PREVALENCE, MOLECULAR CHARACTERIZATION AND RISK FACTORS ASSOCIATED WITH INTESTINAL PARASITES AMONG SCHOOL GOING CHILDREN FROM INFORMAL SETTLEMENTS OF NAKURU TOWN, KENYA

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DECLARATION AND RECOMMENDATION

DECLARATION

This thesis is my original work and has not been presented in this university or any other for the award of a degree.

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RECOMMENDATION

This thesis has been submitted with our approval as University supervisors.

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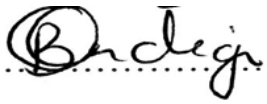
Signature  Date: 10th May 2021

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DEDICATION

I dedicate this thesis to the Almighty God who has been my source of knowledge, hope, strength and good health in accomplishing this study mission. Making it this far, I give God all glory and honour.

I dedicate this thesis to my siblings Margaret Chege and Daniel Chege for their insight and continued believe in me especially during my graduate studies. They have consistently reminded me that no matter how large a task is, it can be accomplished when done one step at a time. Their active support and words of encouragement at times when it seemed impossible to continue has helped me a great deal.

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Finally, I wish to dedicate this work in memory of my loving parents John Chege and Tabitha Mwangi who always believed in my ability to be successful in the academic arena. Although gone, your belief in me has made this journey possible.

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ABSTRACT

Health and economic impact of intestinal parasitic diseases is huge. These diseases are prevalent in regions with poor or inadequate sanitation and sewerage facilities mainly in developing and underdeveloped countries. While most of rural areas have been the focus of national control strategies, urban informal centers have recently emerged as disease foci. However, transmission dynamics in these centers is different from rural foci, making deployment of standard control strategies of provision of clean water, sanitation, and hygiene (WASH) potentially inadequate. Consequently, understanding disease transmission dynamic in urban informal centers is important in deployment of appropriate control strategies. This was a cross-sectional study to determine prevalence of intestinal parasites IPs (soil transmitted helminths (STH) and intestinal protozoan parasites) and associated risk factors in school-going children between the ages of 8-13 years from informal centres in Nakuru town, Kenya conducted 2018. Children from Kaptembwa, Milimani and Prisons primary schools were studied. Socio-demographic variables were collected using a pre-tested structured questionnaire to inform on associated risk factors. A total of 248 stool samples from randomly selected pupils were screened for soil transmitted helminths (STH) using microscopy. A random subset of stool samples (n=96) were also screened for intestinal protozoan parasites using polymerase chain reaction (PCR). The overall prevalence of STH and intestinal protozoan parasites was 1.2%, (n=3) and 41.7% (n=40) respectively. Each school had one case of STH infection. Intestinal protozoan parasites infections prevalence by school was Kaptembwa 47.5% (n=19), Prisons 32.5% (n=13) and Milimani 20% (n=8). Multiple infections with STH and intestinal protozoan parasites were 0.4% (n=1) and 5.2% (n=5) respectively. Risk factors for STH and intestinal protozoan parasites included rearing of goat (p=0.046), earthen floor type (p=0.022), number of household rooms (p=0.035), and source of food (p=0.016). Additionally, sourcing of water from water vendors (p=0.003) and living > 1km from school (p=0.013) were important risk factors to infection in Prisons primary school children. The low prevalence of IPs could be attributed to improvements in sanitation, hygiene, and good health practices.

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LIST OF ABBREVIATIONS AND ACRONYMS

A	Absorbance
AIDs	Acquired Immunodeficiency Syndrome
CMR	Centre for Microbiology Research
DALY	Disability-Adjusted Life Year
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
EPG	Eggs Per Gram
GAHI	Global Atlas of Helminth Infections
GBD	Global Burden of Disease
GDH	Glutamate Dehydrogenase
GIS	Geographical Information System
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
IPIs	Intestinal Parasitic Infections
ID	Identification
IDA	Iron Deficiency Anaemia
IQ	Intelligence Quotient
IL	Interleukin
KEMRI	Kenya Medical Research Institute
MDA	Mass Drug Administration
NTDs	Neglected Tropical Diseases
NHPs	non-human primates
PCR	Polymerase Chain Reaction
RNA	Ribonucleic acid
SAC	School-Aged Children
SERU	Science and Ethics Review Unit
SNPs	Single Nucleotide Polymorphisms
SPSS	Statistical Package for Social Sciences
SSU rRNA	Small Subunit ribosomal Ribonucleic acid
STH	Soil Transmitted Helminths
TDS	*Trichuris* dysentery syndrome

TAE	Tris base, acetic acid and Ethylenediaminetetraacetic acid
TE	Tris-Hydrochloric acid and Ethylenediaminetetraacetic acid
TH-2	Helper T cell 2
UK	United Kingdom
UN	United Nations
UNICEF	United Nations Children Funds
USA	United States of America
WASH	Water quality, Sanitation and Hygiene
WHO	World Health Organisation

CHAPTER ONE
INTRODUCTION

1.1 Background information

Intestinal parasites (IPs) affect over 3.5 billion people worldwide. In developing countries, they rank among the top ten major public health problem and the main cause of diarrhoea in children. Cases of diarrhoea and mortalities attributed to IPs are approximately 800 million and 4.5 million respectively (Tigabu et al., 2010). The endemicity and impact of IPs in tropical and subtropical regions, have attracted an increasing interest among health researchers and donors. Notably, provision of clean water and sanitation, is among United Nations (UN) sustainable development goals (SDGs), towards amongst others, help improve hygiene and control of IPs infections. Another control measure applied is mass deworming of school going children who are disproportionately affected. Intestinal parasitic infections cause mild to severe medical complications, impair physical and mental development of children, promote absenteeism of children in schools and their socio-economic development (Miller et al., 2003). Epidemiology-based surveys have revealed that occurrence of these parasites is influenced by poverty, poor personal and environmental hygiene, inadequate health services access and illiteracy in parasite life cycle and transmission mechanisms (Brooker et al., 2009; Alum et al., 2010). These occur in rural and urban informal settings in developing countries. If hygiene is not upgraded with increased urbanisation, the infection burden is expected to rise significantly.

Rural-urban migration due to economic constraints has caused growth of informal settlements. Low-cost housing attracts low-income earners in the cities. This results to overcrowded unhygienic conditions, with limited access to government sponsored healthcare, administrative and public sanitary services (Dana, 2011; Nguyen et al., 2015). The poor drainage systems, piles of uncollected garbage, indiscriminate disposal of excreta, such as wrap and throw and shared pit latrines pose increased health risks to the inhabitants (Shobha et al., 2013). Contamination of water sources and food with pathogens for example Soil transmitted helminths (STH), *Entamoeba histolytica* and *Giardia intestinalis* leads to risk of contraction by humans through accidental ingestion and skin penetration (Amuta et al., 2010; WHO, 2011). Their impact is further enhanced by unavailability of treatment for some protozoan diseases. In addition, some parasites remain highly resistant to water treatment procedures such as chlorination and other commonly used antiseptics (Singh et al., 2009). Their spread is intensified by asymptomatic carriers who transmit the pathogens contributing to difficulty in control hence increased disease burden.

Intestinal parasites affect persons of all ages. According to World Health Organisation (WHO), pre-school and school-aged children are disproportionately at risk of infection because of less developed immune systems and increased nutritional requirements (WHO, 2012). Moreover, their hyperactivity and behavioural factors such as, playing in contaminated fields and random eating habits increase their likelihood to infections (Houmsou et al., 2016; Steinbaum et al., 2016). Over 270 million pre-school children and an estimated 600 million school-going children living in the tropical and subtropical regions, are at risk of intestinal parasite infections (WHO, 2010). Approximately 89.9 million school-aged children in the sub-Saharan Africa are reported to harbour at least one soil transmitted helminth (STH) (Brooker et al., 2006). In Kenya, out of 12 million people reported to be at risk of STH infection, approximately 20% are children of school-going age (Kihara et al., 2011; Mwinzi et al., 2012). This prevalence is likely an underestimate since the burden in some regions of Kenya is unknown.

The WHO had a target to eradicate morbidities related to STH by 2020 through regular deworming, clean water provision and sanitation and with behavioural change (WHO, 2012). Great improvements have been witnessed in use of these strategies, but the target has not been achieved. Mass deworming program as an example, has significantly decreased STH prevalence (Andereck et al., 2014). Subsequently, high infection levels have been reported in regions initially not considered at risk (Davis et al., 2014; Suchdev et al., 2014) which threaten the net effect of mass deworming. The rapid urbanisation, limited development of infrastructure and service delivery are not keeping pace with the growth of urban centres and consequently there is poor access to sanitation as population increases. There is an urgent need to evaluate the vulnerability of these regions to IPs infections and possibility of their inclusion in the mass deworming programs. Therefore, this study investigated prevalence of IPs and their potentially associated risk factors in informal settlements in Nakuru town that is not considered for national mass deworming program. The study estimated the prevalence, intensity and risk factors associated with IPs infections among school-going children in informal settlements of Nakuru town. Information generated from this study highlights key areas that require public health interventions to interrupt transmission of IPs.

1.2 Statement of the problem

Rapid rural-urban migration due to poverty has contributed to increase of informal settlements (slums) in towns and cities. However, corresponding development of required social and structural amenities is lacking. In Kenya, for example, it is approximated that the number of people in urban centres will exceed those in rural areas (World Bank Report, 2019). Further, 61% of city inhabitants reside in informal settlements in Kenya (UN-Habitat, 2016). Consequently, informal settlements are uninhabitable due to overcrowding and poor hygiene and sanitation. Access to the limited urban council services such as clean and adequate water and sewerage and garbage collection is limited or absent due to poor infrastructure. Such unhygienic conditions are a health risk, more so to pre-school and school-going children who suffer the most severe morbidities. Notably, risk of contracting pathogens responsible for gastrointestinal diseases such as intestinal helminths and protozoa that thrive well in unhygienic environments and transmitted faecal-orally is high. Implementation of appropriate control strategies, therefore, requires accurate knowledge more so where asymptomatic cases occur. The asymptomatic hosts form larger parasite reservoir to permit persistent pathogen transmission. Despite the availability of effective and safe drugs for intestinal parasites, lack of accurate information on the disease burden and transmission dynamics in urban informal centres, hugely limits the effectiveness of deployed control strategies. Therefore, there is need to assess the status of IPs in urban informal settlements to inform on appropriate and effective disease control.

1.3 Objectives

1.3.1 General objective

To determine the prevalence of intestinal parasites and risk factors among school-going children from informal settlements in Kenya, 2018.

1.3.2 Specific objectives

i. To determine the prevalence of IPs among school-going children in informal settlements of Nakuru town, Kenya.
ii. To determine IPs co-infection rates among the school-going children.
iii. To determine the risk factors associated with IPs infections.
iv.

1.4 Hypotheses

i. Intestinal parasites are not prevalent among school-going children in informal settlements of Nakuru town, Kenya.

ii. There are no IPs co-infections among the school children.

iii. There are no risk factors associated with IPs infections among school children.

1.5 Justification of the study

Intestinal parasites are among the most preventable diseases. They are disproportionately endemic among the poorest countries and contribute to economic instability and social marginalization. Children especially those residing in informal settlements of major urban centres of Africa and other developing countries suffer the most severe morbidities with a potential consequence on their physical, psychological and mental health and subsequently poor quality of life and short life expectancy. Despite the impact these infections have on health, growth, and development of children in these regions, there is no firm policy that exists on control of IPs infections in urban informal settlements. Insight on prevalence, and transmission dynamics of IPs is important in formulation and deployment of appropriate foci-based control strategies based on national and WHO guidelines, which are unavailable for majority of informal settlements. Monitoring of IPs infections in school children is essential for improvement of their health outcomes and academic performance and advice the Ministry of Education and Health for the necessary interventions. In addition, this investigation will reveal indirect impact of increased rural to urban migration on health more so for Neglected tropical diseases (NTDs) that are a target for global elimination and eradication. The insights gathered will inform not only on control, but also development of models that can allow control and management of other old and novel infectious diseases caused by such undertaking. This will also offer a strong case for increased investment in urban and peri-urban healthcare systems, sanitation, clean water, and surveillance. In sum, understanding the prevalence of IPs in informal settlements will inform on transmission dynamics and allow knowledge-based deployment of appropriate control strategies.

