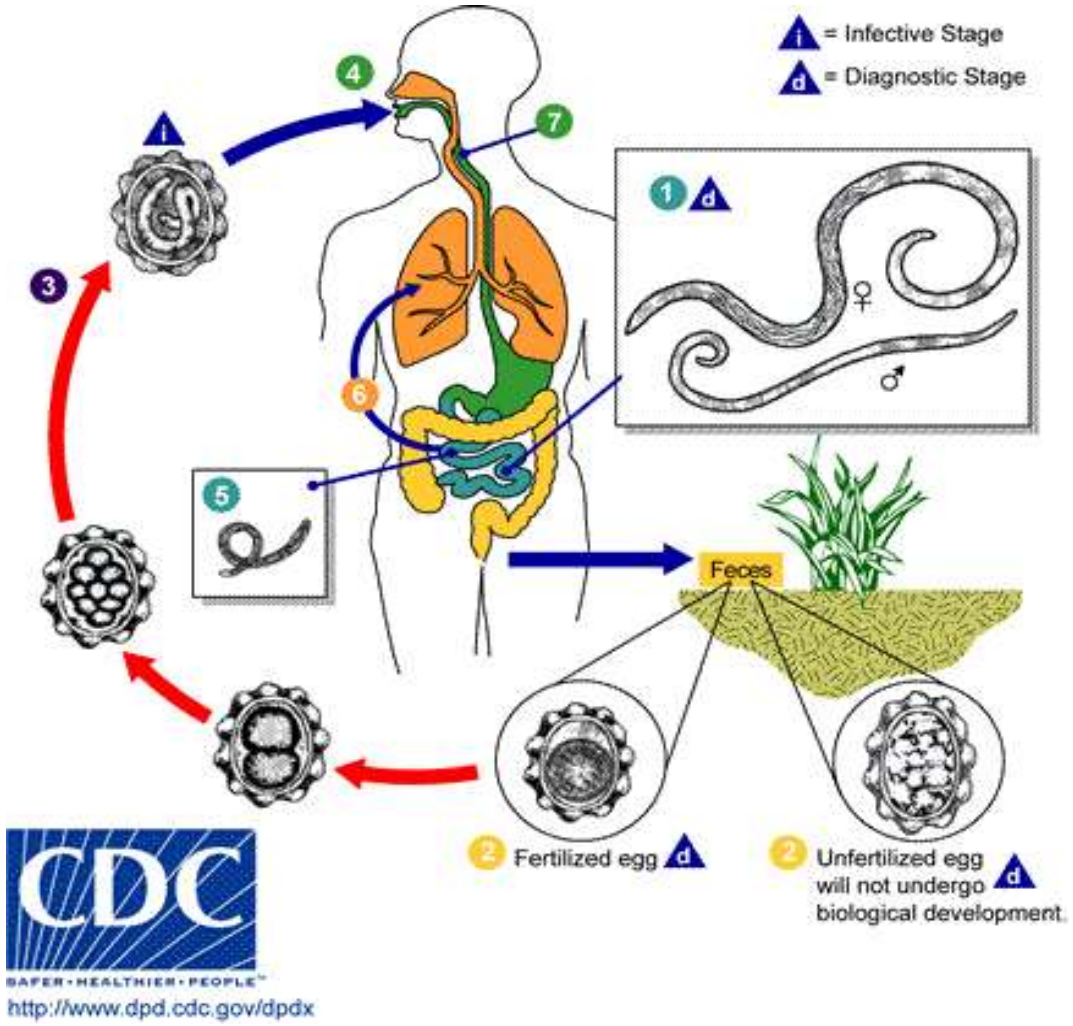

Figure 2.1: Life Cycle of *Ascaris Species*

(b) Pathogenesis

Pit latrines are known to be a source of transfer for soil transmitted helminthes such as *Ascaris species, Trichuris trichiura* and hookworm (Baker, 2010).When juveniles break out of capillaries into respiratory system, they cause hemorrhage and heavy infections can cause pools of blood to accumulate leading to edema and clogging of air space (Robert and Janovy, 2005). When bacterial infections become superimposed, death can result (Nnaemeka et al, 2010). The main food of *Ascaris species* is the liquid contents of the host's intestinal lumen and the theft of nutrients causes malnutrition, under development of young children, abdominal pains, asthma, insomnia often result as

allergic responses to metabolites released by the worm (Brusca and Brusca, 2003). Massive infection can cause fatal intestinal blockage and multiple liver abscesses have resulted from such invasion (Roberts and Janovy, 2005). Worms move about and upon reaching the stomach the acidity causes them to writhe, leading to a nausea feeling and the psychological trauma induced in one who vomits a 45cm ascarid is difficult to quantify and worse still aspiration of vomited worm can cause death (Barnes, 1987).

2.2.2 (a) *Balantidia Species*

This is the largest protozoan parasite of humans although it is primarily a parasite of pigs (Roberts and Janovy, 2005). Its trophozoites are oblong, spheroid or more slender measuring upto 150 micrometers long and 120 micrometers wide and encysted stages found in stools are spheroid or ovoid measuring upto 60 micrometers in diameter (Hulinskaca, 1968). The worm lives in the colon of humans, pigs, rats and many other mammals and luckily, it is not easily transmitted from one host to another since it requires a period of time to adjust to the symbiotic flora of a new host (Kucik et al., 2004). However when successful, the protozoan flourishes and can become a serious pathogen, particularly in humans (Gutiérrez, 2000).

(b) Pathogenesis

Under ordinary conditions, trophozoites feed by ingesting particles through a vestibulum and cytostome (Hulinskaca, 1968). However, the organism can produce enzymes that eat away the host's epithelial wall of the intestines causing ulcers (Kucik et al., 2004). The ciliate's capacity to encyst after being passed through stool increases the number of potential infection from a single reservour host and can remain alive in moist pig faeces for weeks (Roberts and Janovy, 2005).

2.2.3 (a) *Cryptosporidia species*

Human cases of *Cryptosporidium* species have been reported in various parts of the world and the prevalence appears to be highest in the tropics with the majority of the infection in man is by *Cryptosporidium species* (Getachew, 2014).*Cryptosporidium*

species is a widespread pathogen of humans (Paziewska, 2007) These coccidians are very small about 6 micrometers living in the brush border or just under the free-surface membrane of hosts gastrointestinal or respiratory epithelial cells (Getachew, 2014). *Cryptosporidium species*, like other coccidian, sequesters itself inside the host cell during development (Griffiths, 1998). It is protected from the host immune response and the hostile environment of the gut, while accessing the nutritional and energy reservoirs of the host cell (Griffiths, 1998).

Again, like other coccidia, it lies within a parasitophorous vacuole bounded by a parasite phorous vacuolar membrane, which, in other coccidians, is the portal through which nutrients from the host cytoplasm enter the parasite (Griffiths, 1998). Protozoan oocysts are seen only in faeces of the host (Roberts and Janovy, 2005).

(b) Pathogenesis

Exposure to *Cryptosporidia species* protozoan oocysts, either directly through contact with infected humans or animals, or indirectly by drinking or eating food washed with contaminated water, may lead to acute diarrhea (Getachew, 2014). Sanitation is the primary barrier for preventing faecal-oral disease transmission. If excreta disposal is ineffective or non-existent (or other animals serve as sources of excreted pathogens) other measures must be taken to avoid disease transmission (WHO 2006). Removing or destroying infectious agents by disinfecting drinking water prior to consumption or preparation of food; cleaning hands, utensils, and surfaces before food preparation and consumption; and cooking food thoroughly are interventions that will reduce disease transmission (WHO 2006).

Cryptosporidia species causes an acute, self-limiting infection and diarrheal disease (Griffiths, 1998). *Cryptosporidium species* is a common cause of gastroenteritis called cryptosporidiosis which manifests as a watery diarrhea in humans (Griffiths, 1998). The diarrhea may become profuse and prolonged, and in consequence life-threatening,

particularly in immune-compromised (i.e. HIV positive) or immune suppressed persons (Current and Blagburn, 1991).

Infection presumably begins in the small intestine, where the emerging sporozoites infect enterocytes, and after amplification, endogenous forms spread throughout the epithelial surfaces of both villi and crypts and the infection may spread throughout the gut, which includes the gastric mucosa and the small and the large intestines (Griffiths, 1998). The parasite displace the microvillus border and eventually lead to the loss of the mature surface epithelium and the rapid loss of surface epithelium causes marked shortening and fusion of the villi and lengthening of the crypts due to acceleration of cell division to compensate for the loss of cells (Current and Blagburn, 1991).

The combined loss of microvillus border and villus height diminishes the absorptive intestinal surface and reduces uptake of fluids, electrolytes and nutrients from the gut lumen. The loss of the microvillus border in the proximal small intestine leads, in addition, to loss of membrane-bound digestive enzymes, whose role in children, in particular, is crucial, and contributes to marked mal-digestion in addition to the mal-absorption. Diarrhea lasting 7–10 days resulting in serious dehydration and loss of body weight (Griffiths, 1998). In patients with AIDS, the parasites cause profuse, watery diarrhea lasting for several months (Roberts and Janovy, 2005). Bowel-movement frequency can get upto 25 times a day and stool volume can reach 17 liters per day (Current and Blagburn, 1991).

2.2.4 (a) *Enterobius species*

These are pinworms. Females lay upto 16000 ova with partially developed juveniles that develop to infectivity within 6 hours at body temperature (Roberts and Janovy, 2005). The pinworm appears as a white, small and delicate nematode (i.e., roundworm) *Enterobius species* has a cylindrical body (Figure 2.2), and a cuticle with three main outer layers made of collagen and other compounds, secreted by the epidermis

(Gutiérrez, 2000). The cuticle layer protects the nematode so it can invade digestive tracts of animals (Barnes, 1987).

The worms molt four times, the first two before hatching, and then before their adult stage (Bogitsh and Cheng, 1998). Female *E. species*, measuring 8 to 13 mm long by 0.4 mm wide, are characterized by the presence of wing like expansion (alae) of the body wall at the anterior end, distension of the body due to the large number of ova in the uteri, and a pointed tail. Males, smaller in size, are 2 to 5 mm long and possess a curved tail (Bogitsh et al., 2012).The egg measures 50 to 54 micrometer in length by 20 to 27 micrometer in width and has a characteristic shape, flattened on one side. It is almost colourless, with a bean-shaped double contour shell a fully formed embryo (Cook and Zumla, 2009).

The ova have five membranes: one inner, lipoidal layer, three middle layers known as membranalucida, and one outer, albuminous membrane which coats the egg. This membrane makes the ova sticky and therefore itchy to the host, which is important in the life cycle (Garcia and Bruckner, 1997; Brusca and Brusca, 2003).The larvae may be visible inside the egg due to the colorless shell of the embryonated ova (Gutiérrez, 2000; Ridley, 2012).The larvae grow to 140–150 micrometers in length (Cook, 1994).

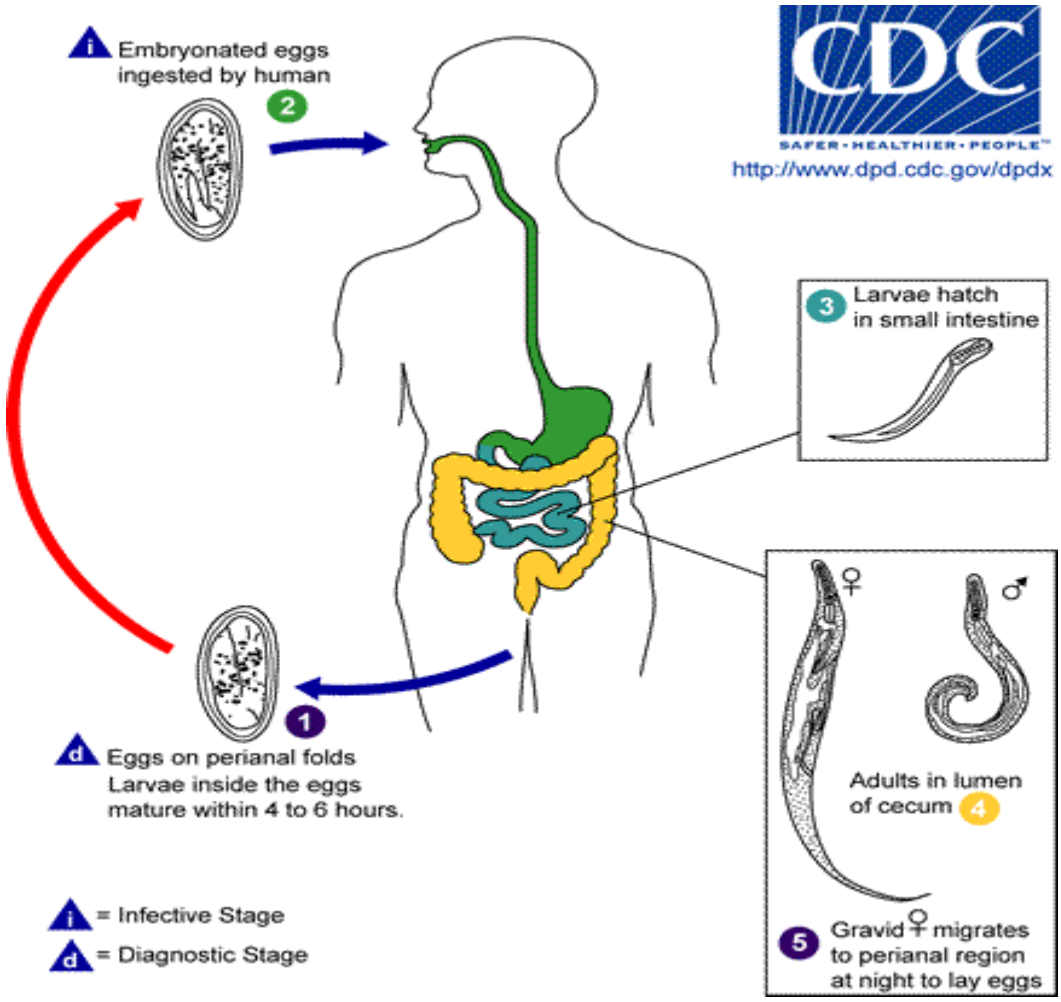

Figure 2.2: Life Cycle of *Enterobius Species*

(b) Pathogenesis

Enterobius species is one of the most common intestinal parasites in human and is the most successful intestinal nematode to thrive among human populations (Kucik et al., 2004).The adult worms inhabit the lumen of the intestine in the region of the ileum, cecum (Hamdona, 2013). *Enterobius species* occur more frequently inflamed appendices than in normal (Hamdona, 2013).In rare instances, pinworms can cause serious gastrointestinal problems and ectopic infections (Petro et al., 2005).

Enterobius species can infect the endometrial cavity and the postulated mechanism involves the gravid female worms migrating from the anus over the perineum and into the vagina and genital tract (Petro et al., 2005).Adult worms congregate mainly in the ileocecal region of the intestine but wander about through the gastrointestinal tract from stomach to anus (Gutiérrez, 2000).They feed on epithelial cells and bacteria however movement of the females around the anus causes the itching and scratching of the perianus, a nightmare of discomfort (Roberts and Janovy, 2005).Clothing and beddings rapidly become seeded with ova when infection occurs including curtains, walls and carpets worse still the pin worm ova can get airborne and infect people when inhaled (Hulinskaca, 1968).

2.2.5 (a) *Giardia species*

This is the most common flagellate of the human digestive tract (Roberts and Janovy, 2005).Intestinal parasite incidence were reported by many parts of the World even the studies in Gaza strip among which were *Giardia species*, *Entamoeba histolytica*, *Ascaris species*, *Trichuris species*, *Enterobius species* and *Strongyloides species* (Shubair et al., 2000; Al-Hindi, 2002).

Giardia species has two major stages in the life cycle; the cyst and vegetative tropozoite (Getachew, 2014). *Giardia* exhibits a simple and direct life cycle meaning that no intermediate host is required in the life cycle (Svärd*et al*., 2003). The trophozoite, during its vegetative (actively feeding and metabolizing) stage, is pear shaped, measuring from 7 to 10μm at its widest part, and 10-15μm long (Barwick*et al*., 2000).

Trophozoites are long rounded at their anterior ends and pointed at the posterior and dorsoventrally flattened and convex on the dorsal surface (Roberts and Janovy, 2005). The flattened ventral surface bears a concave, bilobed adhesive disc (Figure 2.3), which actually is a rigid structure, enforced by microtubules and fibrous ribbons, surrounded by a flexible, apparently contractile, striated rim of cytoplasm (Roberts and Janovy, 2005).The cyst, trophozoites encased in a multilayered cyst wall is oval, measuring from 10

to 15µm in length (Barwick*et al*., 2000). Cysts are excreted in the host's faeces and are transmitted to the next host when cysts contaminate food and water (Barwick *et al*., 2000). Ingested, viable cysts excyst after passage through the stomach and exposure to an acid environment of stomach (Barwick*et al*., 2000). After excystation, trophozoites colonize and reproduce by binary fission in the host's small intestine where, in the presence of bile, they form cysts (Svärd*et al*., 2003).

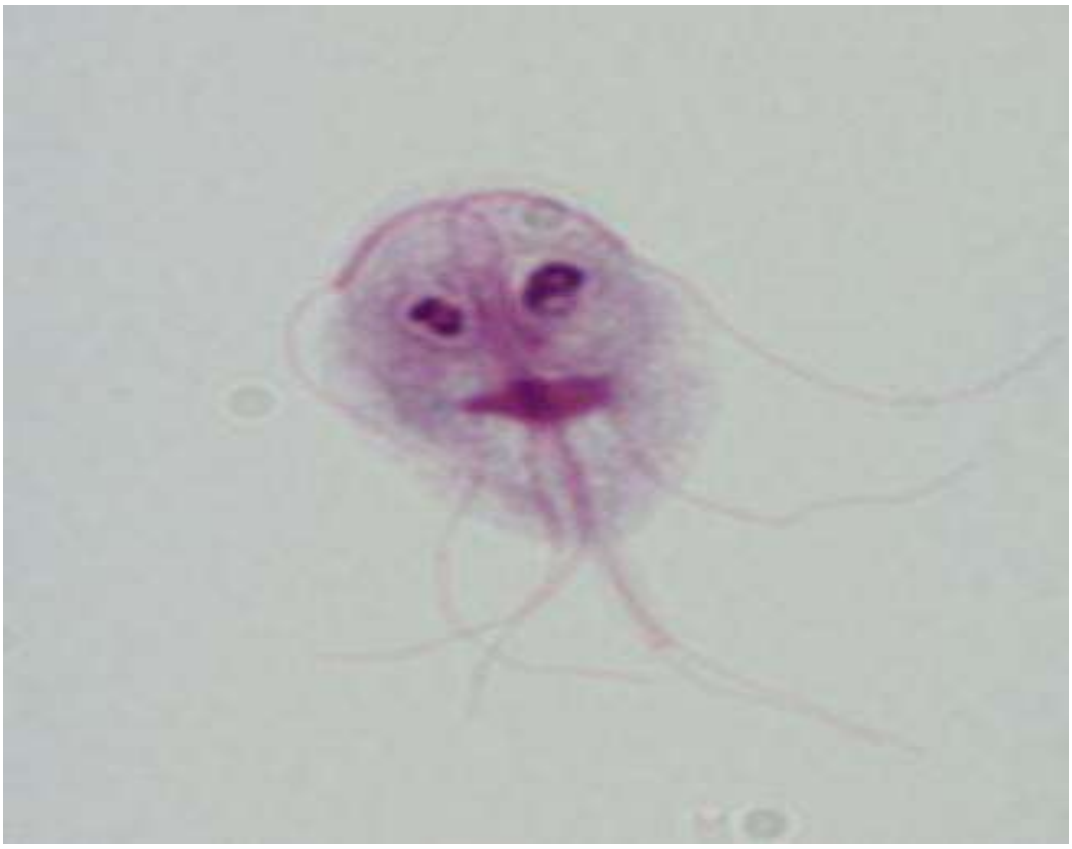

Figure 2.3:Micrograph of *Giardia* showing a cyst stained with Giemsa: Lebbad (2010)

(b) Pathogenesis

Giardia exhibits a typical feacal-oral transmission cycle and infection is acquired by ingesting cysts and factors leading to contamination of food or water with feacal material are usually associated with transmission (Barwick *et al*., 2000). In developing countries, poor sanitation may contribute to the higher levels of giardiasis and water-borne outbreaks

due to inadequate water treatment have been documented (Svärd*et al*., 2003). Giardia species lives in the duodenum, jejunum, and upper ileum of humans with the adhesive disc fitting over the surface of an epithelial cell. The cyst measures about 12 micrometer by 10 micrometers in size (Barwick *et al*., 2000).

Once ingested, the cysts pass safely through the stomach and encyst in the duodenum. Flagella grow out and the parasites are once again at home (Roberts and Janovy, 2005). Giardiasis is highly contagious and in severe infections, the surface of nearly every cell is covered by a parasite leading to increase in mucus production, dehydration, intestinal pain, flatulence, and weight loss. They divide by binary fission, however as faeces enter the colon and begin to dehydrate the parasites begin to become encysted (Roberts and Janovy, 2005). Transmission depends on swallowing of mature cysts. Prevention therefore depends on high level of sanitation (Svärd*et al*., 2003).

2.2.6 (a) *Hymenolempis species*

Commonly called the dwarf tapeworm and also known as the *Vampirolepis species, Hymenolepis species* is one of the common cestodes of humans (Roberts and Janovy, 2005). An intermediate host is optional. It is 4cm long and less than a millimeter wide. The worm can still develop in beetles, fleas, dogs, cats, rats, mice besides humans (Gutiérrez, 2000).

(b) Pathogenesis

When ingested, ova hatch in the duodenum releasing oncospheres which penetrate the mucosa and come to lie in lymph nodes of the villi where they develop into cysticercoids that emerge into the lumen of the small intestines, where they attach and mature causing harm to the host (Gutiérrez, 2000).

2.2.7 (a) *Strongyloides species*

The tiny worms belong to the order Rhabditida, *Strongyloides species*, which is the causative agent of strongyloidiasis, is an opportunistic intestinal threadworm parasite

that infects man, cats, dogs, and can be passed from man to dog/cat or vice versa (Nnaemeka et al, 2010) Most species inhabit decaying organic matter and are common in soil, foul water, decaying fruit et cetera through which they often find their way into the bodies of larger animals whose digestive, reproductive, respiratory and excretory tracts are particularly susceptible (Barnes, 1987).The females can reach a length of about 2.5mm and they burrow their anterior ends into the submucosa of the small intestines. They produce several dozen thin shelled and already embryonated ova that measure upto 58 micrometer by 34 micrometer. The ova hatch as they pass through the lumen into juveniles that measure upto 380 micrometer long and are passed out with faeces (Figure 2.4).

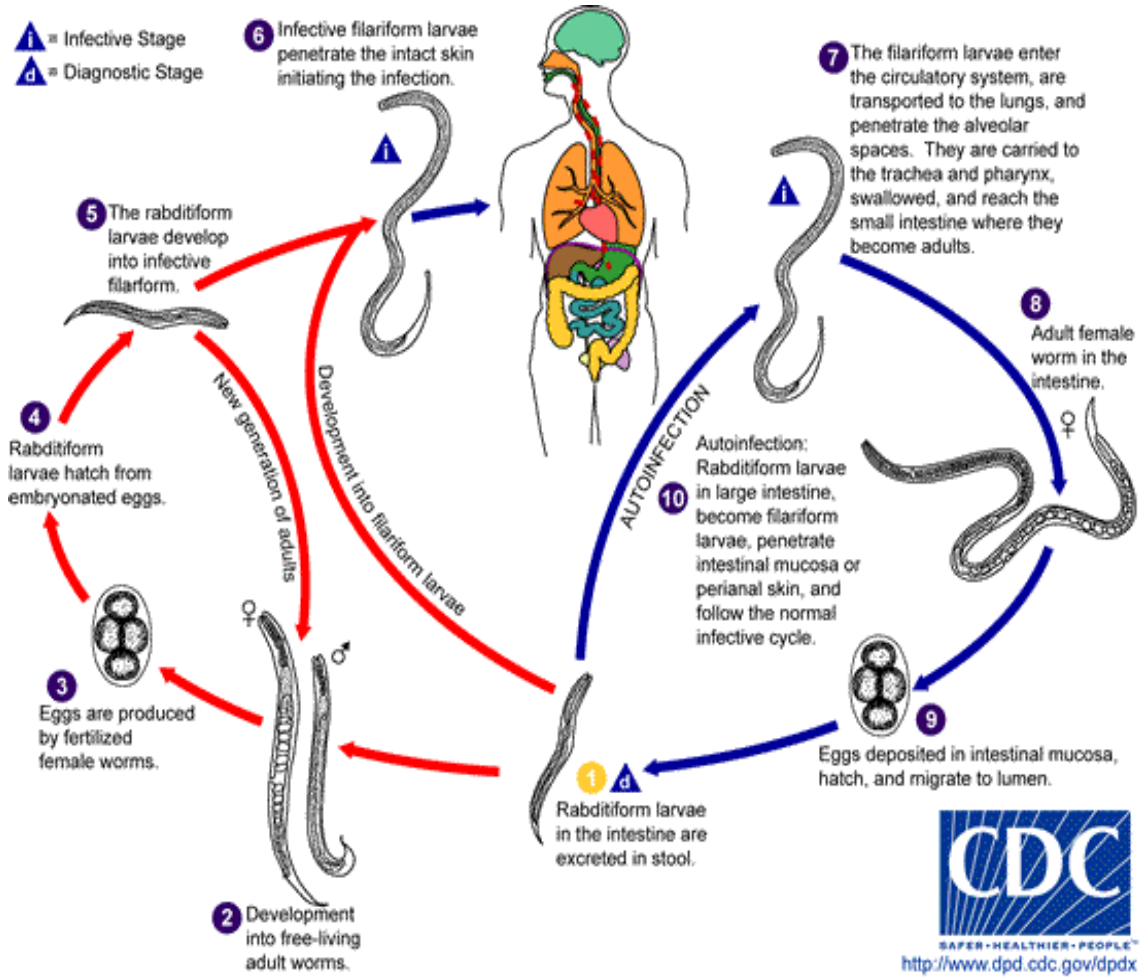

Figure 2.4: Life Cycle of *Strongyloides* Species

(b) Pathogenesis

Poor personal hygiene, insufficient supply of safe drinking water and contemptible sanitary measures has made the spread of infection imminent (Nnaemeka et al, 2010). People become infected by contaminated soil or water and damage to lung tissues result in wheezing during migration (Roberts and Janovy, 2005). Penetration of skin by juveniles results in hemorrhage and swelling with intense itching at site of entry and if pathogenic bacteria were introduced, inflammation results (Nnaemeka et al, 2010).

It is worth emphasizing that in warm moist climates in tropical and sub-tropical countries, where strongyloidiasis is endemic, the appropriate practical preventive measures remain stepping up of health education campaigns on the disease, proper sanitation, regular de-worming, behavioural change through proper disposal of faecal waste and the use of protective foot-wear (Nnaemeka et al, 2010). A burning sensation in the chest with non-productive cough that can be mistaken for asthma (Barnes, 1987).

2.2.8 (a)*Taenia species*

The most dangerous adult tapeworm of humans is the pork tapeworm. The spherical ova are characteristic of Taeniidae. A thin, hyaline, outer membrane is usually lost by the time the egg is voided with the feaces. The embryophore is very thick and riddled with numerous tiny pores, giving it a striated appearance in optical section Figure 2.5). A person can become infected through contamination of food or fingers with ova. It is possible to infect others in the same household by the same means and often with grave results. Human infection is highest in areas where beef is a major food and sanitation is deficient (Roberts and Janovy, 2005).

The scolex of an adult is non retractable taeniid rostellum armed with two circles of upto 32 hooks measuring upto 180 micrometres long. The scolex is 1mm wide with a strobilus that can measure upto 3 meters long. The mature segments are wide with upto 200 testes. Gravid segments are longer than wide and have the typical taeniid uterus, a medial stem with upto 13 lateral branches.

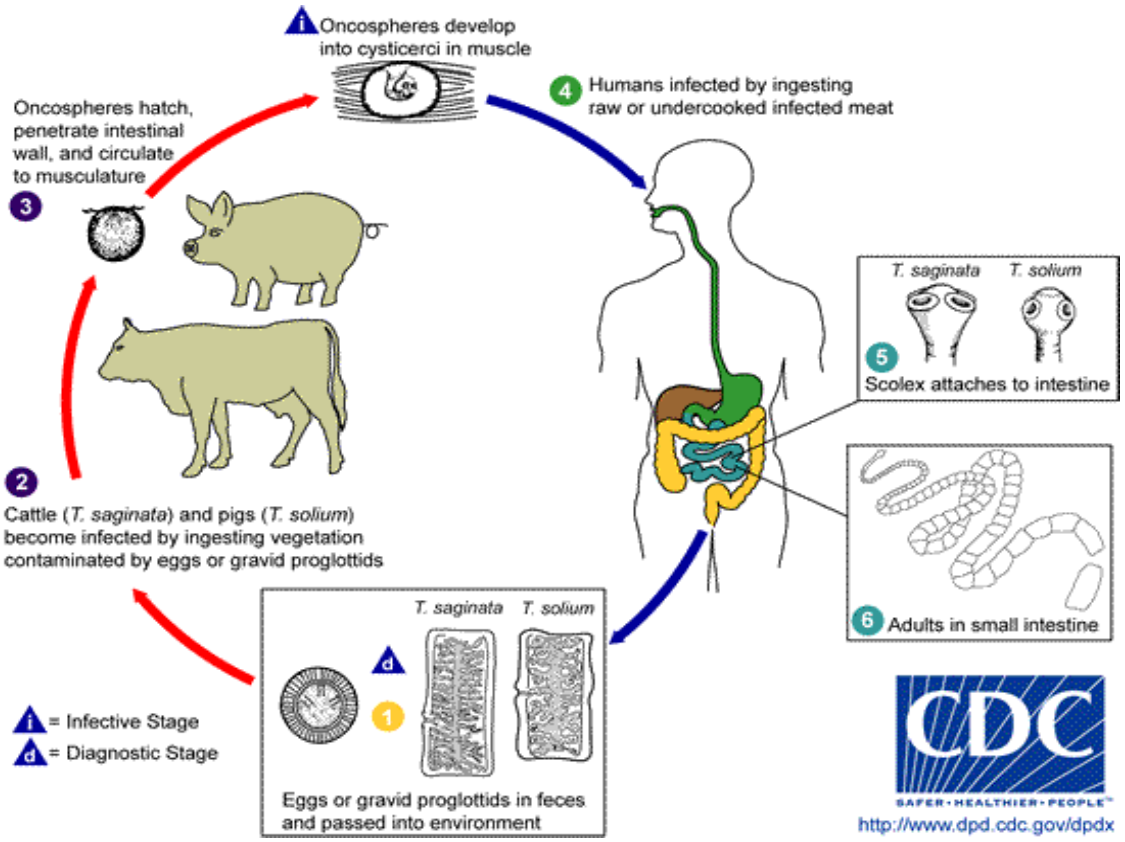

Figure 2.5: Life Cycle of ***Taenia Species***

(b) Pathogenesis

Taenia solium develops easily in humans (Robert and Janovy, 2005). Virtually every organ and tissue of the body may habour cysticeri. Most commonly they are found in the subcutaneous connective tissues. The second most common site is the eye, followed by the brain, muscles, heart, liver, lungs, and colon. The most common symptom is epilepsy of sudden onset. When this happens in an adult with no family history, cysticercosis should be suspected (Roberts and Janovy, 2005).

2.2.9 (a) *Trichuris species*

Trichuris trichuria measures upto 5cm long with the males being smaller than the females. The female worm produces upto 20, 000 ova per day. The ova have lemon

shape with prominent opercular plug at each end. Embryonation is completed 3 weeks in the soil which must be moist and shady because they do not survive direct sun's radiation (Nokes et al, 1992). When ingested the ova hatch in the lumen of the host and when maturing the enlarging posterior portion breaks out of the epithelial wall into the lumen in three months. The slender anterior end remains embedded in the gut mucosa (Figure 2.6). Infective ova are acquired from contaminated soil and a practice of eating the soil. Use of night soil as a fertiliser for vegetables can be an important source of infection. Houseflies, chickens and pigs serve as mechanical vectors (Olsen et al, 2001).

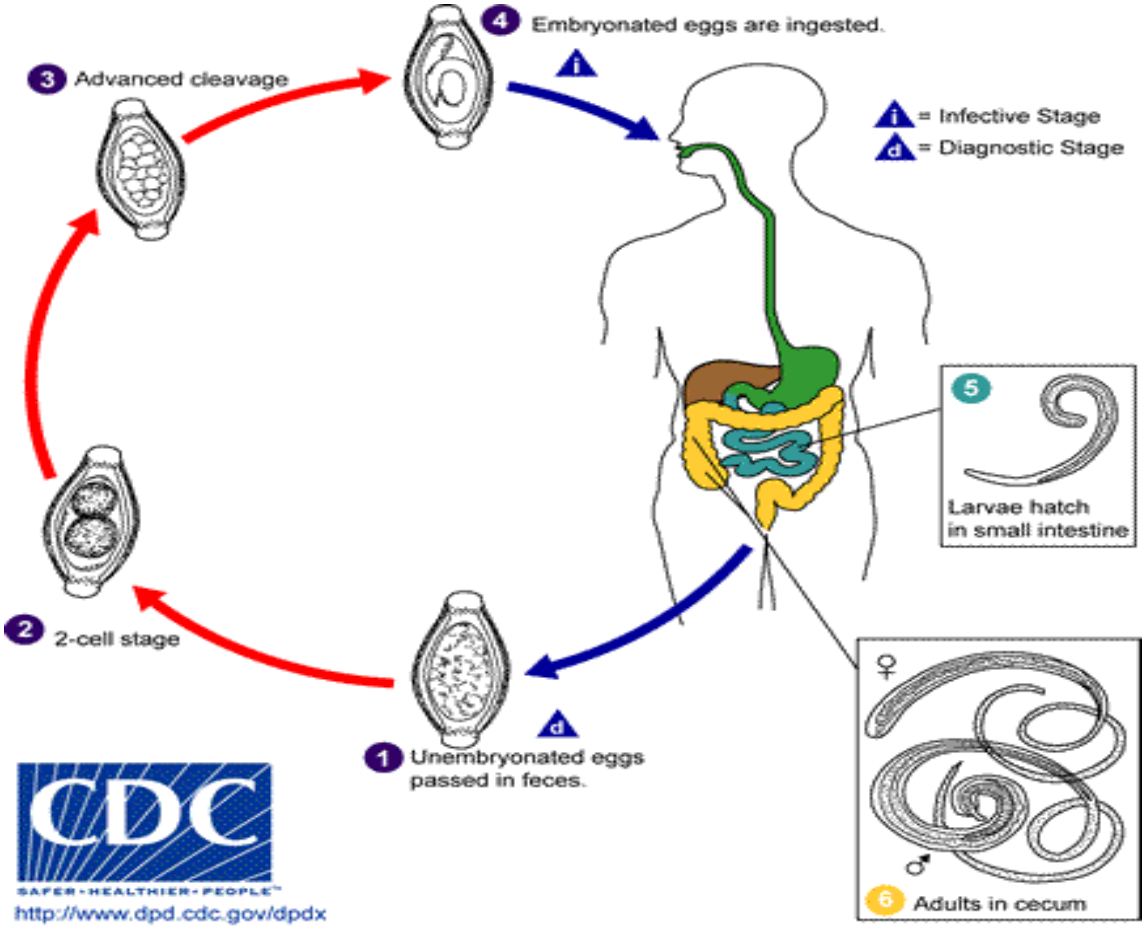

Figure 2.6: Life Cycle of *Trichicuris Species*

(b) Pathogenesis

In intense trichuriasis, dysentery, anemia and growth retardation are very common whereas finger clubbing and rectal prolapse are frequent (Nokes et al, 1992). Trauma to intestinal epithelium and underlying submucosa can cause chronic hemorrhage resulting in anemia (Robert and Janovy, 2005). In children, dysentery, anemia and growth retardation are common with frequent rectal prolapse (Nokes et al, 1992)

2.3 Gaps Identified in the Literature Reviewed

The literature reviewed focused on types and pathogenesis of parasites. Other studies were concerned with Lusaka's geology of karsified marbles that had developed a system of conduits and solution channels increasing the possibility of groundwater and environmental pollution by faecal matter from pit latrines as well as the danger of pit latrine faecal matter to public health (pathogenesis). Still some studies dwelt on the prevalence of the helminthes and protozoa among rural populations, school going children and peri-urban residents. In addition, some research also looked at possible approaches that could encourage demand response approach (DRA) in terms of workable sanitation provision for peri-urban areas. One study brought out issues concerning the reason why pit latrines were not being used in certain rural communities. Further, some study pointed out on how poor sanitation was jeopardizing peoples' lives, deteriorating the environment especially the eutrophication of surface water bodies and pollution of groundwater.

However, none of the studies looked at assessment of the presence, type and viability of helminthes ova and protozoan oocysts and/cysts in Lusaka peri-urban pit latrine faecal matter. And this study agrees with the observations and conclusions of (Phiri et al, 2014) done on LWSC faecal sludge as well as Lusaka ecosan sludge.

CHAPTER THREE: MATERIALS AND METHODS

The chapter outlines the methods and materials used in the data collection process during the course of the research. It focuses on the location of the study areas, data collection procedures, data analysis tools and the limitations of the study.

3.1 Location of the Study Areas

All the project sites are located in Lusaka the capital city of Zambia. Lusaka is situated on an elongated plateau that divides the sub-catchments of Kafue River in the west and Chongwe River in the east. Lusaka is located between longitudes 28^0.0' and 28^0.30' east and between latitudes 15^0.00' and 15^0.30' south of the Equator (Baumle et al, 2012). Figure 3.1 shows the relative locations of Kanyama, George, Madimba and Chaisa compounds of Lusaka where the study was undertaken.

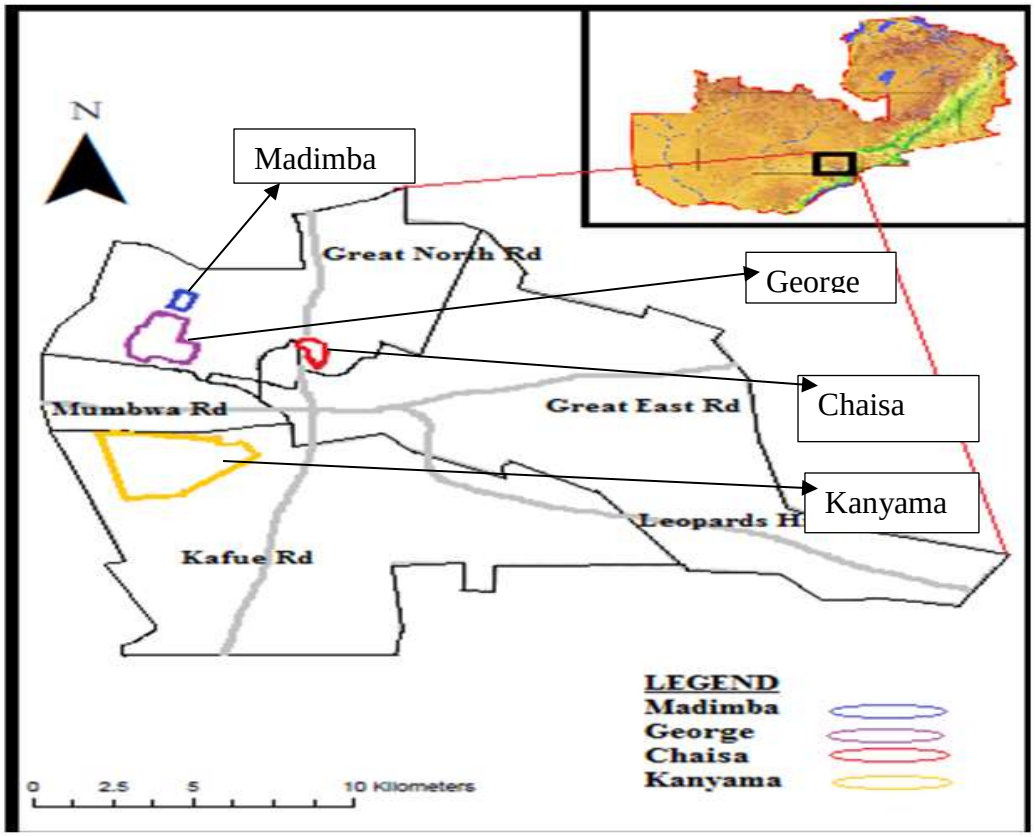

Figure 3.1: A map of Lusaka District showing the relative locations of Kanyama, George,

Madimba and Chaisa compounds, Lusaka, Zambia

Kanyama is located on the west of Lusaka. The township is very near to the Lusaka's Central Business District (CBD) with Mumbwa Road on the North, Light industrial area on the East and Los Angeles Road on the South, occupying an area of about 10 km^2. The water table in Kanyama Compound is normally high because the residential area sits on the highly productive marble aquifer. During the rainy season, flooding is the order of the day (Figure 3.2).

Figure 3.2: Kanyama Compound Floods, Lusaka, Zambia. Photo courtesy of Prof. Nyambe, 2012

Pit latrines are the mode of sanitation (World Bank, 2002). Chaisa is on schist formation and is located 7 km north of Lusaka very close to the Great North Road, which is on its western side. George Compound is on the south of Madimba Compound with similar geophysical characteristics as Madimba Compound. Madimba Compound is located to the northwestern side of the city center; about 10 km away towards

Barlastone Park in Lusaka west and pit latrine is the mode of sanitation in the area (World Bank, 2002).

3.2 Demography

The city has a population of 1.7 million people and about 33 peri-urban residential areas. The peri-urban areas constitute 70% of the city's population (CSO, 2010). Four compounds were purposively selected for this study and included Kanyama, George, Madimba and Chaisa compounds.

(i) Chaisa Compound

The population of Chaisa Compound was 66,205 having 33,294 female and 32,911 male with 11,034 households (CSO, 2010). The settlement is serviced with communal water taps by LWSC.

(ii) George Compound

The population of George Compound was at 62,592 having female 31,079 and 31,513 male with 10,432 households (CSO, 2010). The compound is partly serviced with water supply by LWSC and some households have water taps of their own. However, some sections are supplied by communal taps whereas others rely on shallow wells altogether.

(iii) Kanyama Compound

The population of Kanyama compound was at 364,655 having 182,913 female and 181,742 male with 60,776 households (CSO, 2010). A smaller population of Kanyama is serviced with water kiosks by Lusaka Water and Sewerage Company (LWSC), but the majority relies on shallow wells.

(iv) Madimba Compound

Madimba is one of the areas in Lusaka that started as unplanned settlement with an estimated population of over 3,000 with more than 500 housing units. Out of this population 1410 were male and 1590 female (NECOS, 2005). However, CSO (2010)

has no specific population data for Madimba. Madimba is a local name that means gardens.

The critical problem in the four settlements mentioned above, was that pit latrines, generally pit-latrines with underground substructure having a honey comb design, are often dug within several meters from the wells. During the rainy season, the excreta from the latrines overflow with rainwater and percolation of faecal coli forms into the shallow wells to contaminate groundwater sources occurs (NECOS, 2005). In these peri-urban areas it was noted that some households share the pit latrines hence the number of pit latrines was observed to be less than the number of houses.

3.3 Geology

Lusaka's geology (Figure 4) is mostly marble formation with some muscovite schist in the northern side of the city (Baumle et al, 2012). However, Chaisa Compound is entirely on Schist formation, whereas Madimba Compound to the far west of Chaisa Compound across the Great North Road is on marble just like Kanyama Compound. George Compound sits on marble in the south, but the central including the north east parts are on schist formation (Figure 3.3).

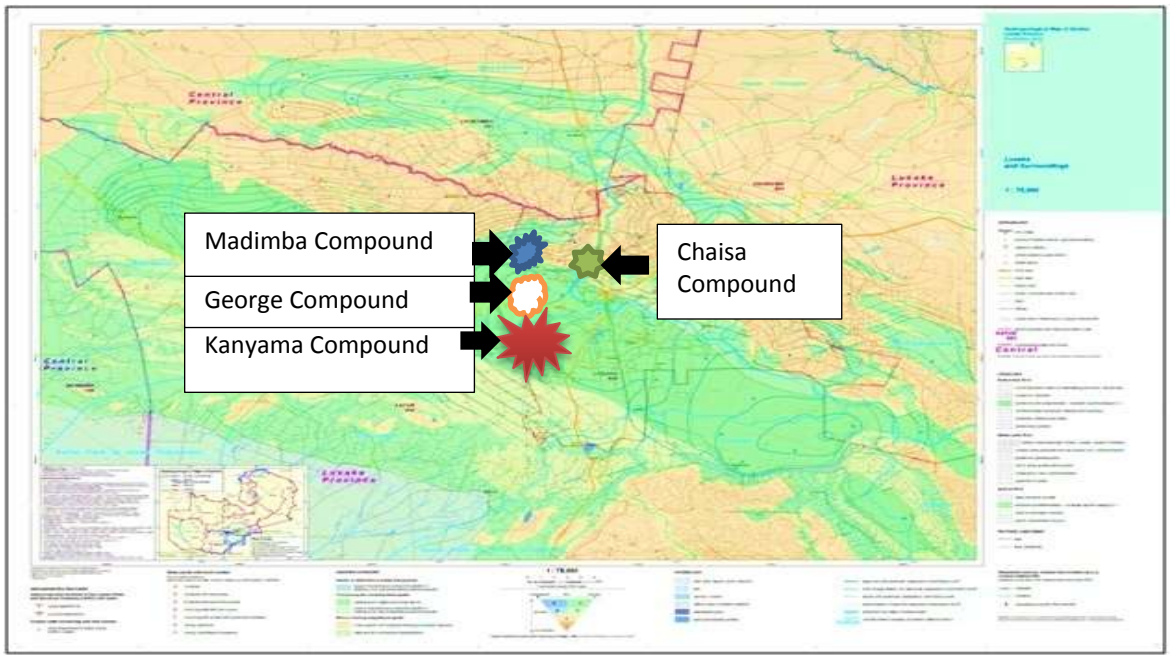

Figure 3.3: A hydro-geological map of Lusaka, Zambia indicating the relative locations of the study areas (Baumle et al, 2012)

3.4 Climate

The study areas being in Lusaka experience the weather patterns of Lusaka, which is sub-tropical, humid with dry winters and hot summers. Commonly, three seasons are distinguished:

(i) Rainy season: a warm wet season from November to April;

(ii) Cold season: a mild to cool, dry season from April to August; and

(iii) Hot season: a hot and dry season from September to November (Baumle et al, 2012).