CHAPTER TWO
LITERATURE REVIEW

2.1 Epidemiology of intestinal helminths

Soil transmitted helminths (STH) are among the most prevalent of chronic human infections. They have a worldwide distribution and are estimated to infect, approximately 1.7 billion people (Baker et al., 2013a). They are mostly endemic in tropical and sub-tropical areas and over 50% the cases reported globally are experienced in South Asia and Sub-Saharan Africa (WHO, 2006). Global Burden of Disease (GBD) study reported that STHs cause approximately 4.98 million disability adjusted years (DALYs) (Baker et al., 2013b). Chronic, moderate to heavy infection intensities have been linked to anaemia, malnutrition, educational loss and cognitive defects (Miller et al., 2003). Asymptomatic cases are also widely reported in subjects with light or moderate intensity (Brooker et al., 2000). Their estimated mortality rate of 12,000-150,000, is minimal compared to other parasitic diseases for example Malaria (Crompton, 1999). This may be the reason why they receive less attention from donors and have been grouped together as NTDs (Hotez et al., 2007). However, the morbidities caused by STH, including malnutrition, impaired physical growth and cognitive development, in pre-school and school-aged children especially in endemic regions like informal settlements of urban centres have attracted the attention of researchers and major health stakeholders.

The most common STHs are *Ascaris lumbricoides, Trichuris trichuris* and hookworm (*Necator americanus* and *Ancylostoma duodenale*) with global infection estimates at 819, 464.6 and 438.9 million respectively (Pullan et al., 2014). According to WHO, children suffer the most burden. About 20% of the global burden of STH is observed in children between the ages of 5 and 14 years (Hailegebriel, 2017), the age group targeted for anthelminthic treatment. About 870 million children are reported to live in areas where STH infections are endemic increasing their likelihood to infection (Brooker *et al.,* 2015). Soil transmitted helminths were previously reported to be predominant in rural areas (Pullan et al., 2011) but are increasingly being reported in urban areas attributed to low socio-economic status, poor sanitary conditions, and improper water supply. *Ascaris lumbricoides, Trichuris trichuris* have increasingly been reported in urban areas and hookworms remain persistently endemic in rural areas (Ngonjo et al., 2012). The high numbers of asymptomatic carriers result in persistent disease endemicity especially in regions with poor sanitation and hygiene are poor.

2.2 Epidemiology of intestinal protozoa

Protozoan infections are a serious public health concerns throughout the globe and are responsible for severe morbidity and mortality (Petri, 2000). They have a worldwide distribution and are particularly common in the tropics and sub-tropics. There are several intestinal protozoa that cause diarrhoea, however, *E. histolytica, G. intestinalis* and *Cryptosporidium species* are the most important (Putignani & Menichella, 2010). They are also the protozoa parasitic species closely associated with socio-economic status, poor sanitation, inadequate medical care, and absence of safe drinking water supplies (WHO, 2009). The 2010 global burden of disease (GBD) reported that *G. intestinalis* and *E. histolytica* account for 0.17 and 0.5 million DALYs, respectively. The median number of cases were also estimated to be 184 and 104 million for giardiasis and amoebiasis respectively (Turkeltaub et al., 2015).

2.2.1 Epidemiology of *Giardia intestinalis*

Giardiasis is the most frequently reported intestinal protozoan disease in the world causing about 280 million symptomatic cases and 2.5 million deaths annually (Kalyoussef & Goldman, 2010). It is reported to infect over 200 million people globally and additional 500,000 new cases each year. The global prevalence in humans ranges between 20-30% in developing countries and 1-8% in developed countries (Alum et al., 2010). Its socio-economic implications and impact among children in developing countries has resulted to its inclusion as an NTD (Osman et al., 2016). Infections with *G. intestinalis* are usually acquired during infancy and they reach up to 30% in children younger than ten years (Laishram et al., 2012). In fact, *Giardia* is the most implicated in non-bacterial diarrhoea in children below the age of 5 years (Mbae et al., 2013). It is a non-invasive parasite that adhere to and colonise the upper small intestine causing acute watery diarrhoea (Miller et al., 2003).

Giardia is perceived to be a re-emerging infection related to travelling and waterborne outbreaks especially in developing countries, where they are common and often underestimated (Cacciò et al., 2005). Its management and treatment have been limited by its resistance to chlorination and ozonolysis and is its viability on cold water surfaces (Singh et al., 2009). Additionally, *G. intestinalis* also has a wide range of vertebrates including pets, livestock, wildlife, and marine animals which act as reservoirs to human infections through close interactions (Cotton et al., 2015). Moreover, children who are known to suffer the most severe morbidities, those living in highly endemic regions are frequently asymptomatic. Unexpectedly, inhabitants of regions with high rates of faecal water contamination and poor

sanitation and hygiene for example in informal settlements of urban areas have higher prevalence rates but few attributable symptoms (Bouwmans et al., 2016). This increases the rate of transmission of infections without their knowledge and contributes to persistence of disease in informal setting of urban centres.

2.2.2 Epidemiology of *Entamoeba histolytica*

Amoebiasis is the second leading cause of death among parasitic diseases after malaria (Stauffer & Ravdin, 2003). The pathogenic species *E. histolytica* is distributed throughout the world and is a major risk in almost all countries where barriers between human faeces, food and water are inadequate. The invasive amoebiasis is more prevalent in sub-Saharan Africa, China, South-East Asia, Mexico, and some parts of South America (Rine et al., 2013). It is estimated to infect 450 million persons each year, leading to approximately 100,000 deaths annually (Petri et al., 2000). Its annual incident rate is over 50 million cases of which about 90% remain asymptomatic (Stanley, 2003). Previously, asymptomatic cases were related to the non-pathogenic species of *Entamoeba spp.*, *E. dispar* and *E. moshkoviskii*, but recent reports confirm *E. histolytica* in both symptomatic and asymptomatic subjects (Ayed et al., 2017). Those who develop symptoms suffer severe gastrointestinal disease that manifest as amoebic colitis or amoebic liver disease (Samie et al., 2012).

Entamoeba histolytica has been reported as an important pathogen in paediatric diarrhoea. More than a third of all children presenting with diarrhoea test positive for *E. histolytica* (Turkeltaub et al., 2015). Infants under a year old rarely get infected, but amoebiasis incidence increases gradually during childhood and reaches its peak in young adulthood (Rine et al., 2013). In Kenya, amoebiasis is prevalent and widespread with prevalence ranging between 0.4 - 44.6% (Table 1). It is an important health problem for children of school-going age recording the highest prevalence attributed to school overcrowding and poor personal hygiene (Otula et al., 2005). Children from informal urban settlements have also recorded high prevalence of *E. histolytica* attributed to the unhygienic conditions of their home residence (Oyiengo et al., 2012). Higher prevalence of *E. histolytica* has also been reported in school-going children residing in informal settlements of Thika (Ngonjo et al., 2012) however, the prevalence information is unavailable for other major urban informal settlements to facilitate the implementation of control intervention.

Table 1: Summary of published studies on prevalence of *Entamoeba histolytica* infections in Kenya among different subjects.

Place	Prevalence	Subjects	References
Kibera	15.2%	Children 2-5years	Oyiengo et al. (2012)
Mukuru	36.7%	Children presenting with diarrhoea	Mbae et al. (2013)
Mathare	19.5%	Children presenting with diarrhoea	Garmie et al. (2016)
Nairobi	10.9%	HIV+ patients	Kirui et al. (2011)
Kyuso	41.2%	School children	Kavili et al. (2016)
Thika	14.6%	School children	Ngonjo et al. (2012)
Bondo	44.6%	School children	Otula et al. (2005)
Muthithi	44.6%	School-going children	Kamande et al. (2015)
Webuye	11.2%	Children ≤5years	Obala et al. (2013)
Mwea	>1%	Pre-schooler's	Sakari et al. (2017)

2.3 Transmission

Different modes of IPs transmission exist. STHs. The process of their transmission involves three major life cycles: the direct, indirect, and percutaneous (Figure 1). The direct life cycle exhibited by *T. trichuris* involves transmission from the soil into the digestive system where maturation of the larvae occurs and later shed in faeces into the soil. Indirect life cycle is similar to the direct life cycle but involves migration of the larvae through the circulatory and respiratory systems, before final migration into the digestive system, where maturation occurs as shown by *Ascaris lumbricoides*. The percutaneous life cycle is exhibited by hookworms. It involves penetration through the skin and migration through the circulatory system, respiratory system and finally into the digestive system where maturation occurs (Brooker et al., 2000).

Intestinal protozoa mode of transmission is direct. Mature cyst of *E. histolytica* and *G. intestinalis* are ingested in contaminated foods and water and are taken to the intestines where they develop into trophozoites and are later released into the large intestines where they live, cause disease and passed through faeces into the environment (Gabbad et al., 2014).

Asymptomatic individuals pass cysts in their stool while those showing symptoms/diarrhoea release trophozoites. In some patients with *E. histolytica* infection however, extraintestinal pathologic manifestation of disease are reported where trophozoites migrate from the intestinal mucosa through the blood, to the liver, brain and lungs and cause

disease. Transmission of these IPs may occur through accidental ingestion in food and water, through skin penetration or zoonosis.

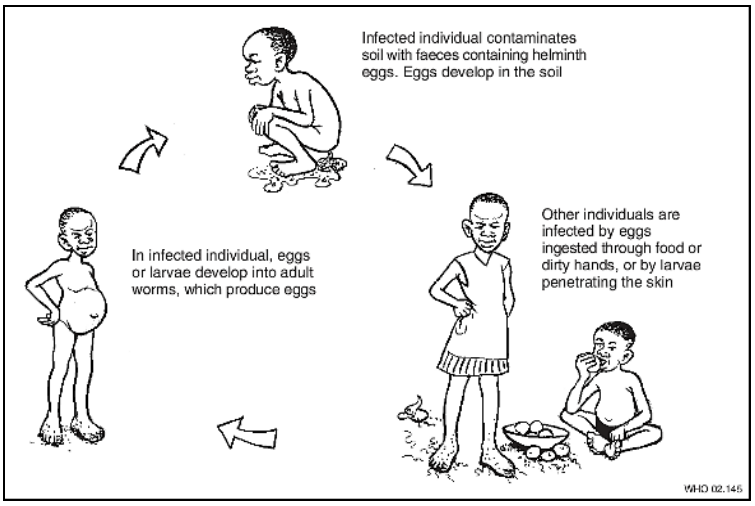

Figure 1: An illustration of how faecal-oral route and skin penetration transmit intestinal parasites from one individual to another. This figure is adopted from Montresor et al. (2002).

2.3.1 Ingestion

Faecal-oral route is the major transmission portal for IPs infection (Figure 1). Contamination of food and water sources with faecal waste containing the infectious larvae and cysts from infected individuals is very frequent in regions where sanitation is poor. Some of the most common water and foodborne pathogens are IPs including C*ryptosporidium spp*., *Giardia intestinalis*, *Cyclospora cayetanensis*. *Giardia intestinalis* is reported to cause approximately 28.2 million cases of diarrhoea annually through ingestion of contaminated food especially raw vegetables and fruits (Kalyoussef & Goldman, 2010). Some of these intestinal parasite's eggs and cysts are very sticky and attached easily to fruits and vegetables and may be easily ingested when they are eaten raw in cases of poor food hygiene (Kamande et al., 2015). On the other hand, clean food may be contaminated by food service worker through improper handling and poor personal hygiene practices (Nyarango, 2008; see also Kamau et al., 2012; Biwott & Ngeiywa, 2014; Gamar et al., 2018).

2.3.2 Skin penetration

Hookworm (*Ancylostoma duodenale* and *Necator americanus*) infection occur mainly through skin penetration by the infective filariform larvae passed from an infected person's stool (WHO, 2011). Some of the highest rates of hookworm transmission occur in the world's coastal regions, where infective third-stage larvae can migrate freely in sandy soils and temperatures and moisture are optimal for larvae viability. Feet and hands appear to be the

major sites of entry for the infective larvae, although some studies have shown that the entire body is vulnerable (Hotez et al., 2004). The stable environmental reservoirs of infective cases complicate eradication of hookworm.

2.3.3 Zoonosis

In rural areas, close contact between animal and humans is part of natural living conditions especially in regions where livestock raising is of economic importance. Similarly, contamination of urban informal settlements streets with animal faeces from dogs, cats and other domestic animals harbouring infective parasitic forms is frequent representing a high risk of infection for the people living in those areas (Xiao & Feng, 2008). *Giardia intestinalis* parasitizes a wide range of mammalian host, such as ruminants, marsupials, rodents and primates may act as important reservoirs of human infections in regions where sanitation is not observed (Bradley & Jackson, 2005). Sticky eggs and oocyst have also been reported to attach to the exoskeleton of houseflies and are transported around where they contaminate food and increases chances of infection. Due to the congested houses, land cover and unhygienic conditions of informal urban settlements, transmission of IPs from domestic animals through close contact with them is expected to be high.

2.4 Implications of intestinal parasitic infections

Intestinal parasites are associated more with morbidity than mortality. Study reports show that people refer IPs as minor diseases that do not require urgent attention (Odhiambo et al., 2014). This is because they remain asymptomatic for long periods of time and, individuals may take up to 10weeks or more before showing any symptoms. Morbidity is associated with intestinal parasite burden rather than the presence or absence of infection (Silver et al., 2018). According to the WHO, appearance of symptoms is also influenced by inoculum size, duration of infection, and host health status and parasite factors (WHO, 1987). The disease burden of IPs relates to chronic and insidious effects on the host health, development and nutritional status.

2.4.1 Nutritional anaemia

The prevalence of anaemia among school children constitutes another public health problem. The World Health Organization (WHO) estimates that school children from low-income countries are the group most severely affected by anaemia (WHO, 2015). Although anaemia is a multifactorial disease, it is not a coincidence that helminths are prevalent in

areas where iron is deficient. Helminth infections are reported to influence iron status through two keyways; reducing the nutrient uptake and interfering directly or indirectly with its metabolism and transport (Robertson et al., 1992). Hookworm infections has been implicated in low haemoglobin and serum ferritin levels which could be explained by chronic intestinal blood loss by its presence in the intestines and by its production of anticoagulant substances which further enhance blood loss (Olsen et al., 1998; Khambalia et al., 2011). It has been reported that *A. duodenale* cause higher amounts of blood loss (0.14-0.4ml) compared to *N. americanus* (0.01-0.03ml) (Parija et al., 2017).

Heavy co-infection of hookworm with *T. trichuris* is associated with extensive loss of blood compared to either of the infections attributed to the pathogen's positioning in the intestines. Adult hookworm inhabits the upper small intestine, and this allows iron to be re-absorbed back as the blood flow down the rest of the gastro-intestinal tract (Crompton, 2000). *Trichuris trichuris* inhabits the colon and re-absorption of iron from blood lost from its invasion is completely impaired (Robertson et al., 1992). Heavy infections with *T. trichiura* cause *Trichuris dysentery syndrome* (TDS) that affect the iron status mainly through loss of erythrocytes from the gut (Robertson et al., 1992; Ok et al., 2009). An estimated 0.005 mL/day/worm of blood is lost from *T. trichuris* infection making it a significant predictor of anaemia for children in Panama (Ngui et al., 2012), Kenya (Brooker et al., 1999) and Malyasia (Osazuwa et al., 2011).

Entamoeba histolytica have also being linked to low haemoglobin levels. Excessive amounts of blood are lost due to disruption of the protective layer overlying the colon mucosa often associated with diarrhoea (Zeki & Al-Warid, 2019). Multiple infections regardless of being light or heavy also increases the chances of anaemia. The rate at which they are able to cause anaemia is dependent on host iron stores, number of pregnancies, the infecting species, intensity and duration of infection and any other concurrent infections (WHO, 2015). Anaemia subsequently impacts on children's physical development, school attendance and their learning abilities.

2.4.2 Diarrhoea and gastrointestinal manifestations

Intestinal parasites are known to be major causes of diarrhoea in developing countries (Coulibaly et al., 2018). Protozoan parasites including *G. intestinalis*, *E. histolytica* and *Cryptosporidium parvum* are the most common causes. Giardiasis and amoebiasis causes acute diarrhoea which can progresses to chronic diarrhoea if not treated while *Trichuriasis* causes persistent chronic diarrhoea (Bethony et al., 2006; Samie et al., 2012). The most

frequent mechanism of diarrhoea is exudation caused by inflammation, ulceration or cellular infiltration of the intestinal mucosa resulting from presence of intestinal parasite (WHO, 2006). *Entamoeba histolytica* produce toxin that may enhance intestinal ulcerations resulting to epithelial bleeding and colitis, bloody diarrhoea, weight loss, fever, gastrointestinal obstruction, and peritonitis (Laughlin & Temesvari, 2005). Extraintestinal symptoms from invasion of *E. histolytica* include abscesses in the liver that may rupture into the pleural space, peritoneum, or pericardium. *Trichuris trichiura*, like *E. histolytica,* leads to abdominal colitis that resembles bowel syndrome associated with diarrhoea and abdominal pain (Parija et al., 2017). *Ascaris lumbricoides,* hookworm and *T. trichiura* has been reported to interfere with digestion and absorption of dietary protein resulting to incomplete digestion and subsequently nutritional oedema syndrome in young children (Albright & Basaric-Keys, 2006; Parija et al., 2017). Infection with *G. intestinalis* may cause nausea, vomiting, malabsorption, diarrhoea, steatorrhea, malaise, mucus, constipation, perianal itching, salivation, fatigue and extensive weight loss (10-20%). The symptoms result from brush border enzyme deficiency and invasion of the intestinal wall by *G. intestinalis* (Gabbad et al., 2014; Doni et al., 2015). The symptoms may persist if the condition is not treated.

Competition for vitamin (A, B_6, B_{12}), mineral (magnesium, calcium and iron) and blockage of nutrient absorption by the intestinal parasitic infection is another fatal strategy that diminishes individual's immunity and predispose them to serious organ damage and sometimes organ death (Bethony et al., 2006). Studies have reported that children may acquire helminth infection early in life which may cause an initial organ damage that can remain subclinical for years and manifest later, in adulthood (WHO, 2006).

2.4.3 Cognitive impairment

Several studies have examined the association between IPs and children academic performance and cognitive function. Some conclusions dispute availability of any evidence that cognitive function is affected by STH while others suggest that the effect on cognition could be even greater than what is reported. The disputes are based on sufficiency of the strategies used to corelate infections to cognitive performance. However, all conclusions agree that control of intestinal parasitic infections are beneficial to children's cognitive performance (Guernier et al., 2017). Improvements in cognitive performance have been previously witnessed with treatment of STH infections (Stephesons et al., 2000). Other studies done on school children in China, Indonesia and Tanzania reported that *A. lumbricoides* and *T. trichiura* of moderate to high intensity are associated with poor verbal

fluency, performance in test and lower intelligence quotient (IQ) levels compared to controls (Ezeamama et al., 2005; Yu et al., 2017). In addition, lower test scores in school children have been associated with *E. histolytica* dysentery and not with other dysenteries (Tarleton et al., 2006). Pregnancy reports support that intestinal parasites, especially STHs are associated with poor cognitive and motor development (Nampijja et al., 2015; Mireku et al., 2015). *Giardiasis* is associated with stunted growth and cognitive development which subsequently influences school performance and thwart educational advancement (Al-Mekhlafi et al., 2005; Ngonjo et al., 2016). Long-term retardation has been reported because of *Giardia intestinalis* infection in the first two years of life (Osman et al., 2016). Soil transmitted helminths and *Giardia* infections, even when asymptomatic, may contribute to growth faltering and impaired cognitive development.

The mechanisms of IPs to cognitive effects can be partly explained by the direct nutritional defects on the brain and the indirect effect on pathophysiological events occurring in the gut environment where STH reside. Evidence shows that there exists a bidirectional communication between the gut and the brain which occurs through hormones, neurons and immune mediators. Infections with intestinal helminths causes gut microbiota dysbiosis causing a disturbance in homeostasis of the healthy gut microbiota hence disrupting gut-brain communication (Guernier et al., 2017).

2.4.4 Evolution of human metabolic genotype

Additionally, nematode parasites have influenced the evolution of human metabolic genotype over time. It is reported that perhaps the exclusion of helminths from the environment has permitted the emergence of immune-mediated disease. Once helminth infections occur, the immune system responds by a strong Th2-mediated immune response that clears nematodes from the digestive track (Moreau & Chauvin, 2010). This immune response against helminths subsequently inhibits production of pro-inflammatory cytokines, promote production of interleukin 4 and 10, and TGF-β, induces CD4+ T cell Foxp3 expression, and generate regulatory macrophages, dendritic cells, and B cells (Elliott & Weinstock, 2012) as shown in Figure 2. These mechanisms in return, protect the host is protected from autoimmune diabetes and allergies.

Therefore, helminth-associated immunomodulation of pro-inflammatory responses prevent onset of diabetes and subsequently improve insulin sensitivity. However, helminths-induced immune responses may worsen any concurrent bacterial infection at the expense of relieving allergic conditions and protecting against high fat diet obesity (Su et al., 2014).

Although this might seem as positive impact, the cognitive development effect on young children in their informative years cannot be compared to these possible protective roles.