Lusaka has a mean annual temperature of 20.7^{0}C. The coldest months are June and July with an average of around 16^{0}C. The highest temperatures occur in the month of October with a mean of 24^{0}C. The mean annual rainfall in Lusaka varies between 750mm and 800mm. The rainfall period is usually from the month of October to the month of May (Baumle et al, 2012).

3.5 Field Survey

The four peri-urban residential areas were identified purposively considering the appreciably sanitation activities taking place in the areas such as biogas generation using unstabilised faecal matter. A faecal matter sampler (Figure 3.4a and 3.4b), (Figure 3.5) and (Figure 3.6) having a sampling cup with a height of about 30cm was employed in the sampling of faecal matter both from top and bottom layers of faecal matter (Figure 3.4). Once fully submerged in the top layer of faecal matter (Figure 3.5), the lid was technically opened to allow the faecal matter to enter and fill the sampler whereas when collecting bottom faecal matter the sampler was pushed down until it reached the bottom of the faecal matter in the pit latrine (Figure 3.6) upon which the lid was opened to allow the bottom faecal matter to fill the sampler Figure 3.7).

Figure 3.4a: Faecal Matter Sampler Designed by the Researcher, 2015

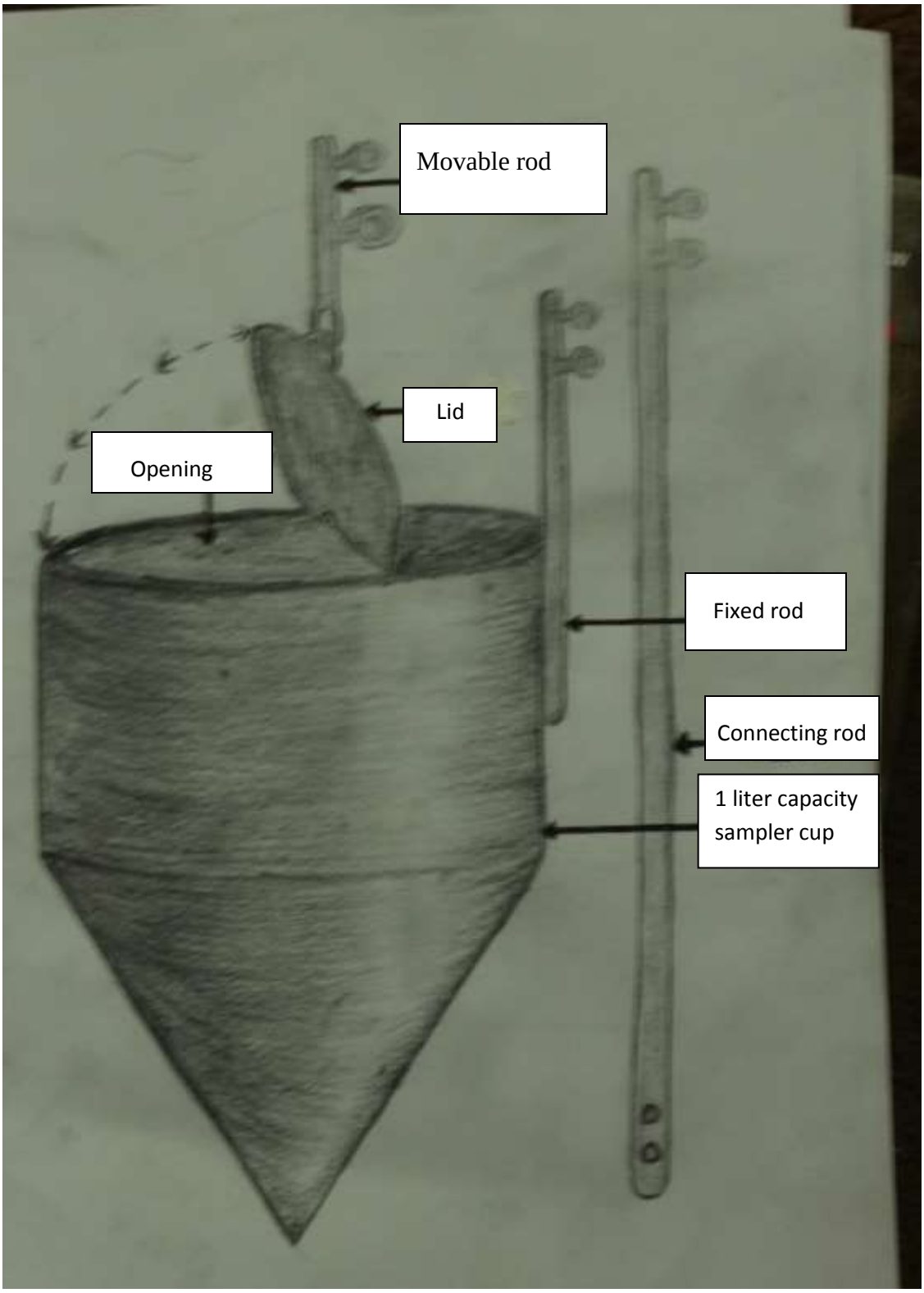

Figure 3.4b: A Sketch of a Faecal Matter Sampler Designed by the researcher, 2015

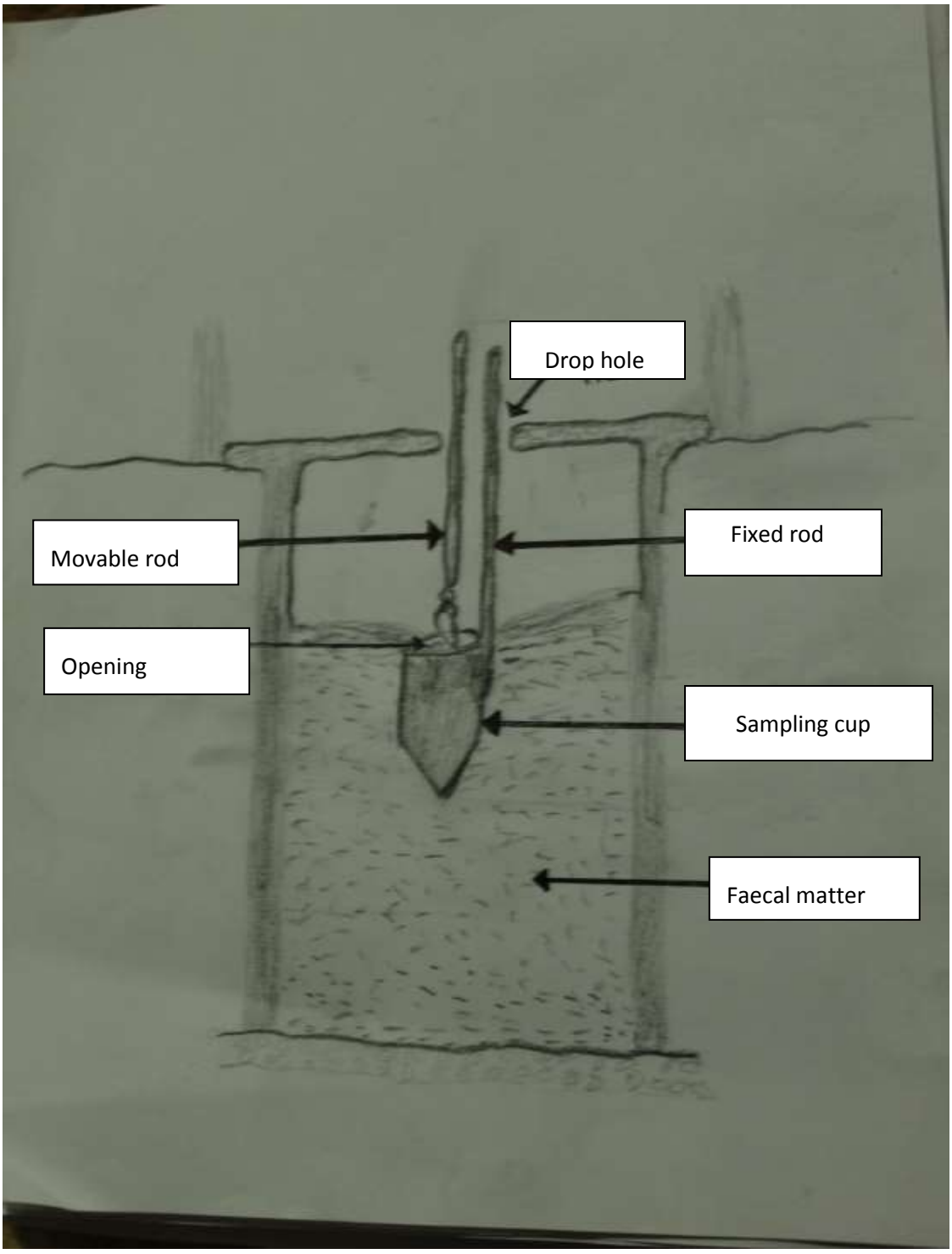

Figure 3.5: Underground Cross Section Sketch Showing the Sampling of Top layer of Faecal Matter.

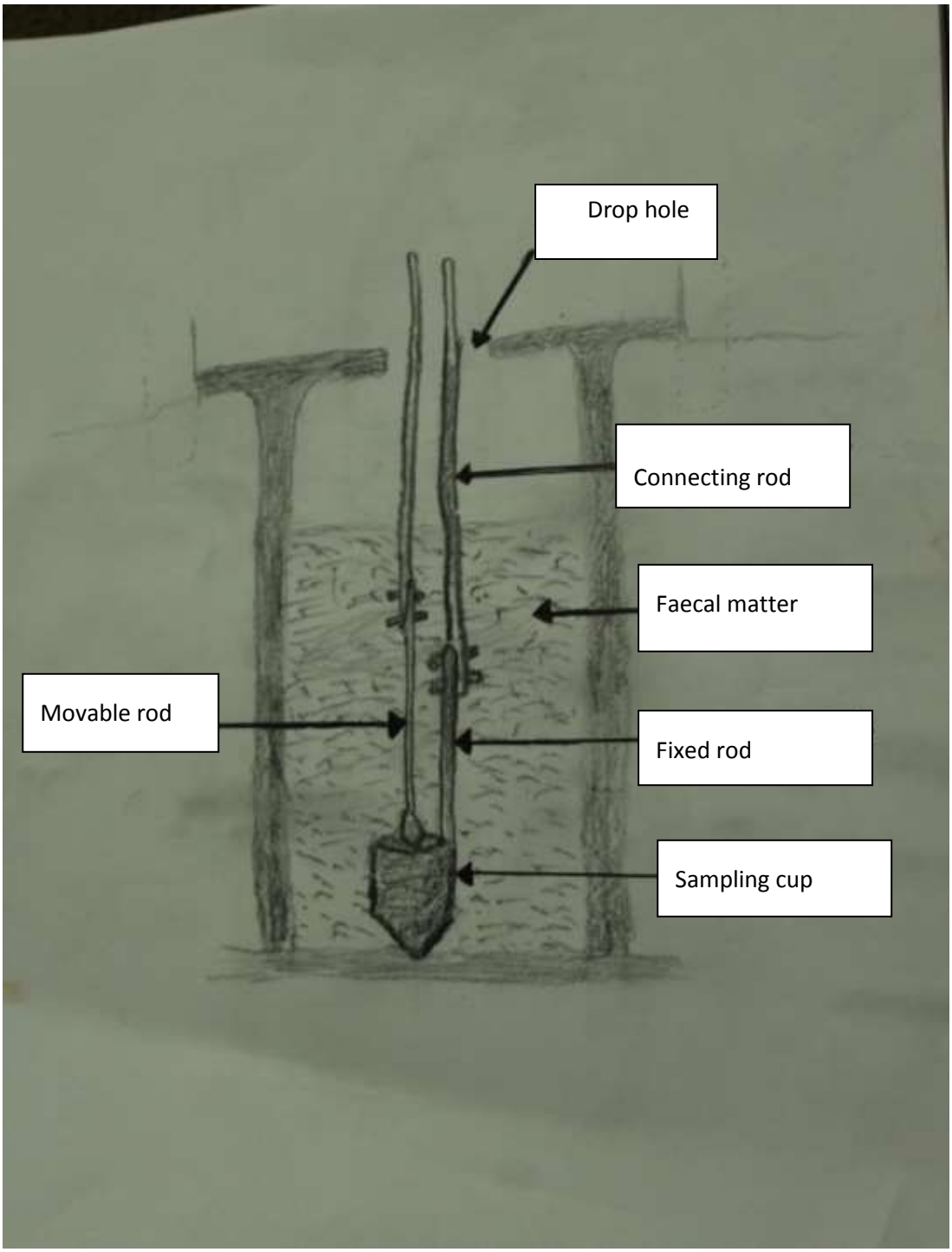

Figure 3.6: Underground Cross Section Sketch Showing the Sampling of Bottom Layer of Faecal Matter.

Figure 3.7: A Faecal Matter Sampler with Scooped Faecal Matter

A total of Twenty-five (25) pit latrines were sampled from in each of the four selected residential areas making a grand total of 100 pit latrines. About 1 litter of faecal matter

from the top layer was put in a 5 liter open container where it was well mixed before filling it into 3 bottles with capacity of 250ml. Another amount of the sample was collected from the faecal matter at the bottom of each pit latrine mixed well in a 5 liter open container before filling 3 sampling bottles with a capacity of 250ml (Figure 3.8) making a total of 6 samples per pit latrine giving a grand total of 600 samples as demanded by the sponsor of the research, the Water Research Commission.

Figure 3.8: Filling of Faecal Matter into 250ml Sampling Bottles

The sponsor demanded for reliable research results and it was required that three tests be done on each layer of faecal matter per pit latrine. About 5 pit latrines were sampled from during each trip and on average, a total of 20 sampling trips were made in the whole field survey. The sampled faecal matter in each sampling bottle was kept at a temperature below 4^0C in ice filled cooler boxes. The low temperature in the cooler box minimized significantly any change in the chemical, physical and biological properties

of the samples. Within a reasonable period of less than eight hours, the samples were collected, transported and subjected to scientific tests that gave results that helped to determine the presence of several types of ova, cysts and protozoan oocysts of helminthes.

3.6 Laboratory Analysis of Collected Faecal Matter Samples

The presence of helminth ova was detected through the use of Ammonium bicarbonate (AMBIC) floatation and sedimentation method, Water Research Commission (2014). The determination of viability of helminth ova by using Crystal violet stain was done.

3.6.1 Helminths Recovery Method

The AMBIC protocol consists of three steps namely:

(i) Sample preparation;

(ii) Mixing with Ammonium bicarbonate; and

(iii) Recovery with an adjusted Zinc sulphate floatation solution.

According to the method by the Water Research Commission (WRC 2014), South Africa, about 1 gram sample of faecal matter was placed into each 15ml test tube. The samples were mixed with a saturated (AMBIC) solution up to the 14ml mark on the tubes and shaken using a Vortex for 3 minutes. Lids were placed on the test tubes and left to stand for an hour (Figure 3.9).

Figure 3.9: Laboratory Preparation of Faecal Matter in Test Tubes

Thirty minutes into the hour, the tubes were again vortexed for 3 minutes and manually shaken for a further 2 minutes. After the hour elapsed, the conical test tubes were centrifuged at 940 Revolutions Per Minute (RPM) for 3 minutes and the supernatant decanted into other empty test tubes. Distilled water was added up to a 14ml mark, the contents mixed and vortexed for two minutes. The tubes were again centrifuged at 940 RPM for 3 minutes and the supernatant discarded. This constituted a wash up step to remove excess AMBIC solution.

Thereafter, Zinc sulphate with specific gravity adjusted to 1.4 was added to the pellet from the previous step up to 12ml mark (WRC, 2014). The mixture was vortexed for 2 minutes then centrifuged at 600 RPM for 3 minutes. The entire supernatant was divided equally among 4 clean conical test tubes. The pellet was retained for a further floatation trial. The 4 conical test tubes were topped up with distilled water and centrifuged for 3 minutes but this time at 1850 RPM. Thereafter, the supernatant was discarded and the

entire pellet was examined under a light microscope and the present ova counted. The retained pellet was subjected to another floatation using the same methodology outlined above and any ova that might have been retained were examined.

3.6.2 Recovery of *Cryptosporidia species* protozoan oocysts

According to this technique, about 1 gram sample of faecal matter was placed into each 15ml test tube. The samples were mixed with a saturated AMBIC solution up to the 14ml mark on the tubes and shaken using a vortex for 3 minutes. Lids were placed on the test tubes and left to stand for an hour. Thirty minutes into the hour, the tubes were again shaken using a vortex for 3 minutes and manually shaken for a further 2 minutes. After the hour elapsed, the conical test tubes were centrifuged at 940 RPM for 3 minutes and the supernatant discarded. Deionised water was added up to a 14ml mark, the contents mixed and shaken using a vortex for two minutes. The tubes were again centrifuged at 940 RPM for 3 minutes and the supernatant discarded. This constituted a wash step to remove excess (AMBIC) solution.

Thereafter, thin smears were mounted on glass slides using the concentrate. The smears were air dried and fixed with absolute Methanol for 3 minutes. The thin smears were then stained with unheated Carbol Fuchsin for 5-10 minutes. The slides were then rinsed with tap water. A solution of 3% Hydrochloric acid in 95% Ethanol was used to decolourise the smears until no more colours could flood from the smears. The decolourisor was rinsed off using tap water. The smears were counter stained for a minute with a 0.25% Methylene Blue. The stain was rinsed with tap water and allowed to dry in a draining rack. Once dry, the smear was examined for identification of protozoan oocysts under a light microscope using x40 and x100 objective lens with oil emulsion.

3.6.3 Method Used to Determine Viability of Ova

The working solutions made up in Ammonium oxalate solution consisting 4gram of Crystal violet (Hindiyeh, 1995**)**, 40ml of 95% alcohol, mixed with 160ml of 1% aqueous Ammonium oxalate (Lillie, 1977).

The *Ascaris* species ova were extracted from faecal matter and mixed with 7% Sodium hypochlorite for 30 minutes to remove the ova shell. The ova were washed at least five times with distilled water until neutral pH was achieved. About 2-3 drops of Crystal violet solution was mixed with the ova suspension. One drop of ova suspension was placed on a clean glass slide. After 10 minutes, a light microscope was used to distinguish between none viable *Ascaris species* ova that had accumulated the stain (blue) whereas viable ova excluded the dye and were colourless. The concentrate was then microscopically examined for the presence and viability of helminth ova.

3.7 Data Analysis

3.7.1 Microsoft Excel

Excel sheets were used to produce an actual presentation and interpretation of the findings for each pit latrine by way of histograms. Data were presented in the form of numbers, percentages, photographs, tables and figures. The stipulated methods were employed on the basis of availability of analytical equipment and the available apparatus (Appendix I).

3.7.2 The Seven Step Paired Sample T-Test

The data collected were analysed and interpreted by comparing the means of two populations using paired sample t-test. In this research, the top layer of faecal matter constituted one population whereas the bottom layer of the faecal matter constituted the second population. Therefore each pit latrine had a pair of samples. The mean for the three top layer samples and the mean for the three bottom layer samples were employed in the paired sample t-test statistical analysis.

Step 1: The null hypothesis stated that there was no difference in the mean concentrations of helminthes and protozoa between the top layer samples and bottom

layer samples; whereas the alternative hypothesis stated that there was a difference in the mean concentrations between top layer samples and bottom layer samples collected from all the four residential study areas;

Step 2: The test was performed at 5% significance level.

Thus $\alpha = 0.05$;

Step 3: The critical value $t_{0.05}$ was 1.711 and since there were 25 pairs in each residential area, the degree of freedom (d_f) was n - 1 = 24;

Step 4: The paired difference between the top layer mean and the bottom layer mean was calculated for each pit latrine samples;

Step 5: The value of the test statistic was calculated as:

$$t = d/(s_d/\sqrt{n})$$

where: **d** is the mean difference between paired samples;

S_d is the standard deviation whereas;

n is the number of paired sample means;

d_f is the degree of freedom (n-1);

t is the calculated t-test statistic; and

$t_{0.05}$ is the tabulated t value at 5% confidence level

$(\sum d^2)$ means square each d and sum them up

$(\sum d)^2$ is the squared sum of all the d

$d = \sum d/n$

$S_d = \sqrt{[n(\sum d^2) - (\sum d)^2]/n(n-1)}$

Substituting the values into $t = d/(s_d/\sqrt{n})$ gives the test statistic;

Step 6: All values of the test statistic that fell into the rejection region led to the rejection

of the null hypothesis and all values that did not fall into the rejection region

provided for the null hypothesis not to be rejected by the researcher; and

Step 7: The conclusion was made depending on whether the null (H_o) hypothesis was rejected or not and the statistic tabulated summary for each residential area was presented in Tables 2, 3, 4 and 5 in the next chapter.

CHAPTER FOUR: RESULTS

4.1 The Enumeration Presentation and Interpretation of the Results

4.1.1 Kanyama Compound:

A cumulative amount of two thousand, two hundred (2200) *Ascaris species* ova (Table 4.1, Figure 4.1 and Figure 4.2) were observed in a total of 150 top layer sample preparations and each sample preparation weighed about 1 gram on average. About one thousand nine hundred (1900) *Strongyloides species* larvae (Table 4.1 and Figure 4.3) were seen in the top layer samples.