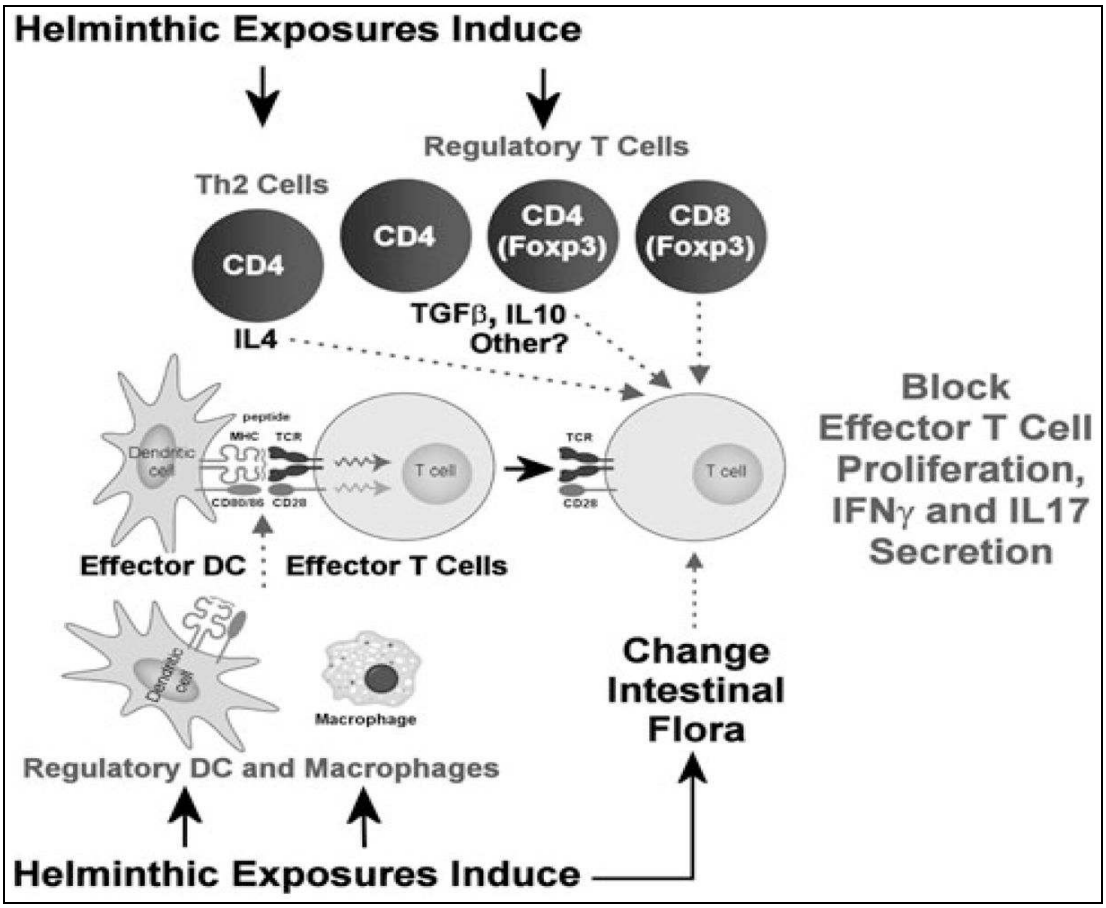

Figure 2: Helminth-induced regulatory mechanisms that prevent inflammatory bowel disease and other immune-mediated disorders. Parasite antigens are recognized by dendritic cells, which as antigen presenting cells to T cells, initiate an immune response. The release of cytokines like IL-4, IL-10 and TGFβ lead to secretion of inflammatory mediators. IL-interleukin; APC, antigen-presenting cells; DC, dendritic cells; INFγ-interferon gamma; TGFβ- transforming growth factor; Th- T helper cells. This figure is adopted from Elliott & Weinstock (2012).

2.5 Diagnosis of intestinal parasites

Reliable estimates of infectious diseases rely heavily on accurate methods of diagnosis. Global estimates of intestinal parasitic infections are conceivably thought to be underestimates due to lack of standard and quality diagnostic techniques (Silver et al., 2018). Microscopy is the most widely used diagnostic method especially in developing countries (Verweij & Stansvold, 2014) despite its low sensitivity (60%) and misleading results (Haque et al., 2003). Development of advanced and sensitive methods have revolutionized intestinal

parasitic detection in both developed and developing countries. However, economic constrains in developing countries and the need of high-tech laboratory and trained personnel limit the use of advanced and sensitive detection techniques.

2.5.1 Diagnosis of soil transmitted helminths

Soil transmitted helminths diagnosis relies on the traditional ova and parasite stool examination using fresh samples to increase sensitivity. These techniques rely on morphology of the pathogen are not very accurate but are dominant in developing countries because of their cost-effectiveness (Assefa et al., 2014). The intensity of infection is measured by the number of eggs per gram (epg) of faeces, generally by the Kato-Katz faecal thick-smear technique. Molecular methods targeting genetic differences are however available and are more accurate (Connell & Nutman, 2016; Meurs et al., 2017).

2.5.2 Diagnosis of *Entamoeba spp.*

The morphological similarity of *Entamoeba species* complicates diagnosis of amoebiasis. *Entamoeba histolytica* (12 - 15 μm), is the only known species to cause amoebiasis. However, other species like *E. dispar, E. moshkovskii,* that are morphologically identical have also been isolated in symptomatic patients suggesting that they could have a role to play in pathogenesis (Ali et al., 2003). Molecular techniques have been used distinguish *Entamoeba spp.* at the gene level (Verweij et al., 2001; see also Ali et al., 2003; Haque et al., 2003; Najafi et al., 2019). Antigen and antibody targeted tests have also been used in detention of *E. histolytica* although the later sensitivity depends on presence or absence of invasive disease (Fotedar et al., 2007). Colonoscopy and flexible sigmoidoscopy have also been useful in patients with colitis where *E. histolytica* is suspected but not detectable in stool (Najafi et al., 2019).

2.5.3 Diagnosis of *Giardia intestinalis*

Giardia intestinalis (8 - 12 μm), a bi-nucleated flagellated protozoan parasite, isolated in human and other hosts including mammals, dogs, cats, reptiles, and birds (Heyworth, 2016). There are over eight reported assemblages of *Giardia intestinalis* (assemblages A-H) with only two infective in human (A & B) (Cacciò et al., 2005). The two assemblages are indistinguishable morphologically. Phenotypically, intermittent diarrhoea and persistent diarrhoea has been associated with B and A respectively while other studies have linked assemblage A in children as asymptomatic (Laishram et al., 2012). Assemblages C to H

infect humans passively through interactions with the animal hosts (Kalyoussef & Goldman, 2010). Molecular characterization is essential to aid in distinguishing the different assemblages and for the accurate diagnosis of giardiasis. Several other immunological-based assays are also available for diagnosis of *giardiasis*. However, microscopy in combination with stool concentration methods are routinely used in medical laboratories being economical compared to the more sensitive molecular and immunological based methods.

2.6 Risks factors for intestinal parasitic infections

Intestinal parasitic infections are predominant in developing countries. The epidemiological patterns of these parasites vary in different geographical region attributed to the different sanitation and hygiene status, age, and other host factors.

2.6.1 Urbanisation, poverty, hygiene, and sanitation

Urbanization has been identified as one of the most important components of global transformations. However, African urbanization has not been accompanied by economic development as compared to other continents. Urbanisation in Africa has been faced with several challenges, including development of high-risk informal settlements (Dana, 2011). Africa urban informal settlements are characterized by construction of disordered houses and unplanned squatter, which are overcrowded with limited access to water, heaps of uncollected garbage, blocked drainage systems, shared pit latrines with no access to exhaust services (Coulibaly et al., 2018). Open defecation is a common occurrence and water sources are often contaminated with faecal waste (WHO, 2009). These factors are attributed to the huge population of individuals that move to urban centres and settle in informal settlements in search for greener pastures. The high levels of illiteracy, poverty, and unemployment drive people to live in these more affordable unhygienic informal settlements (Odhiambo et al., 2014) that harbour intestinal parasitic pathogens and risk their health and survival. With the limited space, there is increased transmission per contact of human-human and human-animal infections. Close contact with domestic animals which act as reservoirs to some IPs occurs increasing the risk of transmission (Ngonjo et al., 2016).

2.6.2 Age

Intestinal parasites affect persons of all ages (Gebretsadik et al., 2019), and are mostly severe in children and in individuals whose cell-mediated immunity has been impaired (Cotton et al., 2015). Evidence suggests that patients infected with human immunodeficiency

virus (HIV) have a higher likelihood of developing severe disease especially with intestinal protozoa infection (Matey et al., 2016). Children are disproportionately at risk of intestinal parasitic infections due to their high nutritional needs and less developed immune systems (Steinbaum et al., 2016). They are among the groups listed by the WHO at high risk of intestinal parasitic infections (Onochie et al., 2013; WHO, 2014). Severe cases are observed in children below 5 years of age especially when maternal hygiene is not strictly observed. The children consequently suffer from exacerbated morbidity which makes then more vulnerable to other infections. Studies in endemic communities have shown that children are more prone to infection after the weaning period and the risk increases by age, and peaks at the age of 9-12 years (Gebretsadik et al., 2019). This is attributed to their activeness, play habits and less hygiene literacy. Older children (>12years) practise hygiene and have a better understanding of disease prevention and control mechanisms and therefore remain less susceptible (Breiman et al., 2015). Schools in developing countries are often overcrowded which increases chances of person to person contact transmission. Water provision and hand washing facilities are not routinely functioning which increases their chances of infection.

2.6.3 Genetics

Genetics have also been linked to susceptibility of some intestinal parasites. Some candidate genes and genetic regions have been linked with susceptibility to *A. lumbricoides* and *T. trichiura* and *Cryptosporidium sp.* in humans (Bradley & Jackson, 2005; Putignani & Menichella, 2010). In pedigree studies carried out in China and Nepal, a substantial genetic variability in susceptibility to *T. trichiura* (28%) and *A. lumbricoides* (30–50%) have been reported (Bradley & Jackson, 2005) suggesting a part played by genetics in IPs parasites infections. The protective mechanisms against helminths, *Ascaris lumbricoides* for instance, have been linked to Th2 immune signalling. Th2 immune activity in the bronchus brings about an episodic symptomatology of asthma but this activity in the gut promotes expulsion and protection against helminths (Moreau & Chauvin, 2010). Genetic variations upregulating Th2 activity enhance protection against helminths including gene variations in IL-13 and IL-4 receptors, and STAT6 (Peisong et al., 2004). Previous studies have linked loci controlling intensity of *A. lumbricoides* on chromosomes 1 and 13 although no gene has been inferred directly (Quinnell, 2003).

In addition, the observed genetic variations in resistance to albendazole and mebendazole in field studies could explain the genetic predisposition and susceptibility in intestinal parasitic infections. Molecular screening of STHs like *T. trichiura,* hookworm and

A. lumbricoides gene recovered in human have identified single-nucleotide polymorphism (SNPs) in codon 167,198 and 200 in β-tubulin that confer resistance to commonly used anti-helminthic drugs (Diwara et al., 2013; Matamoros et al., 2019). This may increase the risk of intestinal parasitic infections in endemic region and have a long-term effect on the use of chemotherapeutic drugs. The predisposition, therefore, may also be partial due to variable environmental factors and exposure, host factors and parasite factors (Connell & Nutman, 2016). There is need to assess and reassess the endemic regions to prevent the misuse of the chemotherapeutic management strategy.

2.6.4 Climatic changes

The global and geographical distribution of IPs is dependent on ecology influencing the parasite's biology. Seasonal variability and climatic conditions affect the viability of the free-living infective stage, its development and survival. The infective stages develop and die at certain specific temperatures (Table 2). The maximum development rates temperature is 35-40°C above which the pathogens may not survive. The optimal temperatures in Kenya are experienced in Western and Coastal regions where significantly high prevalence is reported (Pullan et al., 2011). High soil moisture and humidity enhance faster development of STH ova and consequently high infection and transmission rates (Brooker et al., 2006)

Table 2: Optimal growth parameters and life expectancy of different growth stages of *A. lumbricoides*, *T. trichuris* and hookworm. The table is adopted from Brooker et al. (2006).

Parameter	***A. lumbricoides***	***T. trichiura***	**Hookworm**
Infective stage	Ova	Ova	Larvae
Life expectancy of free-living infective stages	28–84 days	10–30 days	3–10 days
Adult life span	1–2 years	1–2 years	3–4 years
Larvae development time to infective stage	8–37 days	20–100 days	2–14 days
Max. temp. of viable development	35–39°C	37–39°C	40°C

2.7 Control of intestinal parasites

The control and prevention of intestinal parasitic infections has become more feasible over time owing to the discovery of safe and effective drugs, simplicity in diagnosis and advances in parasite population biology. Elimination of morbidities related to intestinal helminths has become a priority and campaigns for preventive chemotherapy in endemic region are ongoing (WHO, 2015). Schools-based targeted deworming has been the corner stone of STH infections control, but the high rate of reinfection rates limits the ability to achieve sustainable reduction. To maintain the long-term success, improvement in sanitation and hygiene need to be re-emphasized (Hotez et al., 2006; WHO, 2008). In resource limited developing countries, preventive chemotherapy is considered compared to more costly and time-consuming sanitation improvements strategy.