Table 4.1: Total helminth ova, larvae and protozoan oocysts seen per 25 grams/smears of faecal matter from each of the residential areas

Residential Areas	Name of Parasite											
	Ascaris *Species* ova			*Strongyloides* *Species* larvae			*Cryptosporidia* *Species* protozoan oocysts			*Enterobius* *Species* ova		
	Top layer	Bottom layer	Total	Top layer	Bottom layer	Total	Top layer	Bottom Layer	Total	Top layer	Bottom layer	Total
Kanyama Compound	2200	2950	5150	1900	1400	3300	512	369	881	250	200	450
George Compound	7500	4550	12000	1000	200	1200	2333	1349	6382	50	1000	1050
Madimba Compound	4450	3400	7850	800	100	900	1291	726	2017	50	100	150
Chaisa Compound	6950	5500	12450	350	250	600	2170	875	3045	0	200	200

Source: Field data

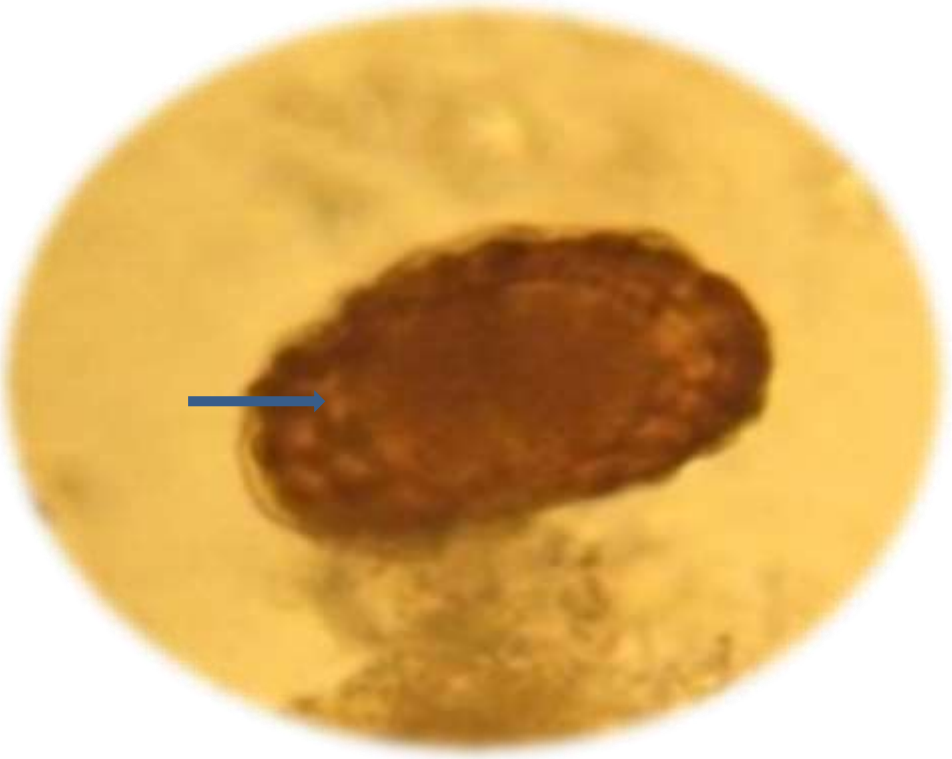

Figure 4.1: A microscopic picture of *Ascaris species* ovum with mammiliated layer at x40 Magnification

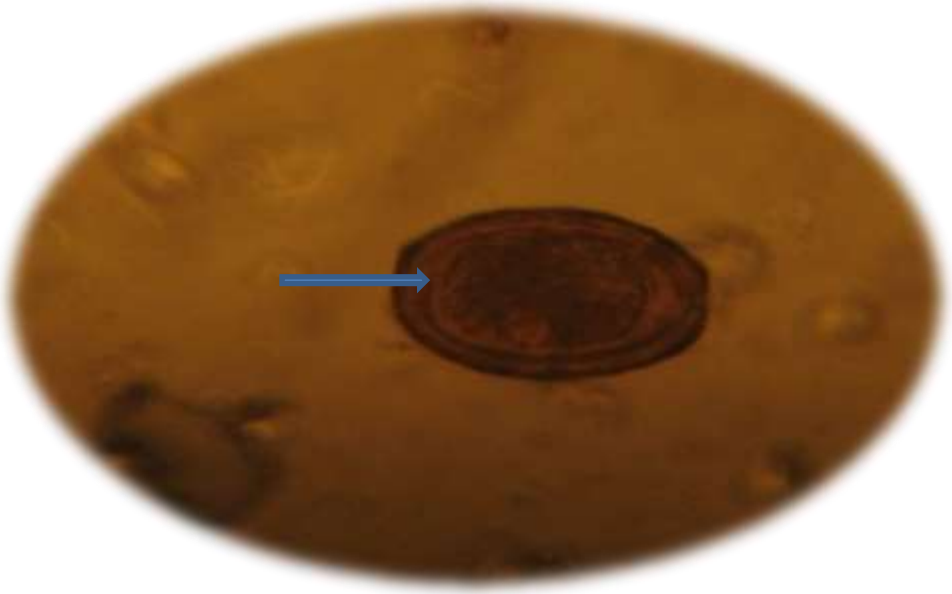

Figure 4.2: A microscopic picture of *Ascaris species* ovum without mammiliated layer at x40 Magnification

Figure 4.3: A microscopic picture of *Strongyloides species* larvae at x40 magnification

Moreover, the top layer samples indicated a presence of five hundred and twelve (512) *Cryptosporidia species* protozoan oocysts (Table 4.1 and Figure 4.4) in a total of the 150 sample preparation smears that were done on samples from Kanyama Compound as well as two hundred and fifty (250) *Enterobius species* ova (Table 4.1 and Figure 4.5) were observed and noted. The bottom layer samples from Kanyama Compound registered a total of two thousand nine hundred (2900) *Ascaris species* ova (Table 4.1). The bottom layer samples also indicated a presence of one thousand four hundred (1400) *Strongyloides species* larvae (Table 4.1) and three hundred and sixty-nine (360) *Cryptosporidia species* protozoan oocysts which were seen in the samples (Table 4.1) as well as two hundred (200) *Enterobius species* ova (Table 4.1).

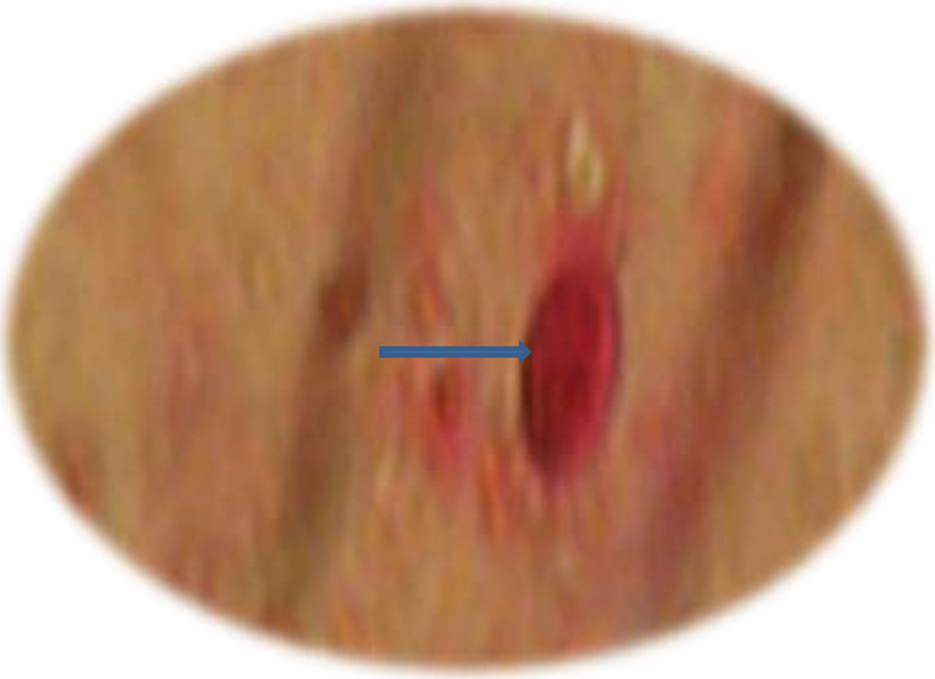

Figure 4.4: A microscopic picture of *Cryptosporidium species* protozoan oocyst, observed at x100 Magnification.

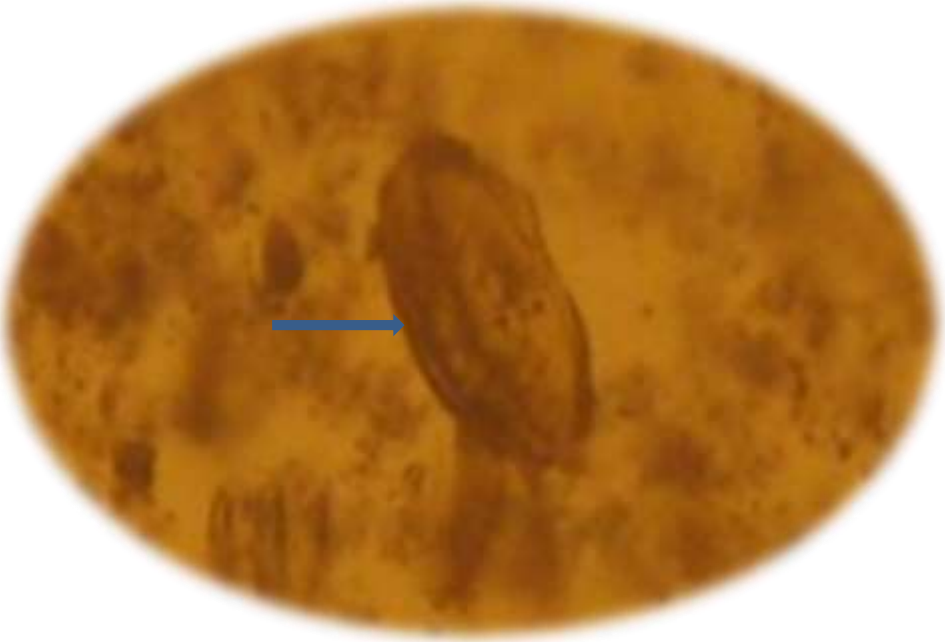

Figure 4.5 *Enterobius species* ovum at x40 microscopic magnification

4.1.2 Chaisa Compound:

A cumulative amount of six thousand, nine hundred and fifty (6950) *Ascaris species* ova were observed in a total of 75 sample preparations that weighed about 1 gram on average including nine hundred and fifty (950) *Strongyloides species* larvae were seen in the top layer samples(Table 4.1). Moreover, the top layer samples also indicated a presence of two thousand, one hundred and seventy (2170) *Cryptosporidia species* protozoan oocysts in a total of the 75 sample preparation smears that were done on samples from Chaisa Compound although *Enterobius species* ova were not observed in all the pit latrines (Table 4.1). Nevertheless, the bottom layer samples from Chaisa Compound registered a total of twelve thousand, four hundred (12,450) *Ascaris species* ova (Table 4.1). The bottom layer samples also indicated a presence of two hundred and fifty (250) *Strongyloides species* larvae and eight hundred and seventy-five (875) *Cryptosporidia species* protozoan oocysts, which were seen in the samples with two hundred (200) *Enterobius species* ova in the bottom layer samples (Table 4.1).

4.1.3 George Compound:

A total amount of Seven thousand, five hundred (7500) *Ascaris species* ova were observed in a total of 150 sample preparations that weighed about 1 gram on average including one thousand (1000) *Strongyloides species* larvae were seen in the top layer samples (Table 4.1). In addition, the top layer samples indicated a presence of two thousand, three hundred and thirty-three (2333) *Cryptosporidia species* protozoan oocysts in a total of the 150 sample preparation smears that were done on samples from George Compound as well as fifty (50) *Enterobius species* ova were seen. Besides, the bottom layer samples from George Compound registered a total of four thousand five hundred and fifty (4550) *Ascaris species* ova and a presence of two hundred (200) *Strongyloides species* larvae (Table 4.1). One thousand, three hundred and forty-nine (1340) *Cryptosporidia species* protozoan oocysts were seen in the samples as well as one thousand (1000) *Enterobius species* ova (Table 4.1).

4.1.4 Madimba Compound:

An amount of four thousand, four hundred (4400) *Ascaris species* ova were recorded in a total of 75 sample preparations that weighed about 1 gram on average which included one hundred (100) *Strongyloides species* larvae (Table 4.1). Moreover, the top layer samples also indicated a presence of one thousand, two hundred and nine-one (1291) *Cryptosporidia species* protozoan oocysts in a total of the 75 sample preparation smears which were done on samples from Madimba Compound as well as fifty (50) *Enterobius species* ova were noted (Table 4.1). The bottom layer samples from Madimba Compound registered about three thousand four hundred (3400) *Ascaris species* ova (Table 4.1). The bottom layer samples also indicated a presence of one hundred (100) *Strongyloides species* larvae and seven hundred and twenty-six (726) *Cryptosporidia species* protozoan oocysts which were seen in the samples as well as one hundred (100) *Enterobius species* ova (Table 4.1).

4.2 The Percentage Presentation and Interpretation of the Results

4.2.1 Chaisa Compound

Chaisa Compound indicated a presence of *Ascaris species* in 56% of the pit latrines; 96% of pit latrines showed a presence of *Cryptosporidia species* protozoan oocysts. About 8% of the pit latrines showed a presence of *Strongyloides species* ova with 4% of the pit latrines indicating *Enterobius species* (Figure 4.6).

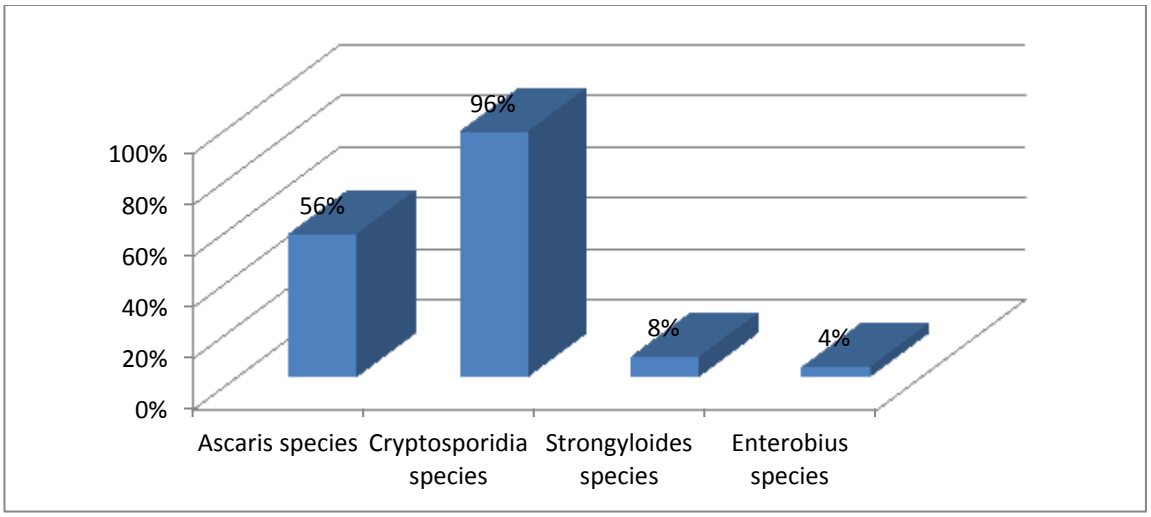

Figure 4.6 : Type and Percentage Presence of Helminth Ova, Larvae and Protozoan Oocysts per 25 pit latrines of Chaisa Compound

4.2.2 George Compound

George Compound indicated a presence of *Ascaris species* in 68% of the pit latrines; 96% of pit latrines showed a presence of *Cryptosporidia species* protozoan oocysts. About 4% of the pit latrines showed a presence of *Strongyloides species* ova with 4% of the pit latrines indicating *Enterobius species* (Figure 4.7)

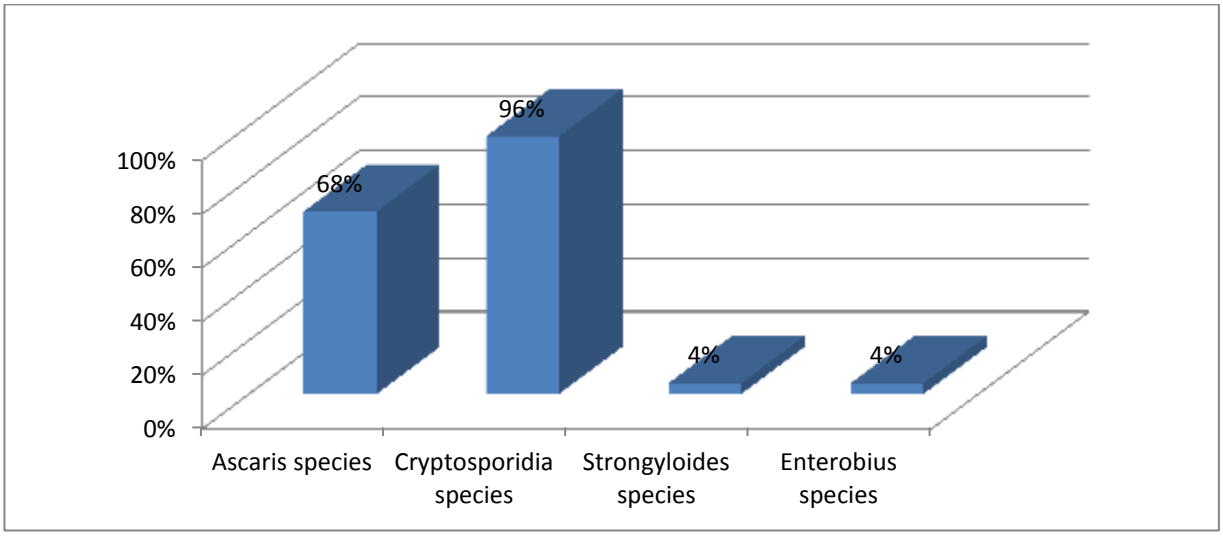

Figure 4.7: Type and Percentage Presence of Helminth Ova, Larvae and Protozoan Oocysts per 25 pit latrines of George Compound

4.2.3 Kanyama Compound

Kanyama Compound indicated a presence of *Ascaris species* in 60% of the pit latrines; 96% of pit latrines showed a presence of *Cryptosporidia species* protozoan oocysts. About 16% of the pit latrines showed a presence of *Strongyloides species* ova with 20% of the pit latrines indicating *Enterobius species* (Figure 4.8)

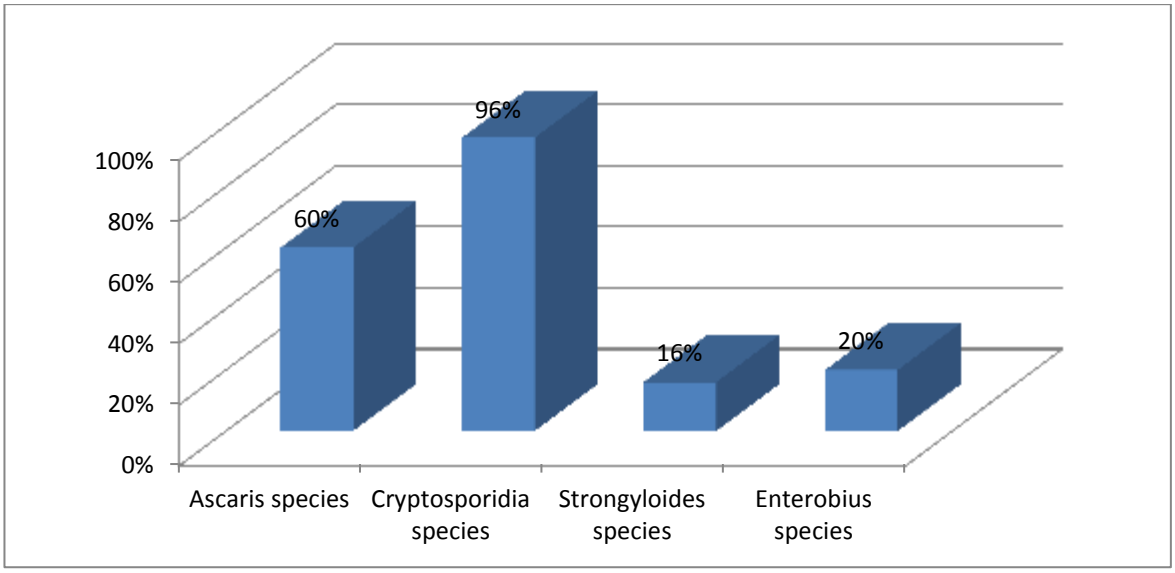

Figure 4.8: Type and Percentage Presence of Helminth Ova, Larvae and Protozoan Oocysts Per 25 pit latrines of Kanyama Compound

4.2.4 Madimba Compound

About 40% of the pit latrines indicated *Ascaris species* with about 92% of the 25 pit latrines in Madimba Compound showed a presence of *Cryptosporidia species* parasite; *Enterobius species* appeared in about 16% of the pit latrines with *Enterobius species* indicated a presence in about 16% and *Cyclospora species* were observed in 40% of the pit latrines. Besides, 20% had viable *Ascaris species* ova in the top layer only (Figure 4.9)

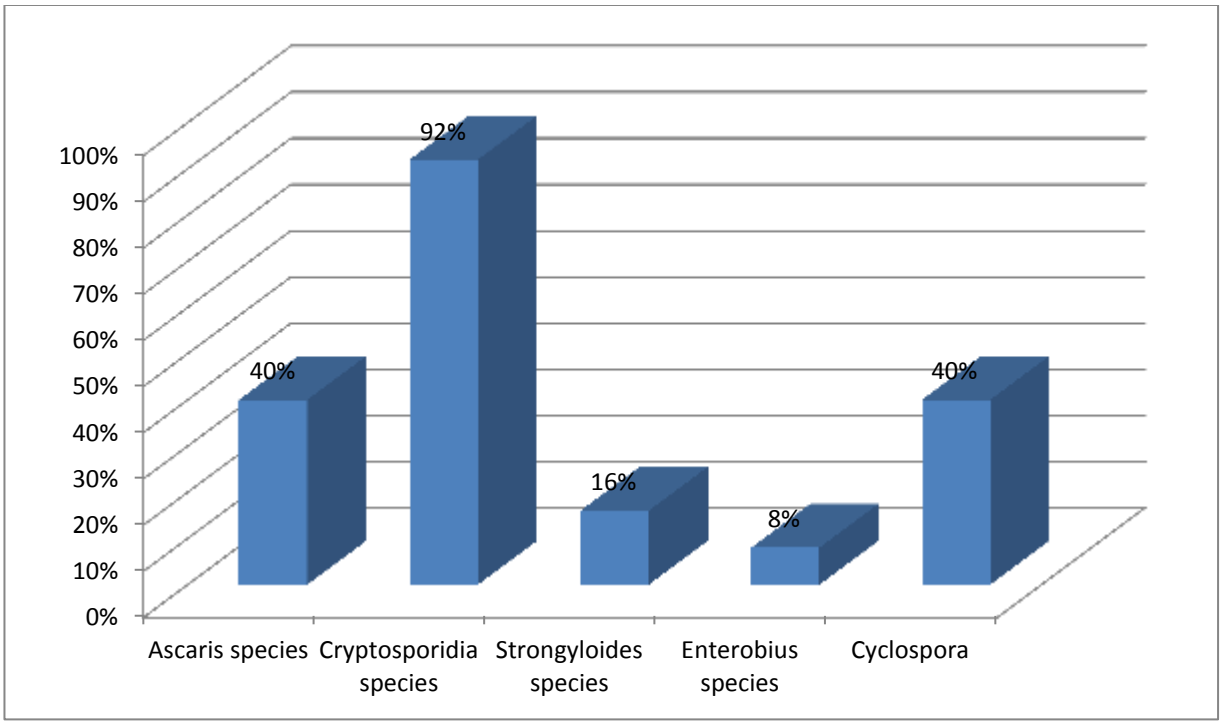

Figure 4.9: Type and Percentage Presence of Helminth Ova, Larvae and Protozoan Oocysts per 25 pit latrines of Madimba Compound

4.3 Presentation of *Ascaris* ova viability Results

4.3.1 Chaisa Compound

About 56% of the 25 pit latrines indicated a presence of *Ascaris species* ova. And out of the number of pit latrines that indicated a presence of Ascaris species, 72% had viable ova in the top layer only where as the bottom layer samples showed non-viable ova that completely absorbed the Crystal violet stain (Figure 4.10).

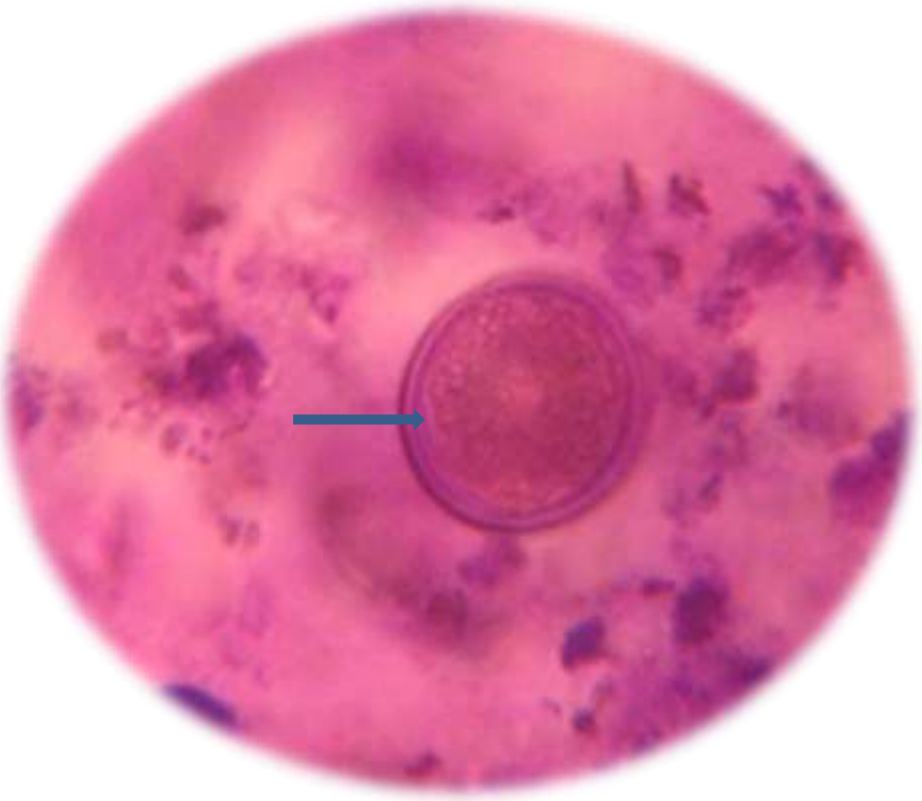

Figure 4.10: A microscopic picture of a nonviable *Ascaris species* ovum at x40 magnification

4.3.2 George Compound

In George Compound, about 68% of the 25 pit latrines indicated a presence of *Ascaris species* ova. And out of the number of pit latrines that indicated a presence of Ascaris species, 75% had viable ova in the top layer only whereas the bottom layer samples showed non-viable ova.

4.3.3 Kanyama Compound

It was observed in Kanyama Compound that about 60% of the 25 pit latrines registered a presence of *Ascaris species* ova. And out of the number of pit latrines that indicated a presence of Ascaris species, 6% had viable ova in both the top and the bottom layer samples.

4.3.4 Madimba Compound

In Madimba Compound, about 40% of the 25 pit latrines indicated a presence of *Ascaris species* ova. And out of the number of pit latrines that indicated a presence of Ascaris species, 60% had viable ova in the top layer only whereas the bottom layer samples showed non-viable ova.

4.4 Statistical Presentation of Paired Sample T-Tests Results

4.4.1 Chaisa Compound

The data provided sufficient evidence to conclude that in Chaisa Compound, the mean concentration of *Ascaris species* ova per gram of faecal matter did vary with increasing depth in Chaisa Compound. It appeared that the increase in depth of the pit latrine/faecal matter did reduce the concentration of *Ascaris species* ova. At confidence level of 90%, the concentration reduced by about 56.63% with increasing depth on average, (Table 4.2). It was observed that the mean concentration of *Strongyloides species* larvae per gram of faecal matter did not vary with increasing depth in Chaisa Compound. It was noted that the increase in depth of the pit latrine/faecal matter did not reduce the concentration of *Strongyloides species* larvae, (Table 4.2).

The data provided sufficient evidence to conclude that the mean concentration of *Cryptosporidia species* protozoan oocysts per gram of faecal matter did not vary with increasing depth in Chaisa Compound. It appeared that the increase in depth of the pit latrine/faecal matter did not reduce the concentration of *Cryptosporidia species* protozoan oocysts, (Table 4.2). The data indicated that the mean concentration of *Enterobius species* ova per gram of faecal matter did not vary with increasing depth in Chaisa Compound. It appeared that the increase in depth of the pit latrine/faecal matter did not reduce the concentration of *Enterobius species* ova, (Table 4.2).

Table 4 .2 : Statistical Presentation and Interpretation of Results from Chaisa Compound				
	Ascaris species ova	*Strongyloides species* larvae	*Cryptosporidia species* protozoan oocysts	*Enterobius species* ova
đ	29.36	2.56	18.36	0
$(\sum d^2)$	158223.33	2777.56	153071.1	0
$(\sum d)^2$	145671.99	1111.56	56953.82	0
S_d	79.69	10.67	79.27	0
n	25	25	25	25
t	**1.84**	**1.2**	**1.16**	**0**
d_f	24	24	24	24
$\mathbf{t_{0.05}}$	**1.711**	**1.711**	**1.711**	**1.711**
Confidence interval	2.09 to 56.63	-1.09 to 6.21	-8.77 to 45.49	0
Conclusion	Reject Ho	Do not reject Ho	Do not reject Ho	Do not reject Ho

Source: Field data

4.4.2 George Compound

The data evidently indicated that in George Compound, the mean concentration of *Ascaris species* ova per gram of faecal matter did vary with increasing depth in George Compound. It appeared that the increase in depth of the pit latrine/faecal matter did reduce the concentration of *Ascaris species* ova (Table 4.3). With 90% confidence level, the ova concentration varied about 100% on average. However, it was observed that the mean concentration of *Strongyloides species* larvae per gram of faecal matter did not vary with increasing depth in George Compound (Table 4.3). It appeared that the increase in depth of the pit latrine/faecal matter did not reduce the concentration of *Strongyloides species* larvae.

The data provided sufficient evidence to conclude that the mean concentration of *Cryptosporidia species* protozoan oocysts per gram of faecal matter did not vary with increasing depth in George Compound (Table 4.3). It appeared that the increase in depth of the pit latrine/faecal matter did not reduce the concentration of *Cryptosporidia species* protozoan oocysts. The data indicated that the mean concentration of *Enterobius species* ova per gram of faecal matter did vary with increasing depth in George

Compound. It appeared that the increase in depth of the pit latrine/faecal matter did reduce the concentration of *Enterobius species* ova. At 90% confidence level, the concentration reduced at 33.68% with increasing depth (Table 4. 3).

Table 4.3: Statistical Presentation and Interpretation of Results from George Compound

	Ascaris species ova	*Strongyloides species* larvae	*Cryptosporidia species* protozoan oocysts	*Enterobius species* ova
đ	61.97	10.67	12.71	-2
$(\sum d^2)$	828889.13	111386.78	9116.95	2500
$(\sum d)^2$	2400361.48	71107.56	100914.23	2500
S_d	174.75	67.25	14.55	10
n	25	25	25	25
t	**1.77**	**0.79**	**4.37**	**-1**
d_f	24	24	24	24
$\mathbf{t_{0.05}}$	**1.711**	**1.711**	**1.711**	**1.711**
Confidence interval	2.17 to 121.77	12.34 to 33.68	7.73 to 17.69	-5.4 to 1.4
Conclusion	Reject Ho	Do not reject Ho	Reject Ho	Do not reject Ho

Source: Field data

4.4.3 Kanyama Compound

The data provided sufficient evidence to conclude that in Kanyama Compound, the mean concentration of *Ascaris species* ova per gram of faecal matter did not vary with increasing depth in Kanyama Compound. It appeared that the increase in depth of the pit latrine/faecal matter did not reduce the concentration of *Ascaris species* ova (Table 4.4). It was observed that the mean concentration of *Strongyloides species* larvae per gram of faecal matter did not vary with increasing depth in Kanyama Compound. It appeared that the increase in depth of the pit latrine/faecal matter did not reduce the concentration of *Strongyloides species* larvae (Table 4.4).

The data provided sufficient evidence to conclude that the mean concentration of *Cryptosporidia species* protozoan oocysts per smear of faecal matter did not vary with increasing depth in Kanyama Compound. It appeared that the increase in depth of the pit latrine/faecal matter did not reduce the concentration of *Cryptosporidia species* protozoan oocysts (Table 4.4). The data indicated that the mean concentration of *Enterobius species* ova per gram of faecal matter did not vary with increasing depth in Kanyama Compound. It appeared that the increase in depth of the pit latrine/faecal matter did not reduce the concentration of *Enterobius species* ova, (Table 4.4).

Table 4.4: Statistical Presentation and Interpretation of Results from Kanyama Compound

	Ascaris species ova	*Strongyloides species* larvae	*Cryptosporidia species* protozoan oocysts	*Enterobius species* ova
đ	-7.3312	6.6664	1.6276	2
$(\sum d^2)$	185830.7	14999.34	739.47	3055.12
$(\sum d)^2$	33591.56	27775.56	1655.68	2500
S_d	87.68	24.06	5.3	11.1
n	25	25	25	25
t	**-0.42**	**1.39**	**1.54**	**0.9**
d_f	24	24	24	24
$\mathbf{t_{0.05}}$	**1.711**	**1.711**	**1.711**	**1.711**
Confidence interval	-37.33 to 22.67	-1.57 to 14.90	-0.19 to 3.44	-1.80 to 5.80
Conclusion	Do not reject Ho	Do not reject Ho	Do not reject Ho	Do not reject Ho

Source: Field data

4.4.4 Madimba Compound

The data provided sufficient evidence to conclude that in Madimba Compound, the mean concentration of *Ascaris species* ova per gram of faecal matter did not vary with increasing depth in Madimba Compound. It appeared that the increase in depth of the pit latrine/faecal matter did not reduce the concentration of *Ascaris species* ova (Table

4.5). It was observed that the mean concentration of *Strongyloides species* larvae per gram of faecal matter did not vary with increasing depth in Madimba Compound. It appeared that the increase in depth of the pit latrine/faecal matter did not reduce the concentration of *Strongyloides species* larvae (Table 4.5).

The data provided sufficient evidence to conclude that the mean concentration of *Cryptosporidia species* protozoan oocysts per gram of faecal matter did vary with increasing depth in Madimba Compound. It appeared that the increase in depth of the pit latrine/faecal matter did reduce the concentration of *Cryptosporidia species* protozoan oocysts (Table 4.5). At 90% confidence level, the protozoan oocysts reduced by 12.11% with increasing depth. However, the data indicated that the mean concentration of *Enterobius species* ova per gram of faecal matter did not vary with increasing depth in Madimba Compound. It appeared that the increase in depth of the pit latrine/faecal matter did not reduce the concentration of *Enterobius species* ova, (Table 4.5).

Table 4.5: Statistical Presentation and Interpretation of Results from Madimba Compound

	Ascaris species ova	*Strongyloides species* larvae	*Cryptosporidia species* protozoan oocysts	*Enterobius species* ova
đ	14.666	10.0004	7.7604	0.6668
$(\sum d^2)$	246108.23	45833.67	5379.5656	277.89
$(\sum d)^2$	134432.22	62505	37639.88	277.89
S_d	100.15	42.49	12.7	3.33
n	25	25	25	25
t	**0.73**	**1.18**	**3.06**	**1**
d_f	24	24	24	24
$\mathbf{t_{0.05}}$	**1.711**	**1.711**	**1.711**	**1.711**
Confidence interval	-19.61 to 48.94	-4.54 to 24.54	3.41 to 12.11	0 to 1.14
Conclusion	Do not reject Ho	Do not reject Ho	Reject Ho	Do not reject Ho

Source: Field data

CHAPTER FIVE: DISCUSSION OF THE FINDINGS

5.1 The Helminthes and Protozoa

Generally, about 13 types of helminthes and protozoa were observed during the laboratory examination of the collected faecal matter samples namely: *Ascaris species, Enterobius species, Strongyloides species, Taenia species, Hymenolempis species, Faschiola species, Balantidia species, Cryptosporidia species, Giardia species, Isospora species, Toxocara species, Cyclospora species* and *Tricho Strongyloides species.* However, only *Ascaris, Cryptosporidia, Enterobius* and *Strongyloides* species were seen in more than 50% of all the 100 pit latrines sampled from and all the four were considered in this research as indicator helminthes and protozoa.

This is in line with (Niwagaba, 2009) who stated that when measuring the treatment efficiency of FS treatment processes, it is too expensive and labour intensive to measure all types of pathogens. Instead, it is common practice to select indicators of pathogenic activity which are measured to provide an indication of the level of pathogen removal during treatment. These indicators can be either pathogenic, or non-pathogenic, but the organisms need to be carefully selected in order to provide adequate information on the inactivation of pathogens. The following requirements should be met when selecting an indicator (Mara, 2004):

- be exclusively of faecal origin;
- be in numbers greater than those of the pathogens of concern;
- have a removal that mimics, and is close to, that of pathogens of concern;
- be simple, inexpensive, accurate, and reliable to measure; and
- indicator organisms should have the ability to survive longer than the pathogen of concern.

George Compound was the most infected and affected by the helminthes and protozoa followed by Kanyama Compound then Chaisa Compound with the list infected being Madimba Compound by use of *Ascaris species* as indicator parasite. The four compounds had a substantial enumeration of helminth ova and protozoan oocysts with top faecal matter showing large numbers with corresponding high viability that reduced significantly with depth. As a result, the faecal matter in the top layers was found not to be safe to the public health, groundwater and the general environment.

5.1.1 *Ascaris species*

5.1.1.1 Chaisa Compound

The parasitic ova for *Ascaris species* registered the presence in 15 out of 25 Chaisa Compound pit latrines. This collaborates with literature by Strauss and Montangero (2002) which elaborated that in many areas of Africa, Asia and Latin America, helminth, notably nematode infections (*Ascaris, Trichuris, Ancylostoma, Strongyloides, etc.*) are highly prevalent. Among the pathogens causing gastrointestinal infections, nematodes, *Ascaris* in particular, tend to be more persistent in the environment than viruses, bacteria and protozoa with the bulk of helminth ova contained in wastewater or in faecal matter end up in the biosolids generated in treatment schemes (Montangero and Strauss 2002). Hence, nematode ova are the indicators-of-choice to determine hygienic quality and safety where biosolids are to be used as a soil conditioner and fertilizer and the concentration of helminth ova in the biosolids is largely dependent on the prevalence and intensity of infection in the population from which faecal matter or wastewater is collected.

Depending on the duration of biosolids storage and type of treatment, a distinct proportion only of the helminth ova remain viable, Montangero and Strauss (2002). Pit latrine C15 had no *Ascaris species* ova in the bottom layer samples although the top layer samples had *Ascaris species* ova indicating that the users might have been different. The top layer faecal matter samples had a higher concentration of ova per gram of faecal matter compared to bottom samples as verified by the statistical t-test

done on the top layer and bottom layer concentrations. The faecal matter samples from the top layers had viable *Ascaris species* ova in 9 pit latrines for Chaisa Compound.

Generally, 23 out of 25 pit latrines indicated a reduced number of ova and protozoan oocysts with increasing depth thus showing a possibility of a die off trend. Besides there were no other parasites that were seen in Chaisa Compound apart from *Strongyloides species, Cryptosporidia species* and *Enterobius species* discussed below.

5.1.1.2 George Compound

George Compound indicated a presence of *Ascaris species* ova in 17 out of 25 pit latrines. The top layer faecal matter samples had a higher concentration of ova per gram of faecal matter compared to bottom layer samples as verified by the statistical t-test done on the top and bottom layer concentrations. The faecal matter samples from the top layers had viable *Ascaris species* ova in 12 pit latrines although bottom layer faecal matter for pit latrine G15 had viable *Ascaris species* ova for George Compound. Generally, 18 out of 25 pit latrines indicated a reduced number of ova and protozoan oocysts with increasing depth indicating a possibility of a die off trend whereas pit latrines number G01, G11 and G12 showed a higher concentration of parasites in the bottom layer samples meaning that the previous users might have been more infected that the users at the time of sampling. No other parasites were seen in George Compound apart from *Strongyloides species, Cryptosporidia species* and *Enterobius species* discussed below.

5.1.1.3 Kanyama Compound

The faecal matter samples from Kanyama Compound had statistically same concentration of ova per gram of faecal matter in both top and bottom layer samples as verified by the statistical paired sample t-test done on the top and bottom layer concentrations. Only K22 pit latrine indicated viable ova in the top layers although no pit latrine indicated some viable ova in the bottom layers of faecal matter for Kanyama Compound. This clearly proved that if subjected to anaerobic conditions over a

considerable period of time, helminthes and protozoa not only reduce in numbers but also get destroyed eventually resulting in safe faecal sludge.

Moreover, 18 out of 25 pit latrines indicated a reduced number of ova and protozoan oocysts with increasing depth thus showing a possibility of a die off trend although pit latrine K12 was seen to be free from any type of parasites indicating that the users were not at all infected by helminthes and protozoa whereas pit latrine number K13 showed a higher concentration of helminthes and protozoa in the bottom samples meaning that the previous users might have been more infected that the users at the time of sampling. Other parasites that were seen in Kanyama Compound included: *Taenia species* in pit latrine K5; *Hymenolempis species* in pit latrine K1 and pit latrine K7; *Cyclospora species* in pit latrine K1; pit latrines; *Balantidia species* in pit latrine K3, K7, K14 and K15; *Isospora* species in pit latrine K1; *TrichoStrongyloides* in pit latrine K14 and *Faschiola species* in pit latrine K16. No other parasites were seen in Kanyama Compound apart from *Strongyloides species, Cryptosporidia species* and *Enterobius species* discussed below.

5.1.1.4 Madimba Compound

Madimba Compound had 10 out of the 25 pit latrines whose samples indicated a presence of *Ascaris species* ova and 5 pit latrines of which had viable ova for *Ascaris species* in the top layer samples only. The top layer faecal matter samples had a similar concentration of ova per gram of faecal matter compared to bottom samples as verified by the statistical t-test done on the top and bottom layer concentrations for Madimba Compound.

Generally, 10 out of 25 pit latrines indicated a reduced number of ova and protozoan oocysts with increasing depth thus showing a possibility of a die off trend although 2 pit latrines were seen to be free from parasites indicating that the users were not at all infected by parasites whereas pit latrine number M13 and M20 showed a higher concentration of parasites in the bottom samples meaning that the previous users might

have been more infected that the current users. Other parasites that were seen in Madimba Compound include: *Cyclospora species* in M01, M09, M10, M11, M12, and M13 pit latrines; *Trichuria species* M13 pit latrine; and *Faschiola species* in M1 pit latrine.

5.1.2 *Strongyloides species*

5.1.2.1 Chaisa Compound

Chaisa Compound indicated a presence of *Strongyloides species* larvae C6 and C17 out of the 25 pit latrines. The top layer faecal matter samples varied in the concentration of larvae per gram of faecal matter compared to bottom samples as verified by the statistical t-test done on the top and bottom layer concentrations. This could be related to the schist type of formation and very low water table hence being a non conducive environment for the survival of the helminth compared to Kanyama, George and Madimba compounds that are on highly productive water bearing marble rock (aquifer) with a generally very high water table.

5.1.2.2 George Compound

George Compound indicated a presence of *Strongyloides species* in G20 pit latrines. There was no variation between the larvae concentration in the top layer faecal matter samples and bottom samples as verified by the statistical t-test done on the top and bottom layer concentrations. This could imply that the users have been the same all along and were found to be infected by the type of helminth. It also indicated that the environment was suitable for the survival of the helminth.

5.1.2.3 Kanyama Compound

The faecal matter samples from Kanyama Compound did not vary significantly in the concentration of *Strongyloides species* larvae per gram of faecal matter in both the top layer samples and bottom layer samples as verified by the statistical t-test done on the top layer and bottom layer concentrations. Being a moist geological environment with a generally high water table, the survival rate of the helminth in the area was high.

5.1.2.4 Madimba Compound

Madimba Compound had M1, M3, M5 and M7 out of the 25 pit latrines whose samples indicated a presence of *Strongyloides species* larvae. The top layer faecal matter samples had a similar concentration of larvae per gram of faecal matter compared to bottom samples as verified by the statistical t-test done on the top and bottom layer concentrations for Madimba Compound. These pit latrines were in the drier and rocky zone of the compound.

5.1.3 *Cryptosporidia species*

5.1.3.1 Chaisa Compound

Chaisa Compound indicated a presence of *Cryptosporidia species* in 24 out of 25 pit latrines. The top layer faecal matter samples had a higher concentration of protozoan oocysts per smear of faecal matter compared to bottom samples as verified by the statistical t-test done on the top and bottom layer concentrations. *Cryptosporidia* proved to be a protozoa that was able to survive and exist in both high water table marble formation and low water table schist formation. The protozoa appeared to infect everybody in all the study areas as it was found in almost all the 100 pit latrines sampled from. It was conclusive to note that the prevalence of diarrhea cases in Lusaka especially during the rainy season could as well be attributed to this protozoa pathogenic nature as supported by literature by Roberts and Janovy (2005).

5.1.3.2 George Compound

Whereas George Compound indicated a presence of *Cryptosporidia species* in 24 out of 25 pit latrines. The top layer faecal matter samples had a significant variation in the concentration of protozoan oocysts per smear of faecal matter compared to bottom layer samples as verified by the statistical t-test done on the top and bottom layer concentrations. This is because George Compound is on non water bearing rock formation to some extent (Baumle et al, 2012).

5.1.3.3 Kanyama Compound

The parasitic protozoan oocysts for *Cryptosporidia species* registered the presence in 24 out of 25 pit latrines in Kanyama Compound. This collaborates with literature by Adamu and Petros (2009) which elaborated that it is well known that geographical location and levels of general hygiene play a significant role in the distribution of *Cryptosporidia species.* Literature indicates that *Cryptosporidium* species and *Giardia* species are wide-spread pathogens of humans and many species of mammals (Paziewska et al, 2007). The faecal matter samples from Kanyama Compound had similar concentrations of protozoan oocysts per smear of faecal matter in both the top samples and bottom samples as verified by the statistical t-test done on the top and bottom layer concentrations. This agrees with the findings by Baumle at el (2012) on the type and nature of geologic formation for Kanyama Compound.

5.1.3.4 Madimba Compound

Madimba Compound had 23 out of the 25 pit latrines whose samples indicated a presence of *Cryptosporidia species.* The top layer faecal matter samples had a significant variation in the concentration of protozoan oocysts per smear of faecal matter compared to bottom samples as verified by the statistical t-test done on the top and bottom layer concentrations for Madimba Compound This is quiet surprising because Madimba Compound is entirely on a high productive water bearing rock formation like Kanyama Compound (Baumle et al, 2012) and the reasons for this will be known in further research.

5.1.4 *Enterobius species*

5.1.4.1 Chaisa Compound

Chaisa Compound indicated a presence of *Enterobius species* ova in C24 samples only out of 25 pit latrines. The top layer faecal matter samples concentration for *Enterobius species* ova did not vary significantly per gram of faecal matter compared to bottom

samples as verified by the statistical t-test done on the top and bottom layer concentrations (Weiss and Hanset, 1991).

5.1.4.2 George Compound

Whereas George Compound indicated a presence of *Enterobius species* ova in G22 only out of 25 pit latrines (Ridlay, 2012). The top layer faecal matter samples had a similar concentration of ova per gram of faecal matter compared to bottom samples as verified by the statistical t-test done on the top and bottom layer concentrations.

5.1.4.3 Kanyama Compound

The parasitic ova for *Enterobius species* registered the presence in 5 out of 25 pit latrines in Kanyama Compound. This is in line with literature by Amoah (2008) which elaborated that *Enterobius species is among the* pathogens that are infective immediately on excretion with a low median infective dose and a transmission of these diseases occurs predominantly in the immediate domestic environment, especially when low standards of personal hygiene prevail, although the survival times of some excreted viruses and protozoa may be long enough to pose a health risk in wastewater use schemes. The faecal matter samples from Kanyama Compound had a similar concentration of ova per gram of faecal matter in both the top samples and bottom samples as verified by the statistical t-test done on the top and bottom layer concentrations. Generally, K5, K7, K17, K 24 and K25 out of 25 pit latrines indicated a presence of *Enterobius species* ova (Bogitsh et al., 2012).

5.1.4.4 Madimba Compound

Madimba Compound had M7 and M8 out of the 25 pit latrines whose samples indicated a presence of *Enterobius species* ova (Bruckner, 2012). The top layer faecal matter samples had a similar concentration of *Enterobius species* ova per gram of faecal matter compared to bottom samples as verified by the statistical t-test done on the top and bottom layer concentrations for Madimba Compound (Weiss and Hasset, 1991).