2.7.1 WHO strategy for control of intestinal parasites

The current WHO guidelines focus on control of STH-related morbidities, targeting testing and treating individuals including school-going and preschool children and women of reproductive age. Currently, preventive chemotherapy is the most recommended control strategy in elimination of STH related morbidities. The WHO NTDs roadmap and the London declaration act accelerated progress towards eliminating selected NTDs and formalised the long-term disease specific goals (NTDs, 2012). The WHO strategy to eliminate morbidity related to STH by 2020 was through administration of annual or biannual dose of albendazole or mebendazole in children between 1-14 years of age for prevalence above 20% and 50% respectively (WHO, 2012; Imtiaz et al., 2019). Pharmaceutical companies and other donor organizations committed to support the global initiative by donating antihelminth drugs to the WHO for distribution in endemic regions. Priority in drug distribution is given to highly endemic region characterized by WHO thresholds (WHO, 2014). Priority is given to people living in sub-Saharan Africa, Asia, and Latin America where it is estimated that a third of them live on less than two dollars a day and are infected with one or more helminths (Hotez et al., 2007).

The WHO 2020 target was to reach a treatment target of 75% of school-age children (SAC). As of 2013, the target was half-way achieved at 39% (Brooker et al., 2015). Seventy per cent of school-going children had benefited from preventive chemotherapy by 2017. This is an indication that the road map target was well within reach by 2020 (Figure 3). However, between 2017 and 2018 (Figure 3), a decrease in treatment coverage was observed. This decline first was attributed to decision by India to expand their program to areas not

considered for preventive chemotherapy program. Secondly, by reduction of treatment campaigns and interruptions in most of the African countries (WHO, 2018). World health organisation public health target is to achieve morbidity control defined by prevalence < 2% of medium to high infection intensities for preschool and school aged children by 2030 (WHO, 2012; Imtiaz et al., 2019).

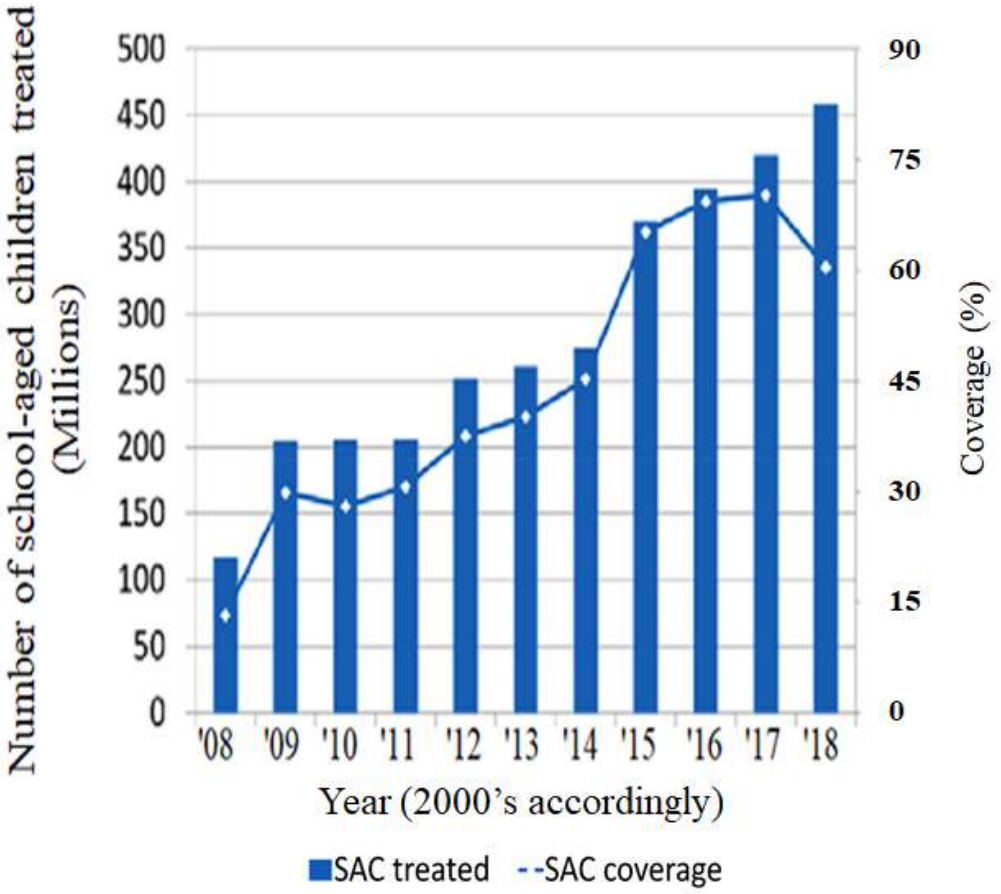

Figure 3: Worldwide coverage and progress in STH treatment and preventive chemotherapy administration measured in numbers and percentages per year between 2008 and 2018 targeting school-aged children (SAC). X-axes represent the year and Y-axis represents the progress in treatment and coverage in percentage and number. This figure is modified from WHO (2018).

2.7.2 Status of preventive chemotherapy in Kenya

A review of global coverage of preventive chemotherapy in endemic countries reported different coverage percentages and effectiveness (WHO, 2018). In a span of 5years, 70 of the endemic countries that had started preventive chemotherapy had achieved effective coverage. Twenty-three other countries had started preventive chemotherapy but with no effective coverage and three countries were yet to implement the strategy. Kenya was among the countries where preventive chemotherapy has been implemented with no effective coverage. This could be explained by the fact that new hotspot to intestinal parasitic infections is increasingly being reported that influence the net impact of effective coverage in previously known endemic regions.

School-going children are the most affected in Kenya as over 5 million (56.8%) are infected with intestinal helminths (WHO, 2013). A national school-based deworming program organised by the ministry of education and public health and sanitation was launched in 135 districts mapped with worm burdens as shown in Figure 4 (Bisanzio et al., 2014). Among the districts recruited were those in endemic regions of the Coastal, Nyanza, Rift valley and Western former provinces who received mass deworming annually or biannually (Riesel et al., 2009) depending on the WHO thresholds. These regions are characterised by poverty, low levels of access to water and sanitation and optimal climatic conditions that sustain the viability of infective larvae/cyst. The regions are also near water masses, which act as an important transmission medium through contamination with faecal matter. By 2014, over 20% and 25% reduction in STH prevalence and school absenteeism had been witnessed (WHO, 2013; Andereck et al., 2014). Between 2012 and 2017, a 58.2% and 77% reduction in STH prevalence and intensity respectively was reported in over 60 schools (Mwandawiro et al., 2019). However, there was heterogeneity in coverage of preventive chemotherapy among different counties and subsequently varied prevalence reduction. Moreover, school children residing in urban informal setting of Kisumu, Kakamega and Thika urban centres reported high prevalence of STHs ranging from 16.2% to 44% (Odiere et al., 2011; Suchdev et al., 2014). The high prevalence requires them to be included in the national deworming program, however there is no enough evidence-based information to guide their inclusion in the program. The status of IPs in particular intestinal helminths needs to be investigated to guide the necessary policy action.

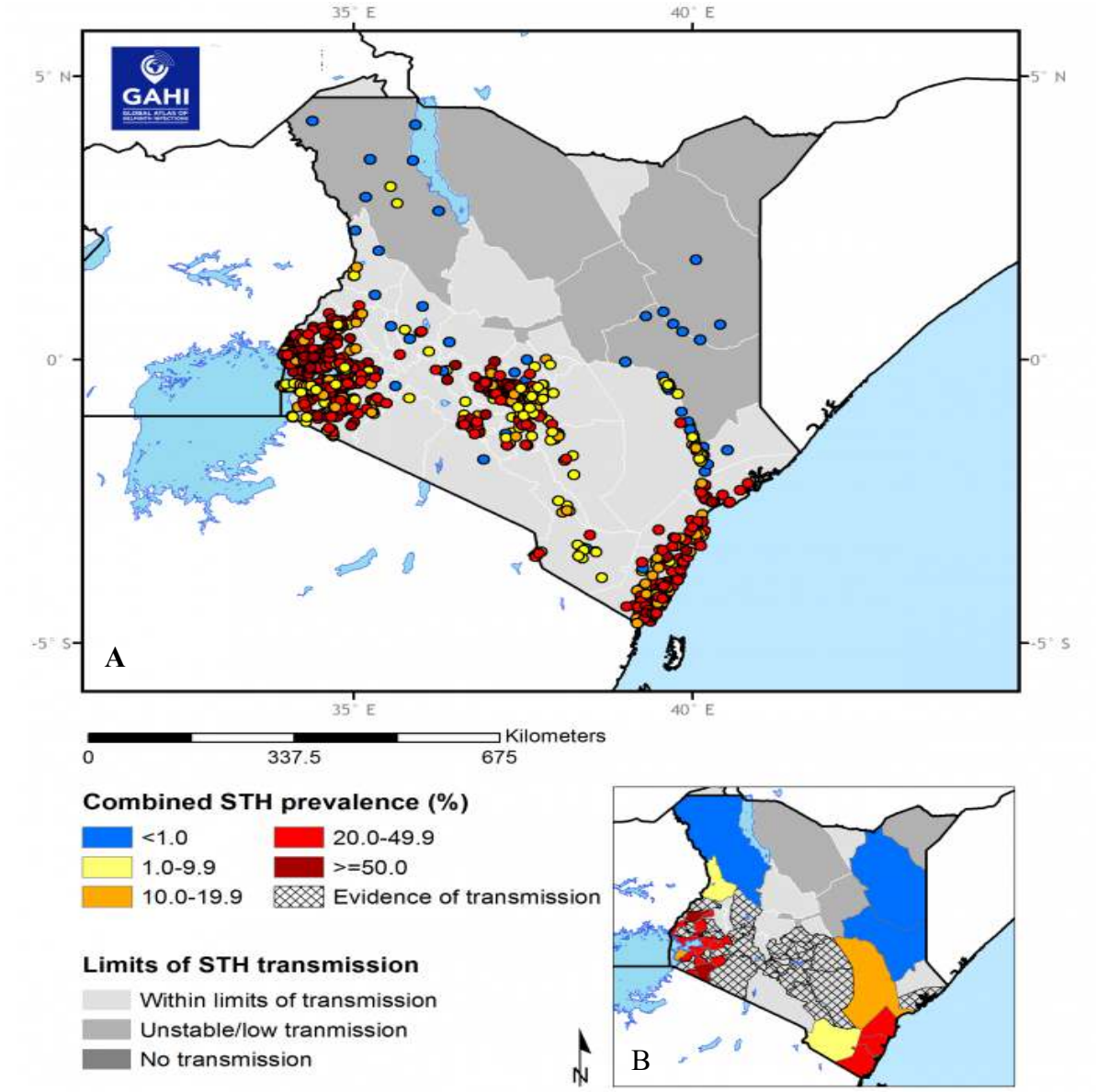

Figure 4: A and B shows the epidemiological based geographical distribution of STH cases in Kenya and their patterns and limits of transmission. The figure shows evidence of clustering of STH infections and high transmission rates in regions near water bodies (Coastal, Nyanza, Rift valley and Western former provinces) and informal urban centres. This figure has been adopted from Global Atlas of Helminth Infections (GAHI, 2020).