CHAPTER SIX: CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The significance of the study was to assess and describe the helminth pathogens and protozoan oocysts in faecal matter in terms of indicator helminthes and indicator protozoa with variation in faecal matter depth. This was essential because sustainable and productive exploitation of available on-site faecal matter is not feasible without adequate knowledge of pathogenic pit latrine faecal matter characteristics. The research aimed at providing scientific information to support decision making in the handling of accumulated faecal matter in pit latrines under Zambian conditions.

There was a knowledge gap in the possible parasitic contamination of groundwater by pit latrine faecal matter from unplanned high density residential areas of Lusaka. Moreover, Integrated Water Resources Management (IWRM) is defined as: a process, which promotes the coordinated development and management of water, land and related resources in order to maximise the resultant economic and social welfare in an equitable manner without compromising the sustainability of vital ecosystems (Technical Advisory Committee, 2000; and SARDC, 2008). This entails that the management of pit latrine faecal matter should be based on the concept of IWRM in terms of safe guarding the quality and quantity of groundwater, surface water and the general environment as well as public health.

All the four compounds enumerated a presence of parasites in a substantial number of pit latrines. A noteworthy die off trend was observed with top layers having large numbers of ova/protozoan oocysts whereas bottom layers showed reduced numbers with

nil in some cases. Chaisa indicated low viability of the *Ascaris species* ova. However, Kayama, Madimba and George compounds showed a relative high viability which could be attributed to the high moisture content of the faecal matter because of the high water table in these three areas of study and this might create conducive environment for the survival of ova, protozoan oocysts and cysts for the parasites.

Using *Ascaris species* as an indicator parasite, George Compound was the most affected by the parasites followed by Kanyama Compound, Chaisa Compound with the least affected being Madimba Compound. However, Kanyama registered 11 types of parasites; Madimba compound indicated 9 types with George and Chaisa compounds having registered 4 types respectively. Although prone to Cholera out breaks, the high presence of *Cryptosporidia species* could be associated with a high occurrence of short term profuse and watery diarrhea with quick bowel movement characteristic of these Lusaka high density residential areas. The faecal matter in all the four study areas is contaminated and not safe for public health, ground water and the general environment.

6.2 Recommendations

It is recommended that in the midterm the possibility of establishing off site water borne sanitation should be considered especially that Kanyama, George and Madimba compounds are on a high productive aquifer;

In the short term, faecal matter digesters could be constructed to allow for the stabilisation of the faecal matter before it could finally be exposed to the open environment;

Well-equipped and competent institutions should be engaged to manage the exhuming, transportation and stabilisation of the hazardous material instead of the poorly equipped casual workers as is the case with the Kanyama Water Trust faecal matter handling programme;

Further research should be done to establish the proper stabilisation approaches before use of faecal matter under Zambian conditions;

Sampling and examination of soil around the pit latrines for the same helminthes and protozoa should be carried out;

Waters from the domestic wells and boreholes in the study area should also be sampled and examined for helminthes and protozoa;

The institutions responsible should ensure provision of protective wear for worker handling raw sewer and faecal matter;

Ministry of Health should take up the lead among the public health practitioners in promoting Information Education and Communication programmes for the community on human intestinal helminthes and protozoa in terms of transmission, pathology, treatment and prevention.

It is further recommended that any review of sanitation related approaches should include these aspects if sustainable sanitation is to be achieved in Zambia. This concept has been applied in Tanzania for promoting sustainable environmental protection using modified pit-latrines (Chaggu, 2004).

The Ministry of Local Government and Housing should revisit the design of the substructure for pit latrines. The Ministry should prepare a policy input to Cabinet in consultation with the University of Zambia, Network for Environmental Concerns and Solutions and Zambia Environment Management Agency as well as Ministry of Water, Sanitation and Environmental Protection. This will ensure that the GRZ standard design for a pit latrine, should be with a sealed and elevated substructure regardless of the geologic formation of any area because, faecal matter is now regarded as a resource both for soil conditioning and biogas generation. Therefore, it should be easier to access

the faecal matter once a pit latrine is full. Underground substructures makes desludging quiet difficult;

National Water and Sanitation Council together with Lusaka Water and Sewerage Company as well as the UNZA IWRM Centre should develop a sensitisation package for users of pit latrine in Lusaka peri-urban areas so that the quality of pit latrine faecal matter could be free from unwanted material such as greywater, bottles and cloths;

The Ministry of Local Government and Housing, the University of Zambia, Ministry of Agriculture and Livestock together with the Ministry of Lands and Natural Resources should develop another sensitisation package for the would be users of the stabilised faecal matter to condition their crop fields so that there could be a ready market for the realised faecal matter;

The Ministry of Commerce, Trade and Industry should lure local and foreign investors into the establishment of faecal matter digesters for biogas generation business. This will provided centers where the desludged unstabilised faecal matter would be transported by Small and Medium Enterprises (SMEs) with suitable transportation mechanisms. This can in turn create employment at this level;

Finally, the Ministry of Health should embark on a serious periodic public deworming campaign of Lusaka as the presence of parasitic ova, cysts and protozoan oocysts in faecal matter showed that the people of Lusaka are infected by parasites; and

Any review of sanitation related policies and legislation should include sealed and elevated pit latrine substructure if sustainable sanitation is to be achieved in Zambia.

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APPENDICES

Appendix I:

The apparatus:

- 2 spray bottles
- 48mm wire mesh, stainless steel, 5 inch in diameter
- A centrifuge machine that could sustain forces of at least 660X G with rotors
- A swinging bucket rotor for holding 15ml conical plastic centrifuge tubes
- Big tissue rolls
- Black plastic rolls
- Conical 15ml centrifuge tubes
- Cover slips
- Digital camera
- Examination gloves
- Surgical gloves
- Glass slides
- GPS
- Ice packs
- Laptop computer
- Large cooler box

- Large test tube rack for 15ml centrifuge tubes
- Mutton cloths
- Pasture pipettes
- Pyrex beakers,
- Refuse plastic bags
- 250ml sampling bottles
- 1 liter capacity faecal matter sampler
- Small test tube rack for 15ml centrifuge tubes
- Teflon spatula
- Tongue depressor

Appendix II: Materials and Reagents

Reagent grade chemicals were used in all tests and these included:

- 1.4 $ZnSO_4$ solution
- 10.1N H_2SO_4
- Crystal violet stain
- 95% alcohol
- 1% aqueous Ammonium oxalate
- 7% Sodium hypochlorite
- 0.25% Methylene blue
- Malachite green
- 3% HCL in 95% Ethanol
- Deionised water
- Ammonium bicarbonate
- Running tap water
- Oil immersion
- Carbol fuchsin
- Detergent
- Jik
- Teepol (Disinfectant)
- Heavy duty disinfectant

Appendix III

CHAISA COMPOUND PAIRED SAMPLE T-TEST TABLE				
Ascaris species Ova Concentration per gram preparation of faecal matter				
Pit Latrine Code	**Top Layer Concentration**	**Bottom Layer Concentration**	**Mean Difference**	**Mean Difference Squared**
C1	0	0	0	0.00
C2	16.67	0	16.67	277.89
C3	0	0	0	0.00
C4	0	0	0	0.00
C5	83.33	50	33.33	1110.89
C6	100	0	0	0.00
C7	566.67	683.33	-116.66	13609.56
C8	83.33	100	-16.67	277.89
C9	0	0	0	0.00
C10	16.67	150	-133.33	17776.89
C11	216.67	66.67	115	13225.00
C12	0	16.67	-16.67	277.89
C13	0	0	0	0.00
C14	0	0	0	0.00
C15	33.33	0	33.33	1110.89
C16	416.67	216.67	200	40000.00
C17	0	0	0	0.00
C18	0	0	0	0.00
C19	33.33	66.67	33.34	1111.56
C20	116.67	66.67	50	2500.00
C21	50	116.67	-66.67	4444.89
C22	550	300	250	62500.00
C23	0	0	0	0.00

C24	0	0	0	0.00
C25	0	0	0	0.00
SUM	**2283.34**	**1833.35**	**381.67**	**158223.3335**
MEAN	**175.6415**	**141.0269**	**29.35923077**	

Strongyloides species larvae concentration per gram preparation of faecal matter

Pit Latrine Code	**Top Mean Concentration**	**Bottom Mean Concentration**	**Mean Difference**	**Mean Difference Squared**
C1	0	0	0	0
C2	0	0	0	0
C3	0	0	0	0
C4	0	0	0	0
C5	0	0	0	0
C6	16.67	0	16.67	277.89
C7	0	0	0	0
C8	0	0	0	0
C9	0	0	0	0
C10	0	0	0	0
C11	33.33	0	33.33	1110.89
C12	0	0	0	0
C13	0	0	0	0
C14	0	0	0	0
C15	0	0	0	0
C16	0	0	0	0
C17	0	33.33	-33.33	1110.89
C18	0	0	0	0
C19	0	0	0	0
C20	0	0	0	0
C21	66.67	50	16.67	277.89
C22	0	0	0	0
C23	0	0	0	0
C24	0	0	0	0
C25	0	0	0	0
SUM	**116.67**	**83.33**	**33.34**	**2777.56**
Mean	**8.974615**	**6.41**	**2.564615385**	

Cryptosporidia species protozoan oocysts concentration per smear preparation of faecal matter

Pit Latrine Code	**Top Mean Concentration**	**Bottom Mean Concentration**	**Mean Difference**	**Mean Difference Squared**
C1	26	16.33	9.67	93.51

C2	3.33	1.33	2	4
C3	62.67	25.67	37	1369
C4	4.33	1	3.33	11.09
C5	5.67	2.67	3	9
C6	14	6	8	64
C7	115	88.33	26.67	711.29
C8	12.33	3.33	9	81
C9	0	0	0	0
C10	12	4.67	8.67	75.17
C11	10.67	6.33	4.34	18.84
C12	35	21.33	13.67	186.87
C13	9.33	6.67	2.66	7.08
C14	0.33	0	0.33	0.11
C15	14.33	5.33	9	81
C16	27.33	13.67	13.66	186.6
C17	0	0	0	0
C18	3	0	3	9
C19	21.33	11	10.33	106.71
C20	21	1.67	19.33	373.65
C21	12.33	11.33	1	1
C22	81	55	26	676
C23	0.33	0	0.33	0.11
C24	14.33	3	11.33	128.37
C25	23	6.67	16.33	266.67
SUM	**528.64**	**291.33**	**238.65**	**4460.07**
MEAN	**40.66462**	**22.41**	**18.35769231**	

Enterobius species ova concentration per gram preparation of faecal matter

Pit Latrine Code	**Top Mean Concentration**	**Bottom Mean Concentration**	**Mean Difference**	**Mean Difference Squared**
C1	0	0	0	0
C2	0	0	0	0
C3	0	0	0	0
C4	0	0	0	0
C5	0	0	0	0
C6	0	0	0	0
C7	0	0	0	0
C8	0	0	0	0
C9	0	0	0	0

C10	0	0	0	0
C11	0	0	0	0
C12	0	0	0	0
C13	0	0	0	0
C14	0	0	0	0
C15	0	0	0	0
C16	0	0	0	0
C17	0	0	0	0
C18	0	0	0	0
C19	0	0	0	0
C20	0	0	0	0
C21	0	0	0	0
C22	0	0	0	0
C23	0	0	0	0
C24	0	0	0	0
C25	0	0	0	0
SUM	**0**	**0**	**0**	**0**
MEAN	**0**	**0**	**0**	

Appendix iv

GEORGE COMPOUND PAIRED SAMPLE T-TEST TABLE				
Ascaris species ova per gram preparation of faecal matter				
Pit Latrine Code	**Top Mean Concentration**	**Bottom Mean Concentration**	**Mean Difference**	**Mean Difference Squared**
G1	100	250	-150	22500
G2	0	0	0	0
G3	0	0	0	0
4G	0	0	0	0
G5	0	0	0	0
G6	50	16.67	33.33	1110.89
G7	50	66.67	-16.67	277.89
G8	0	0	0	0
G9	0	0	0	0
G10	383.33	166.67	216.66	46941.56
G11	33.33	0	33.33	1110.89
G12	133.33	200	66.67	4444.89
G13	50	50	0	0
G14	0	0	0	0
G15	116.67	0	116.67	13611.89
G16	66.67	0	66.67	277.89
G17	0	0	0	0
G18	133.33	83.33	50	2500
G19	0	0	0	0
G20	1016	183.33	832.67	693339.3
G21	133.33	16.67	166.66	27775.56

G22	150	116.67	33.33	1110.89
G23	183.33	116.67	66.66	4443.56
G24	83.33	0	83.33	6943.89
G25	0	50	-50	2500
SUM	**2682.65**	**1316.68**	**1549.31**	**828889.1**
MEAN	**107.306**	**52.6672**	**61.9724**	

Strongyloides species larvae per gram preparation of faecal matter

Pit Latrine Code	**Top Mean Concentration**	**Bottom Mean Concentration**	**Mean Difference**	**Mean Difference Squared**
G1	0	0	0	0
G2	0	0	0	0
G3	0	0	0	0
G4	0	0	0	0
G5	0	0	0	0
G6	0	0	0	0
G7	0	0	0	0
G8	0	0	0	0
G9	0	0	0	0
G10	0	66.67	-66.67	277.89
G11	0	0	0	0
G12	0	0	0	0
G13	0	0	0	0
G14	0	0	0	0
G15	0	0	0	0
G16	0	0	0	0
G17	0	0	0	0
G18	0	0	0	0
G19	0	0	0	0
G20	333.33	0	333.33	111108.9
G21	0	0	0	0
G22	0	0	0	0
G23	0	0	0	0
G24	0	0	0	0
G25	0	0	0	0
SUM	**333.33**	**66.67**	**266.66**	**111386.8**
MEAN	**13.3332**	**2.6668**	**10.6664**	

Cryptosporidia species per smear preparation of faecal matter

Pit	**Top Mean**	**Bottom Mean**	**Mean**	**Mean**

Latrine Code	**Concentration**	**Concentration**	**Difference**	**Difference Squared**
G1	63	52	11	121
G2	15	3.67	11.33	128.37
G3	17.33	17	0.33	0.11
G4	21	12.33	8.67	75.17
G5	106	77.67	28.33	802.59
G6	44.67	13	31.67	1002.99
G7	32	33.67	-1.67	2.79
G8	21	14	7	49
G9	30	12	18	324
G10	0	0	0	0
G11	39.33	5.67	33.66	1133
G12	13.67	5.33	8.34	69.56
G13	5.67	0	5.67	32.15
G14	2	0.33	1.67	2.79
G15	50	31	19	361
G16	15	0	15	225
G17	56.67	18.67	38	1444
G18	13.67	4	9.67	93.51
G19	1	0	1	1
G20	15.33	3.33	12	144
G21	141.67	86	55.67	3099.15
G22	3.33	0.33	2	4
G23	11.33	10	1.33	1.7689
G24	0	0	0	0
G25	0	0	0	0
SUM	**718.67**	**400**	**317.67**	**9116.949**
MEAN	**28.7468**	**16**	**12.7068**	

Enterobius species larvae per gram preparation of faecal matter

Pit Latrine Code	**Top Mean Concentration**	**Bottom Mean Concentration**	**Mean Difference**	**Mean Difference Squared**
G1	0	0	0	0
G2	0	0	0	0
G3	0	0	0	0
G4	0	0	0	0
G5	0	0	0	0
G6	0	0	0	0
G7	0	0	0	0

G8	0	0	0	0
G9	0	0	0	0
G10	0	0	0	0
G11	0	0	0	0
G12	0	0	0	0
G13	0	0	0	0
G14	0	0	0	0
G15	0	0	0	0
G16	0	0	0	0
G17	0	0	0	0
G18	0	0	0	0
G19	0	0	0	0
G20	0	0	0	0
G21	0	0	0	0
G22	0	50	-50	2500
G23	0	0	0	0
G24	0	0	0	0
G25	0	0	0	0
SUM	**0**	**50**	**-50**	**2500**
MEAN	**0**	**2**	-2	

Appendix v

KANYAMA COMPOUND PAIRED SAMPLE T-TEST TABLE				
Ascaris species ova per gram preparation of faecal matter				
Pit Latrine Code	**Top Mean Concentration**	**Bottom Mean Concentration**	**Mean Difference**	**Mean Difference Squared**
K1	16.67	0	16.67	277.89
K2	0	0	0	0
K3	0	0	0	0
K4	66.67	16.67	50	2500
K5	66.67	83.33	-16.66	277.56
K6	16.67	33.33	-16.66	277.56
K7	66.67	16.67	50	2500
K8	16.67	16.67	0	0
K9	0	383.33	-383.33	146941.9
K10	33.33	33.33	0	0
K11	16.67	33.33	-16.66	277.56
K12	0	0	0	0
K13	0	0	0	0
K14	0	0	0	0
K15	166.67	0	166.67	27778.89
K16	16.67	50	-33.33	1110.89
K17	17.67	33.33	-16.66	277.56
K18	183.33	150	33.33	1110.89
K19	0	0	0	0
K20	0	0	0	0
K21	0	0	0	0
K22	66.67	33.33	33.34	1111.56

K23	0	0	0	0
K24	16.67	33.33	-16.66	277.56
K25	0	33.33	-33.33	1110.89
SUM	**767.7**	**949.98**	**-183.28**	**185830.7**
MEAN	**30.708**	**37.9992**	**-7.3312**	

Strongyloides species larvae per gram preparation of faecal matter

Pit Latrine Code	**Top Mean Concentration**	**Bottom Mean Concentration**	**Mean Difference**	**Mean Difference Squared**
K1	0	0	0	0
K2	0	0	0	0
K3	16.67	0	16.67	277.89
K4	0	0	0	0
K5	0	0	0	0
K6	0	0	0	0
K7	0	0	0	0
K8	83.33	16.67	66.66	4443.56
K9	0	0	0	0
K10	0	16.67	-16.67	277.89
K11	0	0	0	0
K12	0	0	0	0
K13	0	0	0	0
K14	0	0	0	0
K15	0	0	0	0
K16	0	0	0	0
K17	0	0	0	0
K18	0	0	0	0
K19	0	0	0	0
K20	0	0	0	0
K21	0	0	0	0
K22	0	0	0	0
K23	0	0	0	0
K24	333.33	233.33	100	10000
K25	200	200	0	0
SUM	**633.33**	**466.67**	**166.66**	**14999.34**
MEAN	**25.3332**	**18.6668**	**6.6664**	

Cryptosporidia species protozoan oocysts per smear preparation of faecal matter

Pit Latrine Code	**Top Mean Concentration**	**Bottom Mean Concentration**	**Mean Difference**	**Mean Difference Squared**

K1	15	5.33	9.67	93.59
K2	13	7	5	25
K3	2.67	9	-6.33	40.07
K4	0.67	1.67	-1	1
K5	7.33	4.33	-3	9
K6	2.33	4	-1.67	2.79
K7	6.33	3.67	2.66	7.08
K8	6.33	12	-5.67	32.15
K9	9.67	5	4.67	21.81
K10	1.33	3	-1.67	2.79
K11	2	0.33	1.67	2.79
K12	0	0	0	0
K13	1.33	3.33	-3	9
K14	22.67	9.33	13.34	177.96
K15	3	2.67	0.33	0.11
K16	6.33	9.67	-3.34	177.96
K17	20	16.33	3.67	13.47
K18	6	6	0	0
K19	5.67	3.33	2.34	5.48
K20	5.67	1.33	4.34	18.84
K21	2	1	1	1
K22	9.67	3.33	6.34	40.2
K23	11	4.33	6.67	44.49
K24	5.67	4.33	1.34	1.8
K25	7.33	4	3.33	11.09
SUM	**173**	**124.31**	**40.69**	**739.47**
MEAN	**6.92**	**4.9724**	**1.6276**	

Enterobius species ova per gram preparation of faecal matter

Pit Latrine Code	**Top Mean Concentration**	**Bottom Mean Concentration**	**Mean Difference**	**Mean Difference Squared**
K1	0	0	0	0
K2	0	0	0	0
K3	0	0	0	0
K4	0	0	0	0
K5	0	16.67	-16.67	277.56
K6	0	0	0	0
K7	0	0	0	0
K8	0	0	0	0
K9	0	0	0	0

K10	0	0	0	0
K11	0	0	0	0
K12	0	0	0	0
K13	0	0	0	0
K14	0	0	0	0
K15	0	0	0	0
K16	0	0	0	0
K17	16.67	0	16.67	277.56
K18	0	0	0	0
K19	0	0	0	0
K20	0	0	0	0
K21	0	0	0	0
K22	0	0	0	0
K23	0	0	0	0
K24	66.67	16.67	50	2500
K25	0	0	0	0
Sum	**83.34**	**33.34**	**50**	**3055.12**
Mean	**3.3336**	**1.3336**	**2**	

Appendix vi

MADIMBA COMPOUND PAIRED SAMPLE T-TEST TABLE				
Ascaris species ova per gram preparation of faecal matter				
Pit latrine Code	**Top Mean Concentration**	**Bottom Mean Concentration**	**Mean Difference**	**Mean Difference Squared**
M1	0	150	-150	22500
M2	166.67	16.67	150	22500
M3	166.67	200	-33.33	1110.89
M4	0	0	0	0
M5	350	66.67	283.33	80275.9
M6	66.67	66.67	0	0
M7	350	166.67	183.33	33609.9
M8	0	0	0	0
M9	33.33	0	33.33	1110.89
M10	0	0	0	0
M11	0	0	0	0
M12	0	0	0	0
M13	0	0	0	0
M14	0	0	0	0
M15	0	0	0	0
M16	0	0	0	0
M17	0	0	0	0
M18	0	0	0	0
M19	116.67	33.33	83.33	6943.89

M20	150	416.67	-266.67	71112.9
M21	83.33	0	83.33	6943.89
M22	0	0	0	0
M23	0	0	0	0
M24	0	0	0	0
M25	0	0	0	0
SUM	**1483.34**	**1116.68**	**366.65**	**246108.2**
MEAN	**59.3336**	**44.6672**	**14.666**	

Strongyloides species larvae per gram preparation of faecal matter

Pit Latrine Code	**Top Mean Concentration**	**Bottom Mean Concentration**	**Mean Difference**	**Mean Difference Squared**
M1	0	0	0	0
M2	16.67	0	16.67	277.89
M3	0	33.33	-33.33	1110.89
M4	0	0	0	0
M5	66.67	0	66.67	4444.89
M6	0	0	0	0
M7	200	0	200	40000
M8	0	0	0	0
M9	0	0	0	0
M10	0	0	0	0
M11	0	0	0	0
M12	0	0	0	0
M13	0	0	0	0
M14	0	0	0	0
M15	0	0	0	0
M16	0	0	0	0
M17	0	0	0	0
M18	0	0	0	0
M19	0	0	0	0
M20	0	0	0	0
M21	0	0	0	0
M22	0	0	0	0
M23	0	0	0	0
M24	0	0	0	0
M25	0	0	0	0
SUM	**283.34**	**33.33**	**250.01**	**45833.7**

MEAN	**11.3336**	**1.3332**	**10.0004**	
Cryptosporidia species protozoan oocysts per smear preparation of faecal matter				
Pit Latrine Code	**Top Mean Concentration**	**Bottom Mean Concentration**	**Mean Difference**	**Mean Difference Squared**
M1	16.67	12	4.67	21.81
M2	10.33	1.67	8.66	74.9956
M3	0.33	0	0.33	0.11
M4	11	8	4	16
M5	8	9.33	-1.33	1.77
M6	0	1.33	-1.33	1.77
M7	7.33	11	4.33	18.75
M8	11	5	6	36
M9	41.67	13	28.67	821.97
M10	11.67	5	6.67	44.49
M11	5	8.33	-6.33	40.07
M12	125	66.33	58.67	3442.17
M13	24	18	6	36
M14	9.67	9	0.67	0.45
M15	38.33	25.33	13	169
M16	19.67	6	13.67	186.87
M17	14.67	6.33	8.33	69.39
M18	17.33	3.67	14	196
M19	10	5	5	25
M20	21.33	20	1.33	1.77
M21	7.33	0	7.33	53.73
M22	0	0	0	0
M23	0	0	0	0
M24	18.67	7.67	11	121
M25	0.67	0	0.67	0.45
SUM	**429.67**	**241.99**	**194.01**	**5379.57**
MEAN	**17.1868**	**9.6796**	**7.7604**	
Enterobius species ova per gram of faecal matter				
Pit Latrine Code	**Top Mean Concentration**	**Bottom Mean concentration**	**Mean difference**	**Squared Difference**
M1	0	0	0	0
M2	0	0	0	0

M3	0	0	0	0
M4	0	0	0	0
M5	0	0	0	0
M6	0	0	0	0
M7	0	0	0	0
M8	16.67	0	16.67	277.89
M9	0	0	0	0
M10	0	0	0	0
M11	0	0	0	0
M12	0	0	0	0
M13	0	0	0	0
M14	0	0	0	0
M15	0	0	0	0
M16	0	0	0	0
M17	0	0	0	0
M18	0	0	0	0
M19	0	0	0	0
M20	0	0	0	0
M21	0	0	0	0
M22	0	0	0	0
M23	0	0	0	0
M24	0	0	0	0
M25	0	0	0	0
SUM	**16.67**	**0**	**16.67**	**277.89**
MEAN	**0.6668**	**0**	**0.6668**	

Appendix vii

Pit Latrine Code/GPS Reading for Chaisa Compound and the Four Indicator Parasite Types with Significant Enumeration Indicating Ova Viability in Some Cases				
CH01 ($15^0$23'01.3" and $028^0$16'54.8")	*Ascaris* species	*Strongyloides* species	*Cryptosporidia* species	*Enterobius* species
Top layer 1 (1.1g)	0	0	28	0
Top layer 2 (1.3g)	0	0	29	0
Top layer 3 (1.3g)	0	0	21	0
Bottom layer 1 (1.3g)	0	0	18	0
Bottom layer 2 (1.4g)	0	0	12	0
Bottom layer 3(1.4g)	0	0	19	0
CH02 ($15^0$23'02.9" and $028^0$16'59.7")				
Top layer 1 (1.2g)	50 Non viable ova	0	4	0
Top layer 2 (1.0g)	0	0	3	0
Top layer 3 (0.9g)	0	0	3	0
Bottom layer 1 (1.4g)	0	0	1	0
Bottom layer 2 (1.4g)	0	0	3	0
Bottom layer 3(1.1g)	0	0	0	0
CH03 ($15^0$23'08.9" and $028^0$17'01.5")				
Top layer 1 (1.2g)	0	0	63	0
Top layer 2 (1.0g)	0	0	68	0
Top layer 3 (1.0g)	0	0	57	0
Bottom layer 1 (0.9g)	0	0	29	0
Bottom layer 2 (1.3g)	0	0	18	0
Bottom layer 3(0.9g)	0	0	30	0

CH04 ($15^0$23'12.0" and 028^{0}17'03.0")				
Top layer 1 (1.0g)	0	0	6	0
Top layer 2 (1.4g)	0	0	5	0
Top layer 3 (1.1g)	0	0	2	0
Bottom layer 1 (1.1g)	0	0	0	0
Bottom layer 2 (1.4g)	0	0	1	0
Bottom layer 3(1.2g)	0	0	2	0
CH05 ($15^0$23'14.6" and 028^{0}17'04.6")				
Top layer 1 (1.4g)	50 Non viable ova	0	5	0
Top layer 2 (1.0g)	200 Non viable ova	0	9	0
Top layer 3 (1.3g)	0	0	3	0
Bottom layer 1 (1.3g)	50 Non-viable ova	0	1	0
Bottom layer 2 (1.0g)	0	0	5	0
Bottom layer 3(1.2g)	100 Non viable ova	0	2	0
CH06 ($15^0$23'10.6" and 028^{0}17'05.8")				
Top layer 1 (1.1g)	0	0	18	0
Top layer 2 (1.0g)	300 Non viable ova	50	14	0
Top layer 3 (1.2g)	0	0	10	0
Bottom layer 1 (1.0g)	0	0	6	0
Bottom layer 2 (1.4g)	0	0	10	0
Bottom layer 3(1.1g)	0	0	2	0
CH07 ($15^0$23'09.4" and 028^{0}17'10.2")				
Top layer 1 (1.2g)	750 viable ova	0	124	0
Top layer 2 (1.1g)	400 viable ova	0	109	0
Top layer 3 (1.3g)	550 viable ova	0	112	0
Bottom layer 1 (1.0g)	650 Non viable ova	0	75	0
Bottom layer 2 (1.0g)	800 Non viable ova	0	100	0
Bottom layer 3(1.0g)	600 Non viable ova	0	90	0
CH08 ($15^0$23'06.8" and 028^{0}17'03.7")				
Top layer 1 (0.9g)	0	0	14	0
Top layer 2 (1.4g)	200 viable ova	0	6	0
Top layer 3 (1.4g)	50 viable ova	0	17	0
Bottom layer 1 (1.3g)	100 viable ova	0	3	0
Bottom layer 2 (1.1g)	150 viable ova	0	2	0
Bottom layer 3(1.2g)	50 viable ova	0	5	0
CH09 ($15^0$23'03.1" and 028^{0}17'05.2")				

Top layer 1 (1.3g)	0	0	0	0
Top layer 2 (0.9g)	0	0	0	0
Top layer 3 (1.2g)	0	0	0	0
Bottom layer 1 (1.g)	0	0	0	0
Bottom layer 2 (1.0g)	0	0	0	0
Bottom layer 3(1.1g)	0	0	0	0
CH10 ($15^0$22'59.5" and $028^0$17'12.0")				
Top layer 1 (1.0g)	0	0	16	0
Top layer 2 (1.0g)	0	0	13	0
Top layer 3 (1.2g)	50 viable ova	0	7	0
Bottom layer 1 (1.3g)	100 Non viable ova	0	9	0
Bottom layer 2 (1.0g)	300 Non viable ova	0	2	0
Bottom layer 3(1.4g)	50 Non viable ova	0	3	0
CH11 ($15^0$22'58.5" and $028^0$17'05.9")				
Top layer 1 (1.1g)	200 viable ova	100	11	0
Top layer 2 (0.9g)	100 viable ova	0	8	0
Top layer 3 (1.3g)	350 viable ova	0	13	0
Bottom layer 1 (1.3g)	0	0	6	0
Bottom layer 2 (1.3g)	150 Non viable ova	0	9	0
Bottom layer 3(1.4g)	50 Non viable ova	0	4	0
CH12 ($15^0$22'55.2" and $028^0$17'10.3")				
Top layer 1 (1.0g)	0	0	41	0
Top layer 2 (1.4g)	0	0	30	0
Top layer 3 (1.3g)	0	0	34	0
Bottom layer 1 (1.0g)	0	0	21	0
Bottom layer 2 (1.1g)	0	0	23	0
Bottom layer 3(1.3g)	50 Non viable ova	0	20	0
CH13 ($15^0$22'51.4" and $028^0$17'14.0")				
Top layer 1 (1.3g)	0	0	13	0
Top layer 2 (0.9g)	0	0	6	0
Top layer 3 (1.0g)	0	0	9	0
Bottom layer 1 (1.1g)	0	0	4	0
Bottom layer 2 (1.3g)	0	0	12	0
Bottom layer 3(1.2g)	0	0	4	0
CH14 ($15^0$22'48.5" and $028^0$17'16.1")				
Top layer 1 (1.2g)	0	0	0	0
Top layer 2 (1.0g)	0	0	0	0
Top layer 3 (1.1g)	0	0	1	0

Bottom layer 1 (1.3g)	0	0	0	0
Bottom layer 2 (1.0g)	0	0	0	0
Bottom layer 3(1.3g)	0	0	0	0
CH15 ($15^0$22'42.9" and 028^{0}17'15.8")				
Top layer 1 (1.2g)	0	0	21	0
Top layer 2 (1.4g)	0	0	16	0
Top layer 3 (1.1g)	100 viable ova	0	16	0
Bottom layer 1 (1.3g)	0	0	2	0
Bottom layer 2 (1.1g)	0	0	8	0
Bottom layer 3(1.4g)	0	0	6	0
CH16 ($15^0$22'45.2" and 028^{0}17'11.8")				
Top layer 1 (1.0g)	100 viable ova	0	24	0
Top layer 2 (1.0g)	500 viable ova	0	21	0
Top layer 3 (1.0g)	650 viable ova	0	37	0
Bottom layer 1 (1.3g)	350 Non viable ova	0	11	0
Bottom layer 2 (1.2g)	150 Non viable ova	0	16	0
Bottom layer 3(1.4g)	150 Non viable ova	0	14	0
CH17 ($15^0$22'42.6" and 028^{0}17'09.5")				
Top layer 1 (1.0g)	0	0	0	0
Top layer 2 (1.1g)	0	0	0	0
Top layer 3 (1.2g)	0	0	0	0
Bottom layer 1 (1.2g)	0	100	1	0
Bottom layer 2 (1.1g)	0	0	0	0
Bottom layer 3(1.0g)	0	0	0	0
CH18 ($15^0$22'46.0" and 028^{0}17'06.2")				
Top layer 1 (1.3g)	0	0	4	0
Top layer 2 (1.3g)	0	0	2	0
Top layer 3 (1.2g)	0	0	0	0
Bottom layer 1 (1.2g)	0	0	0	0
Bottom layer 2 (1.2g)	0	0	0	0
Bottom layer 3(1.4g)	0	0	0	0
CH19 ($15^0$22'48.3" and 028^{0}17'02.4")				
Top layer 1 (1.2g)	0	0	27	0
Top layer 2 (1.2g)	50 viable ova	0	22	0
Top layer 3 (1.4g)	50 viable ova	0	15	0
Bottom layer 1 (0.9g)	150 Non viable ova	0	10	0
Bottom layer 2 (1.0g)	50 Non viable ova	0	12	0
Bottom layer 3(1.1g)	0	0	11	0
CH20 ($15^0$22'48.8" and 028^{0}16'57.5")				
Top layer 1 (1.4g)	50 viable ova	0	17	0

Top layer 2 (1.0g)	200 viable ova	0	20	0
Top layer 3 (1.2g)	100 viable ova	0	26	0
Bottom layer 1 (1.2g)	0	0	2	0
Bottom layer 2 (1.2g)	200 Non viable ova	0	0	0
Bottom layer 3(1.3g)	0	0	3	0
CH21 (15022'52.6" and 028017'02.3")				
Top layer 1 (1.2g)	150 Non viable ova	50	16	0
Top layer 2 (1.0g)	0	100	8	0
Top layer 3 (1.0g)	0	50	13	0
Bottom layer 1 (1.3g)	200 Non viable ova	0	12	0
Bottom layer 2 (1.2g)	50 Non viable ova	0	13	0
Bottom layer 3(1.4g)	100 Non viable ova	150	9	0
CH22 ($15^0$22'55.1" and $028^0$17'04.5")				
Top layer 1 (1.3g)	450 viable ova	0	82	0
Top layer 2 (1.2g)	500 viable ova	0	71	0
Top layer 3 (1.2g)	700 viable ova	0	90	0
Bottom layer 1 (1.1g)	400 Non viable ova	0	60	0
Bottom layer 2 (1.4g)	150 Non viable ova	0	63	0
Bottom layer 3(1.1g)	350 Non viable ova	0	42	0
CH23 ($15^0$22'55.8" and $028^0$17'00.0")				
Top layer 1 (1.1g)	0	0	1	0
Top layer 2 (1.0g)	0	0	0	0
Top layer 3 (1.3g)	0	0	0	0
Bottom layer 1 (1.2g)	0	0	0	0
Bottom layer 2 (1.2g)	0	0	0	0
Bottom layer 3(1.2g)	0	0	0	0
CH24 ($15^0$23'00.9" and $028^0$16'59.5")				
Top layer 1 (1.0g)	0	0	14	0
Top layer 2 (1.3g)	0	0	13	0
Top layer 3 (1.3g)	0	0	17	0
Bottom layer 1 (1.0g)	0	0	1	150
Bottom layer 2 (1.0g)	0	0	2	50
Bottom layer 3(1.4g)	0	0	6	0
CH25 ($15^0$23'04.4" and $028^0$17'03.0")				
Top layer 1 (1.0g)	0	0	27	0
Top layer 2 (1.3g)	0	0	22	0
Top layer 3 (1.3g)	0	0	20	0
Bottom layer 1 (1.2g)	0	0	7	0
Bottom layer 2 (1.2g)	0	0	4	0
Bottom layer 3(1.4g)	0	0	9	0

		600	**2469**	**200**

Appendix viii

Pit Latrine Code/GPS Reading for George Compound and the Four Indicator Parasite Types with Significant Enumeration Indicating Ova Viability in Some Cases				
G01 ($15^0$22'47.8" and $028^0$14'54.4")	*Ascaris* species	*Strongyloides* species	*Cryptosporidium* species	*Enterobius* species
Top layer 1 (0.9g)	200 Non viable ova	0	67	0
Top layer 2 (1.4g)	100 Non viable ova	0	51	0
Top layer 3 (1.4g)	0	0	71	0
Bottom layer 1 (1.2g)	150 Non viable ova	0	52	0
Bottom layer 2 (1.1g)	450 Non viable ova	0	55	0
Bottom layer 3(1.4g)	150 Non viable ova	0	49	0
G02 ($15^0$22'56.0" and $028^0$14'50.4")				
Top layer 1 (1.0g)	0	0	11	0
Top layer 2 (1.4g)	0	0	18	0
Top layer 3 (0.9g)	0	0	16	0
Bottom layer 1 (1.3g)	0	0	4	0
Bottom layer 2 (1.4g)	0	0	6	0
Bottom layer 3(1.3g)	0	0	1	0
G03 ($15^0$23'02.8" and $028^0$14'48.3")				
Top layer 1 (1.1g)	0	0	22	0
Top layer 2 (0.9g)	0	0	19	0
Top layer 3 (1.3g)	0	0	11	0
Bottom layer 1 (0.9g)	0	0	12	0

Bottom layer 2 (1.0g)	0	0	18	0
Bottom layer 3(0.9g)	0	0	21	0
G04 ($15^0$23'10.0" and $028^0$14'48.0")				
Top layer 1 (0.9g)	0	0	24	0
Top layer 2 (1.4g)	0	0	18	0
Top layer 3 (1.4g)	0	0	21	0
Bottom layer 1 (1.1g)	0	0	11	0
Bottom layer 2 (1.1g)	0	0	11	0
Bottom layer 3(1.2g)	0	0	15	0
G05 ($15^0$23'25.0" and $028^0$14'44.5")				
Top layer 1 (1.3g)	0	0	101	0
Top layer 2 (1.3g)	0	0	124	0
Top layer 3 (1.3g)	0	0	93	0
Bottom layer 1 (1.3g)	0	0	80	0
Bottom layer 2 (1.0g)	0	0	86	0
Bottom layer 3(1.3g)	0	0	67	0
G06 ($15^0$23'22.6" and $028^0$14'32.6")				
Top layer 1 (1.4g)	50 viable ova	0	42	0
Top layer 2 (1.4g)	0	0	43	0
Top layer 3 (1.4g)	100 viable ova	0	49	0
Bottom layer 1 (1.3g)	0	0	19	0
Bottom layer 2 (1.1g)	0	0	24	0
Bottom layer 3(1.2g)	50 Non viable ova	0	26	0
G07 ($15^0$23'16.4" and $028^0$14'31.7")				
Top layer 1 (1.1g)	150 viable ova	0	35	0
Top layer 2 (1.3g)	0	0	32	0
Top layer 3 (1.3g)	0	0	29	0
Bottom layer 1 (1.3g)	0	0	37	0
Bottom layer 2 (1.0g)	50 Non viable ova	0	31	0
Bottom layer 3(1.2g)	150 Non viable ova	0	33	0
G08 ($15^0$23'13.4" and $028^0$14'35.2")				
Top layer 1 (0.9g)	0	0	20	0
Top layer 2 (1.4g)	0	0	24	0

Top layer 3 (1.4g)	0	0	19	0
Bottom layer 1 (1.3g)	0	0	14	0
Bottom layer 2 (1.1g)	0	0	16	0
Bottom layer 3(1.0g)	0	0	12	0
G09 ($15^0$23'05.7" and $028^0$14'36.2")				
Top layer 1 (0.9g)	0	0	33	0
Top layer 2 (1.4g)	0	0	34	0
Top layer 3 (1.0g)	0	0	23	0
Bottom layer 1 (1.3g)	0	0	15	0
Bottom layer 2 (1.1g)	0	0	11	0
Bottom layer 3(1.4g)	0	0	10	0
G10 ($15^0$23'03.4" and $028^0$14'39.0")				
Top layer 1 (1.0g)	200 viable ova	0	0	0
Top layer 2 (1.4g)	500 viable ova	0	0	0
Top layer 3 (1.3g)	450 viable	0	0	0
Bottom layer 1 (1.3g)	200 Non viable ova	150	0	0
Bottom layer 2 (1.0g)	0	50	0	0
Bottom layer 3(1.3g)	300 non viable ova	0	0	0
G11 ($15^0$22'52.8" and $028^0$14'40.8")				
Top layer 1 (1.0g)	0	0	41	0
Top layer 2 (0.9g)	0	0	32	0
Top layer 3 (1.3g)	100 viable ova	0	45	0
Bottom layer 1 (1.3g)	300 Non viable ova	0	6	0
Bottom layer 2 (1.0g)	150 Non viable ova	0	3	0
Bottom layer 3(1.0g)	400 Non viable ova	0	8	0
G12 ($15^0$22'47.3" and $028^0$14'38.1")				
Top layer 1 (1.0g)	0	0	15	0
Top layer 2 (1.4g)	300 Non viable ova	0	15	0
Top layer 3 (1.3g)	100 Non viable ova	0	11	0
Bottom layer 1 (1.4g)	0	0	8	0
Bottom layer 2 (1.0g)	0	0	2	0
Bottom layer 3(1.3g)	600 Non viable ova	0	6	0

G13 ($15^0$22'56.2" and $028^0$14'31.8")				
Top layer 1 (1.0g)	50 viable ova	0	5	0
Top layer 2 (0.9g)	100 viable ova	0	9	0
Top layer 3 (1.3g)	0	0	3	0
Bottom layer 1 (1.3g)	50 Non viable ova	0	0	0
Bottom layer 2 (1.0g)	50 Non viable ova	0	0	0
Bottom layer 3(1.0g)	50 Non viable ova	0	0	0
G14 ($15^0$23'02.9" and $028^0$14'24.2")				
Top layer 1 (0.9g)	0	0	3	0
Top layer 2 (1.4g)	0	0	0	0
Top layer 3 (1.2g)	0	0	3	0
Bottom layer 1 (1.3g)	0	0	0	0
Bottom layer 2 (0.9g)	0	0	0	0
Bottom layer 3(1.3g)	0	0	1	0
G15 ($15^0$23'12.2" and $028^0$14'21.0")				
Top layer 1 (1.1g)	50 viable ova	0	50	0
Top layer 2 (1.4g)	200 viable ova	0	55	0
Top layer 3 (1.3g)	100 viable ova	0	45	0
Bottom layer 1 (1.4g)	0	0	36	0
Bottom layer 2 (1.0g)	0	0	31	0
Bottom layer 3(1.2g)	0	0	26	0
G16 ($15^0$23'15.6" and $028^0$14'16.5")				
Top layer 1 (1.0g)	50 viable ova	0	12	0
Top layer 2 (1.1g)	150 viable ova	0	19	0
Top layer 3 (1.0g)	0	0	14	0
Bottom layer 1 (0.9g)	0	0	0	0
Bottom layer 2 (1.2g)	0	0	0	0
Bottom layer 3(1.1g)	0	0	0	0
G17 ($15^0$23'15.0" and $028^0$14'06.5")				
Top layer 1 (1.3g)	0	0	58	0
Top layer 2 (1.0g)	0	0	53	0
Top layer 3 (1.1g)	0	0	59	0
Bottom layer 1 (1.4g)	0	0	21	0

Bottom layer 2 (1.0g)	0	0	19	0
Bottom layer 3(1.4g)	0	0	16	0
G18 ($15^0$23'07.8" and 028^{0}14'08.5")				
Top layer 1 (1.1g)	100 viable ova	0	11	0
Top layer 2 (1.0g)	250 viable ova	0	17	0
Top layer 3 (1.4g)	50 viable ova	0	13	0
Bottom layer 1 (1.1g)	0	0	5	0
Bottom layer 2 (1.2g)	100 Non viable ova	0	0	0
Bottom layer 3(1.2g)	150 Non viable ova	0	7	0
G19 ($15^0$22'47.4" and 028^{0}14'15.1")				
Top layer 1 (1.1g)	0	0	2	0
Top layer 2 (1.2g)	0	0	0	0
Top layer 3 (1.4g)	0	0	4	0
Bottom layer 1 (1.2g)	0	0	0	0
Bottom layer 2 (1.1g)	0	0	0	0
Bottom layer 3(1.2g)	0	0	0	0
G20 ($15^0$22'56.7" and 028^{0}14'11.8")				
Top layer 1 (1.40g)	700 viable ova	600	20	0
Top layer 2 (1.2g)	900 Viable ova	350	10	0
Top layer 3 (1.1g)	1450 viable ova	50	16	0
Bottom layer 1 (1.4g)	150 Non viable ova	0	6	0
Bottom layer 2 (1.1g)	250 Non viable ova	0	4	0
Bottom layer 3(1.2g)	150 Non viable ova	0	0	0
G21 ($15^0$22'34.0" and 028^{0}14'13.0")				
Top layer 1 (1.1g)	0	0	152	0
Top layer 2 (1.0g)	0	0	161	0
Top layer 3 (1.2g)	400 viable ova	0	112	0
Bottom layer 1 (1.2g)	0	0	91	0
Bottom layer 2 (1.0g)	0	0	81	0
Bottom layer 3(1.2g)	50 Non viable ova	0	86	0
G22 ($15^0$22'45.7" and 028^{0}14'07.6")				
Top layer 1 (1.1g)	250 viable ova	0	6	0
Top layer 2 (1.4g)	100 viable ova	0	4	0