CHAPTER THREE
MATERIALS AND METHODS

3.1 Study area

The children targeted were from Kaptembwa, Milimani and Prisons primary schools from Kaptembwa, Gioto and London informal settlement areas respectively of Nakuru town. Nakuru town is the fourth-largest urban centre in Kenya, covering an estimated area of 7496.5km (Kenya National Bureau of Statistics & Society for International Development, 2013). The town hosts over half a million people and nearly 50% of them live in low-income settings/informal settlements. In these informal settlement areas, majority of the single room hosting at least 4 individuals, and most often over 40 households sharing the same pit latrine (Basweti, 2009). The high housing density reduces the available space for recreation. Sanitary amenities such as garbage disposition and collection, clean water and health facilities are inadequate. In addition, no specific social facilities such as children playing grounds are available. The schools they attend are overcrowded, with no stable water supply and hand washing facilities (Gacheiya & Mutua, 2009).

3.2 Ethical clearance

The study was conducted in conformity to the Declaration of Helsinki. The protocol and accompanying documents were reviewed and approved by Kenya Medical Research Institute (KEMRI) Science and Ethics Review Unit (SERU) protocol number 7/3/1 (Appendix i) from Kenya Medical Research Institute. *Informed Consent Process:* Written informed consent was obtained individually from each study participant parent/guardian. A written informed consent form was provided to potential subjects in the language of their choice (English or Swahili) (Appendices ii & iii) and its contents were carefully and fully explained to the participants. The verbal consent process involved explaining the study purpose, any anticipated consequences of the research, the anticipated uses of the data, possible benefits of the study and possible harm or discomfort that might affect them, and the option to end the interview or study involvement at any time. In addition, it was explained that the information they were to give for the study will be kept secure and confidential and all the participants were given access to their aggregate final study results. *Subject Confidentiality:* Data collected from participants was de-identified at the time of data collection and was only linked to identifying information through the unique study identification. All the identifying information and study datasheets were kept in separate locked file cabinets located in a locked room dedicated only for research activities. All the

study staff signed a confidentiality form (Appendix iv). Permission to carry out the study was obtained from county education officers, county public health officers, chiefs and village executive officers and the school administration. Participants found infected for any intestinal parasite received treatment as per ministry of health recommendation by certified health worker.

3.3 Study design

This was a cross-sectional study on school-going children between the ages of 8-13 years in the month of June 2018. The children were from Kaptembwa, Milimani and Prisons primary schools. After obtaining the relevant approvals, prior to brief potential study participants and teachers on the purpose and significance of this study was undertaken. The inclusion criteria were the children must be residence of one of the three informal settlements, had not been dewormed in the last three months and were within the study's age bracket (8-13 years). Children who fulfilled the study criteria but could not get stool sample at the time of sample collection were also excluded from the study. Those who fulfilled the study criteria were given consent form based on randomized approach using a random number generator to present to their parents/guardians for consent signing.

With the help of the school administration, the consent forms returned were collected, sorted and used to prepare for the sample collection day. Stool samples were collected from the participant and each responded to a structured questionnaire seeking to establish the correlates of IPs infections.

3.4 Sample size calculation

The sample size was calculated based on Cochran's 1977 formula and the 16.2% STH prevalence from informal settlements reported in Kisumu (Odiere et al., 2011). The choice of Kisumu as reference was since the study was done in public primary school children from informal settings with similar aspects.

$$n = \frac{z^2(p)\,(1-p)}{(d)^2} \qquad n = \frac{1.962\,(0.162)\,(1-0.162)}{(0.05)^2}$$

Where

n= sample size

p= prevalence of infection in the region (%)

d= absolute precision required

z=1.96 at 95% confidence level

Therefore, sample size = 210 pupils.

210 + 42 = 252

The study factored in 20% (42 samples) of the total samples to cater for those pupils who might not return samples or might be absent from school on the day of sample collection.

3.5 Stool sample collection

The sterile aluminium bags were issued to each participant labelled with a unique code. An oral description on use and proper handling of the stool bag and sample were given and each participant was instructed to bring a fresh stool sample. The stool specimen was examined physically for possible contamination and quantity. The samples were transported in a cool box within 6 hours of collection to the field laboratory at Langalanga county hospital and tested for intestinal helminths using Kato Katz. An aliquot of the sample was preserved in DNAzol for molecular analysis at the Centre for Microbiology Research (CMR) KEMRI laboratories in Nairobi.

3.6 Risk factors for infection

Each participant who provided a stool sample, filled a questionnaire. The schoolteachers and staffs were briefed on what information is required from the questionnaire before the actual data collection to facilitate and enhance data integrity. The participants were asked questions in Swahili and answers recorded in a questionnaire (Appendix v) that was later used to identify factors that could predispose the children to intestinal parasitic infections. The questions related to water, sanitation, and hygiene in households were captured in the questionnaire.

3.7 Laboratory analysis

The samples collected were subjected to microscopic detection using Kato Katz technique (n=248) and molecular analysis (n=96) for STHs and intestinal protozoa respectively.

3.7.1 Microscopic detection of intestinal helminths

An approximate of 41.7mg of each sample faeces was subjected to Kato-Katz examination. Two Kato-Katz thick smears were prepared from each stool sample and analysed for helminths eggs (*T. Trichiura*, *A. lumbricoides* and hookworm) using a

microscope (M10 series, Swift, Schertz, TX) by two independent microscopists under 40x magnification. Since hookworm eggs clear very rapidly, microscopy was done within 6 hours from sample collection. The faeces were first passed through a 250-micrometer metal sieve to remove fibrous material. A template, containing approximately 41.7 mg of faeces, was filled with stool, and used to place the sample on microscope slides. The double slides were then covered with a piece of cellophane soaked in glycerine malachite green solution and turned upside down to allow even spread of the sample on the slide and dry of the excess solution on a blot paper. The slides were then observed for STH within one hour under the microscope. Observed eggs were counted and any discrepancy in the results between the two microscopists were reconciled. The intensity of infection was determined based on parasite-specific egg counts and the count adjusted to eggs per gram (epg) of faeces by multiplying by a factor (24). Intensity of infection were categorized according to WHO proposed thresholds; *A. lumbricoides*; low (1-4,999), moderate (5,000-49,999) or high (>50,000), *T. trichuris*; low (1-999), moderate (1,000-9,999), high (>=10,000), hookworm; low (1-1,999), moderate (2000-3999) (WHO, 2011).

3.7.2 DNA extraction

Approximately 0.2g of faeces were preserved in 0.8mL DNAzol® (Molecular Research Centre, Inc., OH, USA) to make a stock solution for molecular analyses of intestinal protozoa: *Entamoeba spp*. (*E. histolytica*, *E. dispar*, *E. hartmanni*, and *E. coli*) and *Giardia intestinalis.* The samples were subjected to DNA extraction, quantification, and amplification.

DNA extraction and purification were done form preserved stool samples using the DNAzol® as previously described by Matey et al. (2016). In brief, the cell lysis was done physically via three freeze and thaw cycles and chemically using proteinase K. PCI (phenol: chloroform: isoamyl alcohol) was added to denature and precipitate the proteins separating them into an aqueous and organic phase. Absolute ethanol and ethachinmate were added to the supernatant (aqueous phase) to precipitate the DNA. The DNA was then washed using 75% ethanol, air-dried and resuspended in TE buffer and stored in -20°C until use. The DNA quantity was estimated using Nanodrop spectrophotometer.

3.7.3 PCR amplification

All PCR for intestinal protozoa parasites (n=96) were carried out in a 10-µl reaction volume containing 1x LA Taq® PCR buffer, 0.5 U of LA Taq® polymerase (TaKaRa Bio

Inc., Shiga, Japan), approx. 100ng of extracted DNA as a template, 0.4µM of forward and reverse primers, and 0.5mM of dNTPs with 0.1% dimethyl sulfoxide. Laboratory stock of samples confirmed positive from previous studies via sequencing, were included in each amplification cycle as positive controls. Molecular water was used as negative control.

3.7.4 Molecular screening of *Entamoeba spp.*

Detection of *Entamoeba species* was done in two PCR steps as previously described by Matey et al. (2016). The initial nested PCR using primers shown in Table 3, screened generally for the presence of *Entamoeba spp.* in the sample. The optimal conditions for the run were set at 94°C for 3 min, followed by 30 cycles of 94°C for 30 sec, 58°C for 30 sec, 72°C for 3 min, and a final extension at 72°C for 5 min for denaturation, annealing, elongation, and extension.

The second PCR was species-specific and was only done on samples that were positive for the initial nested PCR. *Entamoeba coli*, *E. histolytica*, *E. hartmani* and *E. dispar* were screened using the *Entamoeba spp.* PCR amplicons as template. The cycle parameters for PCR were optimized at: 94°C for 1 min, followed by 35 cycles of 94°C for 30 sec, 54°C for 30 sec, 72°C for 26 sec, and a final extension at 72°C for 2 min. The primers and product sizes used for detection of all *Entamoeba spp*. are shown in Table 3. Laboratory stock of samples confirmed positive from previous studies via sequencing, were included in each amplification cycle as positive controls. Molecular water was used as negative control.

3.7.5 Molecular screening of *Giardia intestinalis*

Giardia intestinalis was detected using nested PCR primers (Table 3) targeting the (glutamate dehydrogenase gene) GDH gene as described by Mbae et al. (2016). The amplification parameters were optimized at 94°C for 1 min, followed by 30 cycles of 94°C for 30 sec, 56°C for 30 sec, 72°C for 26 sec, and a final extension at 72°C for 2 min. Laboratory stock of samples confirmed positive from previous studies via sequencing, were included in each amplification cycle as positive controls. Molecular water was used as negative control.

Table 3: List of oligonucleotides and gene targets used for molecular identification and characterization of intestinal protozoa and their expected product sizes

Target protozoa	Target gene	Primer	Sequences (5'to 3')	Product size (bp)
Intestinal Protozoa	(18S rRNA)	Prot-A-F(TN21′)	TAAAGATTAAGCCATGCATGT(C/G)(G/T)	1384
		Prot-B-F TN27′)	AGGACACAAGCCATGCATGC	
		Prot-R (TN14′)	GATACCTTGTTACGACTTCT(C/T)	2525
Entamoeba spp	(18S rRNA)	Ent-F (MA115′)	GACATCGGAGAGGGAGCT	1199
		Ent-R (SA12′)	GCGTGC(A/G)GCCCAAGATG	
E. Coli	**(18S rRNA)**	Ec-F (MA113′)	GCCAAGAGAATTGTAGAAATCG	350
		Ent-R2 (TA28′)	CACTATTGGAGCTGGAATTAC	
E. hartmanni	**(18S rRNA)**	Er-F (MA67′)	TTGGATGTAGAGATACATTC	420
		Ent-R2 (TA28′)	CACTATTGGAGCTGGAATTAC	
E. histolytica	**(18S rRNA)**	Eh-L′	ACATTTTGAAGACTTTATGTAAGTA	427
		Eh-R′	CAGATCTAGAAACAATGCTTCTCT	
E. dispar	**(18S rRNA)**	Ed-L′	GTTAGTTATCTAATTTCGATTAGAA	195
		Ed-R′	ACACCACTTACTATCCCTACC	
G. intestinalis	**GDH**	KN41-L'	TCAACGT(C/T)AA(C/T)CG(C/T)GG(C/T)TTCCGT	457
		KN43-L'	CAGTACAACTC(C/T)GCTCTCGG	431
		KN40-R'	GTT(A/G)TCCTTGCACATCTCC	

Source of primers: TaKaRa Bio Inc., Shiga, Japan

3.7.6 DNA detection

All PCR products were resolved on an ethidium bromide-stained 1.5% agarose gel in Tris-Acetate-Ethylenediaminetetraacetic acid (TAE) buffer, and the amplicons visualized under ultraviolet light. Intestinal protozoa parasites detection was based on band size.