Top layer 3 (1.1g)	100 viable ova	0	0	0
Bottom layer 1 (1.0g)	150 Non viable ova	0	0	50
Bottom layer 2 (1.1g)	150 Non viable ova	0	0	100
Bottom layer 3(1.0g)	50 Non viable ova	0	1	0
G23 ($15^0$22'59.5" and $028^0$14'00.9")				
Top layer 1 (1.2g)	0	0	0	0
Top layer 2 (1.4g)	150 viable ova	0	15	0
Top layer 3 (1.0g)	400 viable ova	0	19	0
Bottom layer 1 (1.3g)	100 Non viable ova	0	6	0
Bottom layer 2 (1.2g)	200 Non viable ova	0	13	0
Bottom layer 3(1.4g)	50 Non viable ova	0	11	0
G24 ($15^0$23'08.6" and $028^0$13'58.4")				
Top layer 1 (1.0g)	100 Non viable ova	0	0	0
Top layer 2 (1.0g)	150 Non viable ova	0	0	0
Top layer 3 (1.2g)	0	0	0	0
Bottom layer 1 (1.4g)	0	0	0	0
Bottom layer 2 (1.1g)	0	0	0	0
Bottom layer 3(1.2g)	0	0	0	0
G25 ($15^0$23'12.9" and $028^0$13'54.6")				
Top layer 1 (1.1g)	0	0	56	0
Top layer 2 (1.0g)	0	0	51	0
Top layer 3 (1.3g)	0	0	67	0
Bottom layer 1 (1.2g)	0	0	42	0
Bottom layer 2 (1.1g)	100 Non viable ova	0	39	0
Bottom layer 3(1.0g)	50 Non viable ova	0	38	0
		1200	**3682**	**150**

Appendix ix

Pit Latrine Code/GPS Reading for Kanyama Compound and the Four Indicator Parasite Types with Significant Enumeration Indicating Ova Viability in some Cases				
K1(S:15o26'25.0'' and E:028o14'41.9"); Elevation: 1272m	*Ascaris* species	*Strongyloides* species	*Cryptosporidia* species	*Enterobius* species
Top layer 1 (1.2g)	50 Non viable ova	0	14	0
Top layer 2 (1.0g)	0	0	19	0
Top layer 3 (1.2g)	0	0	12	0
Bottom layer 1 (1.0g)	0	0	7	0
Bottom layer 2 (1.4g)	0	0	6	0
Bottom layer 3(0.9g)	0	0	3	0
K2 (S:15o26'24.1'' and E:028o14'31.1); Elevation: 1272m				
Top layer 1 (1.4g)	0	0	8	0
Top layer 2 (1.0g)	0	0	0	0
Top layer 3 (1.4g)	0	0	31	0
Bottom layer 1 (1.0g)	0	0	8	0
Bottom layer 2 (1.1g)	0	0	0	0
Bottom layer 3(0.9g)	0	0	13	0
K3 (S:15o26'33.6" and E:028o14'49.1"); Elevation: 1279m				
Top layer 1 (1.3g)	0	0	0	0
Top layer 2 (1.0g)	0	50	5	0
Top layer 3 (1.3g)	0	0	3	0
Bottom layer 1 (1.4g)	0	0	0	0
Bottom layer 2 (1.1g)	0	0	12	0
Bottom layer 3(1.1g)	0	0	6	0
K4 (S:15o26'46.1" and E28o14'42.6"); Elevation: 1272m				
Top layer 1 (1.3g)	50 Non viable ova	0	0	0
Top layer 2 (1.2g)	100 Non viable ova	0	2	0
Top layer 3 (1.0g)	50	0	0	0
Bottom layer 1 (1.2g)	0	0	0	0

Bottom layer 2 (1.0g)	50 Non viable ova	0	2	0
Bottom layer 3(1.0g)	0	0	3	0
K5 (S:15o26'42.8" and 028o14'38.7"); Elevation: 1278m				
Top layer 1 (1.3g)	200 Non viable	0	6	0
Top layer 2 (1.3g)	0	0	7	0
Top layer 3 (1.3g)	0	0	*9*	0
Bottom layer 1 (1.2g)	150 Non viable ova	0	4	0
Bottom layer 2 (1.0g)	100 Non viable ova	0	11	0
Bottom layer 3(1.2g)	0	0	*6*	50
K6 (S:15o26'40.6" and E:028o14'32.2"); Elevation: 1273m				
Top layer 1 (1.3g)	50 Non viable ova	0	*5*	0
Top layer 2 (1.1g)	0	0	*2*	0
Top layer 3 (1.4g)	0	0	*0*	0
Bottom layer 1 (1.2g)	50 Non viable ova	0	*6*	0
Bottom layer 2 (1.0g)	50 Non viable ova	0	*5*	0
Bottom layer 3(1.0g)	0	0	*1*	0
K7 (S:15o26'33.2" and E:028o14'27.0"); Elevation: 1271m				
Top layer 1 (1.1g)	50 Non viable ova	0	*1*	0
Top layer 2 (1.2g)	100 Non viable ova	0	*3*	0
Top layer 3 (1.2g)	50 Non viable ova	0	*15*	0
Bottom layer 1 (1.2g)	50 Non viable ova	0	*6*	0
Bottom layer 2 (1.3g)	0	0	*4*	0
Bottom layer 3(1.4g)	0	0	*1*	50
K8 (S:15o26'06.9" and E:028o14'24.7"); Elevation: 1280m				
Top layer 1 (1.0g)	0	50	*8*	0
Top layer 2 (1.2g)	50 Non viable ova	200	*11*	0
Top layer 3 (1.3g)	0	0	0	0
Bottom layer 1 (1.0g)	0	0	*13*	0
Bottom layer 2 (1.0g)	50 Non viable ova	0	*14*	0
Bottom layer 3(1.4g)	0	50	*9*	0
K9 (15o26'13.4" and 028o15'17.3"); Elevation: 1280m				
Top layer 1 (1.2g)	0	0	*13*	0
Top layer 2 (1.4g)	0	0	*5*	0
Top layer 3 (1.4g)	0	0	*11*	0
Bottom layer 1 (1.0g)	1150 Non viable ova	0	*6*	0
Bottom layer 2 (1.2g)	0	0	*0*	0
Bottom layer 3(1.2g)	0	0	*9*	0
K10 (S:15o26'14.9" and E:028o15'23.2"); Elevation: 1288m				
Top layer 1 (1.0g)	0	0	*0*	0

Top layer 2 (1.0g)	100 Non viable ova	0	*0*	0
Top layer 3 (1.0g)	0	0	*4*	0
Bottom layer 1 (0.9g)	50 Non viable ova	0	*0*	0
Bottom layer 2 (1.1g)	50 Non viable ova	0	*6*	0
Bottom layer 3(1.3g)	0	50	*3*	0
K11 (S:15o26'12.2" and E:028o15'20.4"); Elevation: 1284m				
Top layer 1 (1.3g)	0	0	*4*	0
Top layer 2 (1.1g)	0	0	*0*	0
Top layer 3 (1.2g)	50 Non viable ova	0	*2*	0
Bottom layer 1 (1.1g)	0	0	*1*	0
Bottom layer 2 (1.4g)	100 Non viable ova	0	*0*	0
Bottom layer 3(1.0g)	0	0	*0*	0
K12 (S:15o26'14.4" and E:028o15'14.7"); Elevation: 1284m				
Top layer 1 (1.4g)	0	0	0	0
Top layer 2 (1.2g)	0	0	0	0
Top layer 3 (1.2g)	0	0	0	0
Bottom layer 1 (1.1g)	0	0	0	0
Bottom layer 2 (1.0g)	0	0	0	0
Bottom layer 3(1.1g)	0	0	0	0
K13 (S:15o26'17.0" and 028o15'06.9"); Elevation: 1273m				
Top layer 1 (1.0g)	0	0	0	0
Top layer 2 (1.4g)	0	0	1	0
Top layer 3 (1.3g)	0	0	3	0
Bottom layer 1 (1.0g)	0	0	0	0
Bottom layer 2 (1.0g)	0	0	3	0
Bottom layer 3(1.0g)	0	0	7	0
K14 (S:15o26'20.2" and 028o15'05.0")Elevation: 1276m				
Top layer 1 (1.1g)	0	0	22	0
Top layer 2 (1.1g)	0	0	29	0
Top layer 3 (1.3g)	0	0	17	0
Bottom layer 1 (1.0g)	0	0	0	0
Bottom layer 2 (1.1g)	0	0	14	0
Bottom layer 3(1.0g)	0	0	14	0
K15 (15o26'16.9" and E:028o15'00.3"); Elevation:1276m				
Top layer 1 (0.9g)	500 Non viable ova	0	2	0
Top layer 2 (1.1g)	0	0	4	0
Top layer 3 (1.2g)	0	0	3	0
Bottom layer 1 (1.0g)	0	0	3	0
Bottom layer 2 (1.2g)	0	0	5	0

Bottom layer 3(1.4g)	0	0	0	0
K16 (S:15o26'18.5" and 028o14'55.3"); Elevation: 1279m				
Top layer 1 (1.4g)	0	0	7	0
Top layer 2 (1.0g)	0	0	9	0
Top layer 3 (1.2g)	50 Non viable ova	0	3	0
Bottom layer 1 (1.4g)	50 Non viable ova	0	8	0
Bottom layer 2 (1.4g)	50 Non viable ova	0	11	0
Bottom layer 3(1.1g)	50 Non viable ova	0	10	0
K17 (15o26'21.8" and 028o14'57.4"); Elevation: 1281m				
Top layer 1 (1.2g)	0	0	19	0
Top layer 2 (1.2g)	0	0	25	50
Top layer 3 (1.0g)	0	0	16	0
Bottom layer 1 (1.3g)	50 Non viable ova	0	18	0
Bottom layer 2 (0.9g)	50 Non viable ova	0	10	0
Bottom layer 3(1.4g)	50 Non viable ova	0	21	0
K18 (15o26'33.0" and 028o14'59.8"); Elevation: 1277m				
Top layer 1 (1.0g)	300 Non viable ova	0	0	0
Top layer 2 (1.g)	50 Non viable ova	0	*18*	0
Top layer 3 (1.3g)	200 Non viable ova	0	0	0
Bottom layer 1 (0.9g)	0	0	18	0
Bottom layer 2 (1.2g)	250 Non viable ova	0	0	0
Bottom layer 3(1.4g)	200 Non viable	0	0	0
K19 (15o26'10.3" and 028o14'51.3");Elevation: 1274m				
Top layer 1 (1.2g)	0	0	*7*	0
Top layer 2 (1.1g)	0	0	3	0
Top layer 3 (1.4g)	0	0	7	0
Bottom layer 1 (0.9g)	0	0	3	0
Bottom layer 2 (0.9g)	0	0	3	0
Bottom layer 3(1.2g)	0	0	1	0
K20 (S:15o26'10.3" and 028o14'45.3"); Elevation: 1284m				
Top layer 1 (1.4g)	0	0	3	0
Top layer 2 (1.0g)	0	0	4	0

Top layer 3 (1.2g)	0	0	7	0
Bottom layer 1 (0.9g)	0	0	2	0
Bottom layer 2 (1.4g)	0	0	2	0
Bottom layer 3(1.0g)	0	0	0	0
K21 (S:15o26'10.1" and E:028o14'39.1"); Elevation: 1285m				
Top layer 1 (1.1g)	0	0	2	0
Top layer 2 (1.2g)	0	0	0	0
Top layer 3 (1.3g)	0	0	4	0
Bottom layer 1 (1.0g)	0	0	3	0
Bottom layer 2 (1.1g)	0	0	0	0
Bottom layer 3(1.3g)	0	0	0	0
K22 (S:15o26' and E:028o14'); Elevation:1282m				
Top layer 1 (0.9g)	150 (100 of the ova were viable)	0	10	0
Top layer 2 (1.1g)	0	0	11	0
Top layer 3 (1.2g)	50 Non viable ova	0	8	0
Bottom layer 1 (0.9g)	0	0	4	0
Bottom layer 2 (1.0g)	100 (50 of the ova were viable)	0	2	0
Bottom layer 3(1.3g)	0	0	4	0
K23 (S:15o26'53.3" and E:028o14'37.7") Elevation: 1282m				
Top layer 1 (1.2g)	0	0	10	0
Top layer 2 (1.4g)	0	0	9	0
Top layer 3 (1.0g)	0	0	14	0
Bottom layer 1 (1.3g)	0	0	5	0
Bottom layer 2 (1.1g)	0	0	2	0
Bottom layer 3(1.2g)	0	0	6	0
K24 (S:15o25'57.6" and E:028o14'43.7") Elevation: 1276m				
Top layer 1 (1.3g)	50 Non viable ova	100	6	200
Top layer 2 (1.1g)	0	900	4	0
Top layer 3 (0.9g)	0	0	7	0
Bottom layer 1 (1.1g)	100 Non viable ova	450	0	50
Bottom layer 2 (1.1g)	0	0	8	0
Bottom layer 3(1.0g)	0	250	5	0
K25 (S:15o26'02.0" and E:028o14'50.5") Elevation: 1283m				
Top layer 1 (1.0g)	0	0	8	0
Top layer 2 (1.4g)	0	250	7	0

Top layer 3 (1.3g)	0	350	7	0
Bottom layer 1 (1.3g)	0	50	0	0
Bottom layer 2 (1.0g)	100 Non viable ova	200	12	0
Bottom layer 3(1.3g)	0	350	0	0
		3300	**885**	**400**

Appendix x

Pit Latrine Code/GPS Reading for Madimba Compound and the Four Indicator Parasite Types with Significant Enumeration Indicating Ova Viability in some Cases				
MD01 ($15^0$21'37.0" and $028^0$14'30.8")	*Ascaris* Species ova	*Strongyloides* species larvae	*Cryptosporidia* species protozoan oocysts	*Enterobius* species ova
Top layer 1 (0.9g)	0	0	21	0
Top layer 2 (1.3g)	0	0	2	0
Top layer 3 (1.4g)	0	0	27	0
Bottom layer 1 (1.1)	200 Non viable ova	0	9	0
Bottom layer 2 (1.0g)	100 Non viable ova	0	16	0
Bottom layer 3(1.4g)	150 Non viable ova	0	11	0
MD02 ($15^0$21'39.4" and $028^0$14'38.1")				
Top layer 1 (1.1g)	200 viable ova	0	8	0
Top layer 2 (1.4g)	0	0	16	0
Top layer 3 (0.9g)	300 viable ova	50	7	0
Bottom layer 1 (1.4g)	0	0	1	0
Bottom layer 2 (1.4g)	50 Non viable ova	0	3	0
Bottom layer 3(1.3g)	0	0	1	0
MD03 ($15^0$21'36.7" and $028^0$14'46.5")				
Top layer 1 (1.1g)	0	0	1	0
Top layer 2 (0.9g)	450 Non viable ova	0	0	0
Top layer 3 (1.2g)	50 Non viable ova	0	0	0
Bottom layer 1 (0.9g)	300 Non viable ova	0	0	0
Bottom layer 2 (1.0g)	0	100	0	0

Bottom layer 3(0.9g)	300 Non viable ova	0	0	0
MD04 (15^{0}21'37.6" and 028^{0}14'38.4")				
Top layer 1 (0.9g)	0	0	12	0
Top layer 2 (1.4g)	0	0	7	0
Top layer 3 (1.4g)	0	0	14	0
Bottom layer 1 (1.1g)	0	0	7	0
Bottom layer 2 (1.1g)	0	0	13	0
Bottom layer 3(1.2g)	0	0	4	0
MD05 (15^{0}21'36.6" and 028^{0}14'36.8")				
Top layer 1 (1.0g)	100 viable ova	100	11	0
Top layer 2 (0.9g)	550 viable ova	50	10	0
Top layer 3 (1.3g)	400 viable ova	100	3	0
Bottom layer 1 (1.0g)	150 Non viable ova	0	7	0
Bottom layer 2 (1.0g)	0	0	12	0
Bottom layer 3(1.0g)	50 Non viable ova	0	9	0
MD06 (15^{0}21'33.9" and 028^{0}14'32.1")				
Top layer 1 (1.4g)	150 Non viable ova	0	0	0
Top layer 2 (1.0g)	50 Non viable ova	0	0	0
Top layer 3 (1.4g)	0	0	0	0
Bottom layer 1 (1.3g)	0	0	0	0
Bottom layer 2 (1.0g)	200 Non viable ova	0	2	0
Bottom layer 3(1.2g)	0	0	2	0
MD07 (15^{0}21'32.2" and 028^{0}14'31.9")				
Top layer 1 (1.1g)	600 viable ova	150	7	0
Top layer 2 (1.2g)	0	200	9	0
Top layer 3 (1.3g)	450 viable ova	150	6	0
Bottom layer 1 (1.3g)	500 Non viable ova	0	17	50
Bottom layer 2 (1.1g)	0	0	0	50
Bottom layer 3(1.2g)	0	0	16	0
MD08 (15^{0}21'30.8" and 028^{0}14'30.9")				

Top layer 1 (0.9g)	0	0	17	50
Top layer 2 (1.4g)	0	0	0	0
Top layer 3 (1.0g)	0	0	16	0
Bottom layer 1 (1.3g)	0	0	4	0
Bottom layer 2 (1.4g)	0	0	9	0
Bottom layer 3(1.0g)	0	0	2	0
MD09 ($15^0$21'29.0" and $028^0$14'31.1")				
Top layer 1 (1.1g)	100 viable ova	0	7	0
Top layer 2 (0.9g)	0	0	5	0
Top layer 3 (1.3g)	0	0	113	0
Bottom layer 1 (1.2g)	0	0	17	0
Bottom layer 2 (1.0g)	50 Non viable ova	0	22	0
Bottom layer 3(1.0g)	0	0	0	0
MD10 ($15^0$21'31.6" and $028^0$14'36.1")				
Top layer 1 (1.4g)	0	0	5	0
Top layer 2 (1.4g)	0	0	11	0
Top layer 3 (1.3g)	0	0	19	0
Bottom layer 1 (1.3g)	0	0	0	0
Bottom layer 2 (1.4g)	0	0	3	0
Bottom layer 3(1.3g)	0	0	12	0
MD11 ($15^0$21'25.3" and $028^0$14'32.3")				
Top layer 1 (1.3g)	0	0	8	0
Top layer 2 (0.9g)	0	0	5	0
Top layer 3 (1.3g)	0	0	2	0
Bottom layer 1 (1.4g)	0	0	8	0
Bottom layer 2 (1.0g)	0	0	6	0
Bottom layer 3(1.0g)	0	0	11	0
MD12 ($15^0$21'27.0" and $028^0$14'35.4")				
Top layer 1 (1.1g)	0	0	128	0
Top layer 2 (1.2g)	0	0	114	0
Top layer 3 (1.1g)	0	0	133	0
Bottom layer 1	0	0	86	0

(1.4g)				
Bottom layer 2 (1.1g)	0	0	81	0
Bottom layer 3(1.0g)	0	0	32	0
MD13 ($15^0$21'27.5" and $028^0$14'39.1")				
Top layer 1 (1.3g)	0	0	27	0
Top layer 2 (0.9g)	0	0	14	0
Top layer 3 (1.3g)	0	0	31	0
Bottom layer 1 (1.3g)	0	0	16	0
Bottom layer 2 (1.2g)	0	0	24	0
Bottom layer 3(1.0g)	0	0	14	0
MD14 ($15^0$21'28.3" and $028^0$14'40.5")				
Top layer 1 (1.0g)	0	0	8	0
Top layer 2 (1.4g)	0	0	4	0
Top layer 3 (1.2g)	0	0	17	0
Bottom layer 1 (1.3g)	0	0	0	0
Bottom layer 2 (0.9g)	0	0	15	0
Bottom layer 3(1.3g)	0	0	12	0
MD15 ($15^0$21'19.2" and $028^0$14'36.4")				
Top layer 1 (1.1g)	0	0	47	0
Top layer 2 (1.4g)	0	0	38	0
Top layer 3 (1.4g)	0	0	30	0
Bottom layer 1 (1.0g)	0	0	27	0
Bottom layer 2 (1.1g)	0	0	21	0
Bottom layer 3(1.0g)	0	0	28	0
MD16 ($15^0$21'22.7" and $028^0$14'38.6")				
Top layer 1 (1.0g)	0	0	17	0
Top layer 2 (1.1g)	0	0	22	0
Top layer 3 (1.0g)	0	0	20	0
Bottom layer 1 (0.9g)	0	0	5	0
Bottom layer 2 (1.2g)	0	0	2	0
Bottom layer	0	0	11	0

3(1.1g)				
MD17 ($15^0$21'23.4" and $028^0$14'40.9")				
Top layer 1 (1.3g)	0	0	15	0
Top layer 2 (1.3g)	0	0	21	0
Top layer 3 (1.1g)	0	0	8	0
Bottom layer 1 (1.4g)	0	0	0	0
Bottom layer 2 (1.0g)	0	0	6	0
Bottom layer 3(1.0g)	0	0	13	0
MD18 ($15^0$21'22.5" and $028^0$14'43.2")				
Top layer 1 (1.1g)	0	0	17	0
Top layer 2 (1.4g)	0	0	14	0
Top layer 3 (1.g)	0	0	21	0
Bottom layer 1 (1.0g)	0	0	0	0
Bottom layer 2 (1.4g)	0	0	5	0
Bottom layer 3(1.0g)	0	0	6	0
MD19 ($15^0$21'18.0" and $028^0$14'37.8")				
Top layer 1 (1.3g)	350 Non viable ova	0	13	0
Top layer 2 (1.2g)	0	0	10	0
Top layer 3 (1.4g)	0	0	7	0
Bottom layer 1 (1.4g)	0	0	7	0
Bottom layer 2 (1.1g)	100 Non viable ova	0	2	0
Bottom layer 3(1.3g)	0	0	6	0
MD20 ($15^0$21'19.6" and $028^0$14'41.0")				
Top layer 1 (1.2g)	100 viable ova	0	18	0
Top layer 2 (1.2g)	300 viable ova	0	17	0
Top layer 3 (1.1g)	50 viable ova	0	29	0
Bottom layer 1 (1.g)	500 Non viable ova	0	16	0
Bottom layer 2 (1.1g)	400 Non viable ova	0	28	0
Bottom layer 3(1.3g)	350 Non viable ova	0	16	0
MD21 ($15^0$21'2048" and $028^0$14'43.4")				
Top layer 1 (1.1g)	250 viable ova	0	4	0

Top layer 2 (1.0g)	0	0	2	0
Top layer 3 (1.0g)	0	0	16	0
Bottom layer 1 (1.4g)	0	0	0	0
Bottom layer 2 (1.4g)	0	0	0	0
Bottom layer 3(1.2g)	0	0	0	0
MD22 ($15^0$21'22.1" and $028^0$14'45.4")				
Top layer 1 (1.3g)	0	0	0	0
Top layer 2 (1.4g)	0	0	0	0
Top layer 3 (1.2g)	0	0	0	0
Bottom layer 1 (1.0g)	0	0	0	0
Bottom layer 2 (1.4g)	0	0	0	0
Bottom layer 3(1.0g)	0	0	0	0
MD23 ($15^0$21'15.2" and $028^0$14'40.2")				
Top layer 1 (1.2g)	0	0	0	0
Top layer 2 (1.2g)	0	0	0	0
Top layer 3 (1.0g)	0	0	0	0
Bottom layer 1 (1.0g)	0	0	0	0
Bottom layer 2 (1.2g)	0	0	0	0
Bottom layer 3(1.1g)	0	0	0	0
MD24 ($15^0$21'20.4" and $028^0$14'48.7")				
Top layer 1 (1.2g)	0	0	15	0
Top layer 2 (1.4g)	0	0	24	0
Top layer 3 (1.3g)	0	0	17	0
Bottom layer 1 (1.0g)	0	0	12	0
Bottom layer 2 (1.4g)	0	0	7	0
Bottom layer 3(1.0g)	0	0	4	0
MD25 ($15^0$21'23.6" and $028^0$14'49.7")				
Top layer 1 (1.2g)	0	0	0	0
Top layer 2 (1.2g)	0	0	2	0
Top layer 3 (1.0g)	0	0	0	0
Bottom layer 1 (1.1g)	0	0	0	0

Bottom layer 2 (1.2g)	0	0	0	0
Bottom layer 3(1.3g)	0	0	0	0
		900	**2015**	**150**