3.8 Data analysis

Data were entered into Microsoft Excel sheet and crosschecked with questionnaires to ensure accuracy. All statistical analyses were performed using SPSS software system, version 20 (IBM Corp., N.Y., USA). Frequencies and proportions were used to describe the demographic characteristics of the study population. A comparison between children infection status and risk factors for intestinal parasitic infections was performed using crosstabs. Pearson's correlation, chi-square, univariate, and multivariate analysis were used to determine significance of risk factors to intestinal parasitic infections. One-way ANOVA was used to examine the statistical significant of different intestinal parasitic infections reported from different informal settlements. Stepwise multiple linear regression was done on all risk factors analysed against intestinal parasitic infections. A statistical significance level of $p<0.05$ was used.

CHAPTER FOUR

RESULTS

4.1 Socio-demographic characteristics of the study participants

In total, 248 children, aged 8-13yrs [median ($\tilde{x}$) =10 years; IQR = 9.5 - 10.5] took part in the study. The mean age was 10.2±1.048 with the mode age being 10years (Figure 5). Stool samples were collected from 92, 88 and 68 children from Kaptembwa, Milimani and Prisons primary schools, respectively. One hundred and forty-one children were females (56.9%) and 107 (43.1%) were males. Fifty eight percent (n=144) participants parents/guardians were either formally employed or businesspersons, while the remaining, 40 (16.1%) were in informal employment, 42 (16.9%) unemployed and 7 (2.8%) farmers. For 182 (73%) participants, the main source of water for drinking was tap water with 35 (14.1%) of the inhabitants depending on rainwater, 22 (8.9%) on water vendors and 5 (2%) water from wells. Boiling was reported as the preferred means of water treatment for 139 (56%) participants. A third of the population (82) used the water directly from source. Use of commercial water treatments or bottled water was quite uncommon in the three informal settlements. Nearly two-thirds (159) and a third (73) of the participants used a shared toilet among different plots and shared toilet within a single plot respectively majority of which were either pit latrine (160) or flush toilets (75). One study participant from Prisons primary school reported to practise open defecation. Majority of children lived near their schools ≤1km and could walk alone from home to school and back. When outside the house, majority of the participants (201) reported that they wear open shoes. Over two-thirds (68.1%) of the children were on a school-based feeding program. Most participants lived in a nuclear family (181) and their households were between 1 and 3 rooms (219), with cemented floors (213) and majority hosting between 1 to 10 persons per household (235). The participants parents/guardians reared poultry, dogs, cattle, goats, rabbit, sheep, and cats (Table 4).

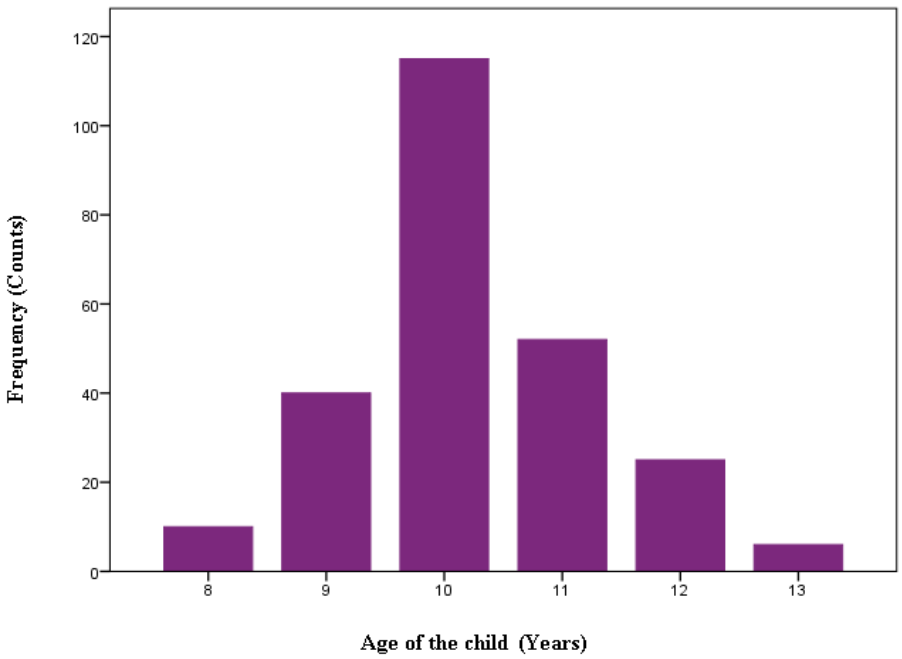

Figure 5: Distribution of age in years among school going children from informal settlements of Nakuru town. X-axis is age in years, Y-axis shows the frequency of age occurrence in the population.

Table 4: Demographic characteristics of the study participants from informal settlements of Nakuru town

Characteristic	Kaptembwa n(%)	Milimani n (%)	Prisons n(%)	N (total)	N/248 (%)
Age groups (in years)					
8-9	15(30)	15(30)	20(40)	50	20.2
10-11	69(39)	71(40.1)	37(20.9)	177	71.4
12-13	8(25.8)	12(38.7)	11(35.5)	31	12.5
Sex					
Female	48(34)	53(37.5)	40(28.4)	141	56.9
Male	44(41.1)	35(32.7)	28(26.2)	107	43.1
Occupation of the parent					
Unemployed	15(35.7)	20(47.6)	7(16.7)	42	16.9
Farmer	1(14.3)	57(1.4)	1(14.2)	7	2.8
Formal employment	26(36.1)	29(40.2)	17(23.6)	72	29.0
Businessman	31(43.1)	22(30.6)	19(26.4)	72	29.0
Informal	15(37.5)	6(15)	19(47.5)	40	16.1
Do not know	0	1(50)	1(50)	2	0.8
Source of water					
Piped water indoors	7(16.2)	28(65.1)	8(18.6)	43	17.3
Piped water outdoor	79(56.8)	25(18)	35(25.2)	139	56.0
Wells	2(40)	1(20)	2(40)	5	2.0
Water vendors	4(18.2)	13(59.1)	5(22.7)	22	8.9
Rainwater	0	17(48.6)	18(51.4)	35	14.1
Pre-treatment of water used for drinking					
Used direct from the sources	35(42.7)	21(25.6)	26(31.7)	82	33.1
Treated using commercially available water treatments	3(21.4)	8(57.1)	3	14	5.6
Boiled	50(36)	52(37.4)	37(26.6)	139	56.0

Bought bottled water for use instead	3(33.3)	4(44.4)	2(22.2)	9	3.6
Means of faecal waste disposal					
Private toilet	15(20.5)	34(46.6)	24(32.9)	73	29.4
Shared toilet	75(47.2)	42(26.4)	42(26.4)	159	64.1
Open defecation	0	0	1(100)	1	0.4
Type of toilet					
Pit latrine	63(39.4)	46(28.75)	51(31.9)	160	64.5
Flush toilet	28(37.3)	34(45.3)	13(17.3)	75	30.2
Non-functional flush toilet	0	8(72.7)	3(27.3)	11	4.4
Source of food					
School prepared	48(28.4)	71(42)	50(29.6)	169	68.1
Home prepared	43(55.8)	17(22.1)	17(22.1)	77	31.0
Distance of school from home				0	0.0
<500m	41(34.2)	36(30)	43(35.8)	120	48.4
500m-1km	38(48.7)	21(26.9)	19(24.4)	78	31.5
>1km	9(34.6)	13(50)	4(15.4)	26	10.5
Means of going home					
Walk alone with other children	83(38.8)	73(34.1)	58(27.1)	214	86.3
Walk accompanied by an adult	6(46.2)	2(15.4)	5(38.5)	13	5.2
Public transport	2(12.5)	12(75)	2(12.5)	16	6.5
Shoes worn outside the house					
Closed shoes	17(39.5)	19(44.2)	7(16.3)	43	17.3
Slippers	75(37.3)	65(32.3)	61(30.3)	201	81.0
Barefoot/others	0	4(100)	0	4	1.6
Type of family					
Nucleus	76(42)	58(32)	47(26)	181	73.0

Extended	2(11.8)	4(23.5)	11(64.7)	17	6.9
Single parent	12(30)	18(45)	10(25)	40	16.1
Religion					
Christian	86(37.4)	78(33.9)	66(28.7)	230	92.7
Muslim	4(44.4)	5(55.6)	0	9	3.6
Pagans	1(100)	0	0	1	0.4
Type of floor					
Cement	85(39.9)	72(33.8)	56(26.3)	213	92.7
Tiles	4(20)	10(50)	6(30)	20	8.1
Earthen	2(16.7)	4(33.3)	6(50)	12	4.8
Number of rooms					
1-3 rooms	86(39.3)	77(35.2)	56(25.6)	219	88.3
4-6 rooms	2(9.5)	10(47.6)	942.9)	21	8.5
>6 rooms	2(33.3)	1(16.7)	3(50)	6	2.4
Number of people living in the house					
1-5 people	50(34.7)	46(31.9)	48(33.3)	144	58.1
6-10 people	41(45.1)	30(33)	20(22)	91	36.7
>10	0	1(100)	0	1	0.4
Rearing of animals					
Dog	35(53)	15(22.7)	16(24.2)	66	26.6
Poultry	27(35.5)	19(25)	30(39.5)	76	30.6
Cow	10(45.4)	5(22.7)	7(31.8)	22	8.9
Goat	9(60)	1(6.7)	5(33.3)	15	6.0
Rabbit	1(20)	3(60)	1(20)	5	2.0
Sheep	1(100)	0	0	1	0.4
Cat	22(40)	23(41.8)	10(18.1)	55	22.2

4.2 Prevalence and intensity of intestinal parasites among study participants

The overall prevalence of STH among the study participants was 1.2% (n=3). *Trichuris trichiura*, *Ascaris lumbricoides* and hookworm prevalence was 1.2% (n=3), 0.4% (n=1) and 0.4% (n=1) respectively (Table 5). The mean infection intensities were 240, 1656, and 334 eggs per gram of faeces for *T. trichiura*, *A. lumbricoides,* and hookworms, respectively. A triple infection involving the three STHs was observed in one of the study participants.

The overall prevalence of intestinal protozoan parasites was 41.7% (n=40). The prevalence of individual protozoan parasites was 0%, 0%, 4.2%, 6.3% and 38.5% for *E. histolytica*, *E. hartmani, G. intestinalis, E. dispar* and *E. coli* respectively (Table 5). Double infections were observed between *E. coli/E. dispar* 3.1% (n=3) and *E. coli/G. intestinalis* 1% (n=1). Triple infections were also observed among *E. coli/E. dispar/G. intestinalis* 1% (n=1). Some of the gel images from the PCR run are shown in Figure 6 and 7, for *Entamoeba spp*. universal and *E. coli* specific PCRs.

Table 5: Prevalence, diversity, and intensity of intestinal parasites among school children from informal settlements of Nakuru town

Type of Intestinal parasite	**Number +ve (%)**	**Infection intensity (epg)**
Soil transmitted helminths (n=248)		
Single infection (n=2)		
Trichuris trichiura	2(0.8)	Light <1000
Triple infection (n=1)		
Trichuris trichiura/A. lumbricoides/hookworm	1(0.4)	Light <5000
Intestinal Protozoa (n=96)		
Single infection (n=35)		
Entamoeba coli	31(32.3)	
Entamoeba dispar	2(2.1)	
Giardia intestinalis	2(2.1)	
Double infection (n=4)		
Entamoeba coli/E. dispar	3(3.1)	
Entamoeba coli/G. intestinalis	1(1.0)	
Triple infection (n=1)		
Entamoeba coli/E. dispar/G. intestinalis	1(1.0)	
No infection		
Entamoeba histolytica	0(0)	
Entamoeba hartmani	0(0)	

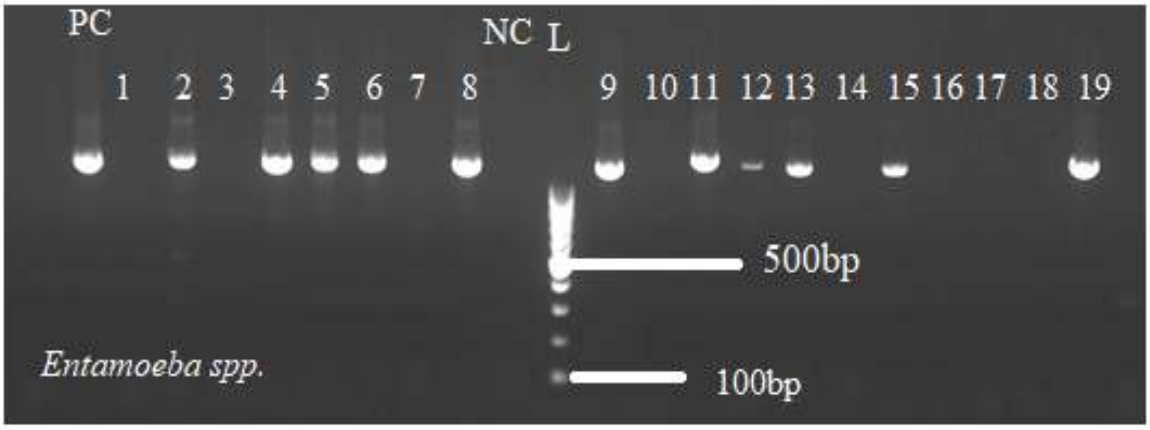

Figure 6: Molecular detection of *Entamoeba species* by nested PCR amplification. *An image of 1.5% agarose gel separated amplification products from DNA extracted from stool samples using* TN14′/TN27′ and MA115′/ SA12′ *primers targeting the 18S rRNA show products of 1200-1400 bp i.e positive samples of Entamoeba spp.* These products were gel purified and used as template for species-specific detection. NC- negative control; L-ladder (100bp); PC- positive control.

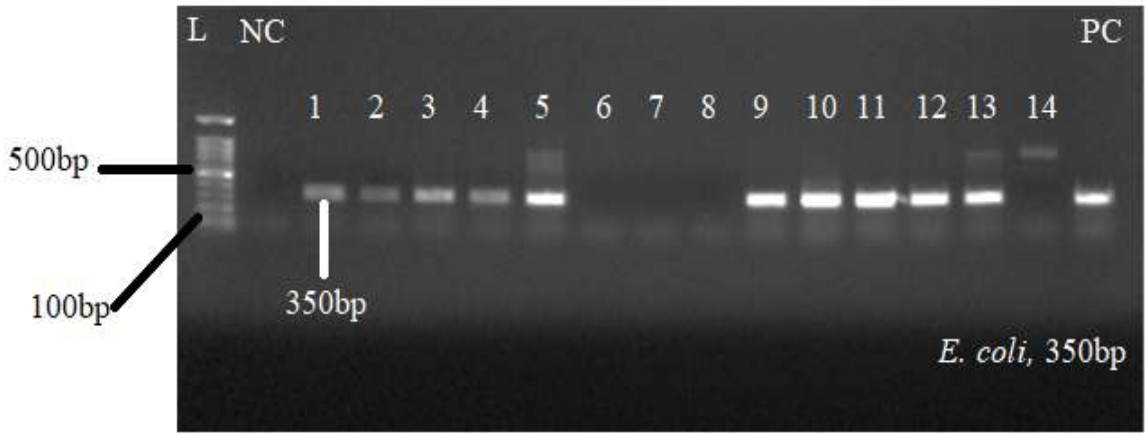

Figure 7: Molecular detection of *Entamoeba coli* by PCR amplification. *An image of 1.5% agarose gel separated amplification products using* TA28′ *and* MA113′ specific *primers targeting 18s rRNA show products of 350 bp i.e positive samples of Entamoeba coli.* NC- negative control; L-ladder (100bp); PC- positive control.

4.3 Intestinal parasitic infections prevalence among the different schools

All three primary schools reported an equal prevalence of STH 0.4% (n=1). Kaptembwa primary school reported the highest prevalence of intestinal protozoan parasites 21.7% (n=20) compared to Prisons 20.5% (n=14) and Milimani 10.2% (n=9) primary schools. The difference in infection prevalence among the three schools however was not statistically significant for both STH (p=0.833) and intestinal protozoa infections (p=0.734).

The distribution of the different STHs and intestinal protozoan parasite is shown in Figure 8. *Entamoeba coli*, *E. dispar* and *T. trichiura* were diagnosed in all three primary schools. *Giardia intestinalis* was detected in both Kaptembwa and Milimani primary schools. Hookworm and *A. lumbricoides* were detected only in Kaptembwa primary school (Figure 8).

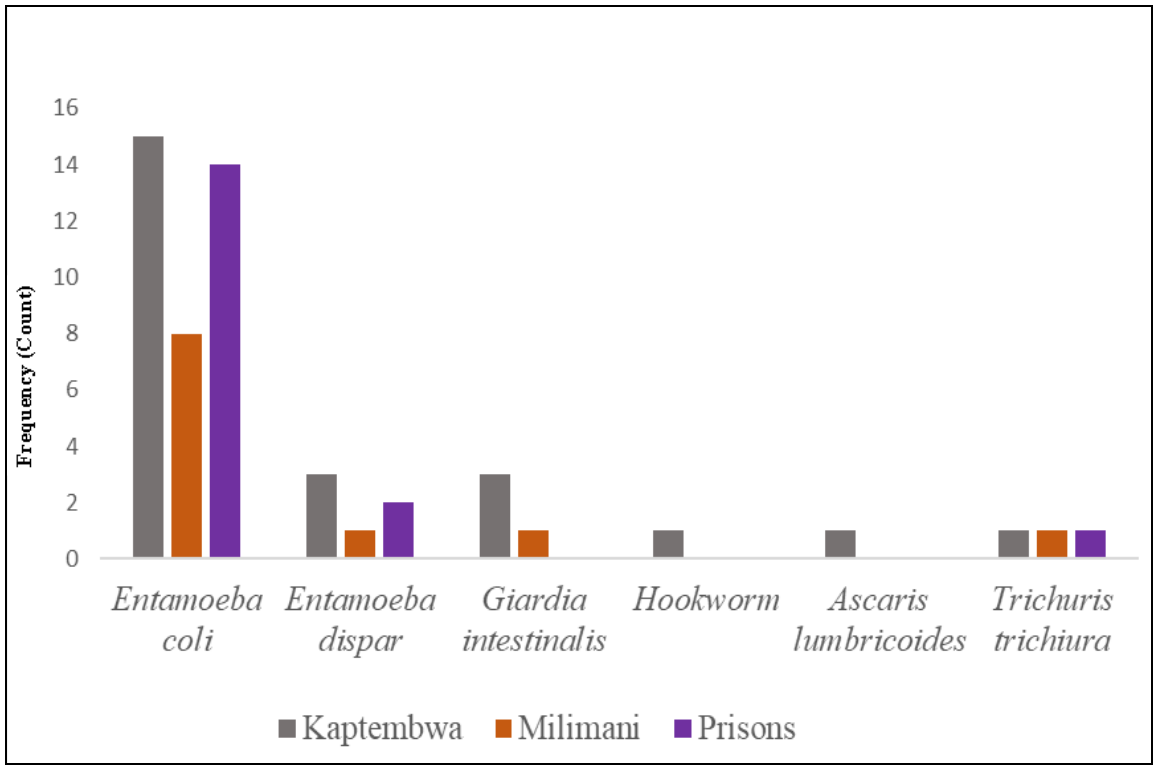

Figure 8: Frequency (n) and distribution of the intestinal parasites among the three primary schools from the three informal settlements. Grey-Kaptembwa; Orange-Milimani; Purple-Prisons primary schools. Y-axis is the frequency (count) and X-axis show the intestinal parasite.

4.4 Multiple infections among study participants

The overall number of multiple infections from STH and intestinal protozoan parasites were (0.4%) (n=1) and 5.2% (n=5) respectively. Triple infections were observed in Kaptembwa (*T. trichiura, A. lumbricoides,* hookworm) n=1 and Milimani primary schools (*E. dispar, E. coli, G. intestinalis)* n=1. Double infections between *E. coli* and *E. dispar* were observed in Kaptembwa (n=1) and Prisons primary schools (n=2) respectively. *Entamoeba coli, G. intestinalis* co-infection was observed in Kaptembwa primary school (Table 6). In total, Kaptembwa, Prisons and Milimani primary schools reported 3, 2 and 1 multiple infections, respectively.

Table 6: Distribution of multiple infections among the three school in informal settlements of Nakuru town

Multiple infections	Kaptembwa	Milimani	Prisons	Total n(%)
Soil transmitted helminths				
Trichuris trichiura, A. lumbricoides, hookworm	1	0	0	1(0.4%)
Total percent STH multiple infections per school	1.1%	0%	0.0%	
Intestinal protozoa parasites				
Entamoeba coli, E. dispar	1	0	2	3(3.1%)
Entamoeba coli, G. intestinalis	1	0	0	1(1%)
Entamoeba dispar, E. coli, G. intestinalis	0	1	0	1(1%)
Total percent intestinal protozoa parasite multiple infections	2.2%	1.1%	2.9%	

4.5 Risk factors associated with intestinal parasitic infections.

The occurrence of IPs in females and males (16.3% and 15.9%; p=0.928) was similar. Although not statistically significant, infection was higher in children aged 8–9 years (20%) and those aged 12–13 years (19.4%) as compared to those 10–11 years (14.4%) among the different age-groups (Table 7). The prevalence of IPs decreased with an increase in the number of household rooms (Figure 9) (p=0.035) with more infections being detected in households with rooms between 1 and 3. Children who had lunch prepared in school reported half the number of infections (12.4%) reported by those who ate a home-packed lunch (24.7%) (p=0.016). Children who lived in houses with earthen floors (p=0.022) had a higher chance of being diagnosed with STH as compared to those who lived in houses with

cemented floor. Similarly, those who reared goats (p=0.046) had a higher like hood to STH infections compared to those who did not. Subsequently, children who used rainwater for drinking were more likely to have intestinal protozoan parasite infections (p=0.052) than those who used tap water, bought water from vendors, or used water from wells.

Analysis of factors related to specific intestinal protozoa (Table 8) confirmed that rearing of goats increased chances of *E. dispar* ($p=0.001$) and *T. trichiura* (p=0.046) infections. Male children (p=0.042) and children who alone or accompanied by other children home (p=0.04) from school reported a higher prevalence of *G. intestinalis.* Children between the ages of 12 and 13 years significantly reported higher prevalence of hookworm (p=0.008) and *A. lumbricoides* (p=0.008). Smaller households accommodating higher number of pupils reported a high prevalence of hookworm and *A. lumbricoides* (p=0.025). Wearing of slippers increased the likelihood of STH diagnosis (hookworm ($p=0.038$) and *A. lumbricoides* ($p=0.038$) *T. trichiura* ($p=0.029$).

Additional risk factors were evident from analysis of risk factors among the three informal settlements. Source of water and distance of home from school were important factors for children attending Prisons primary school. There was an increased likelihood to infection for children whose main source of water was water vendors (80%) compared to those sourcing from taps (11.6%), wells (0%) or rainwater (11.1%) (p=0.003). Children whose households were nearer the school schools <500m (38.2%) reported significantly low levels of infections schools (p=0.013) as compared to those living far away from school 500m-1km (36.8%) and >1km (25%).

Table 7: Univariable and multivariable analysis for association between IPs infection and risk factors among school-going children of Nakuru town informal settlements (statistically significant variables are bolded)

Risk factor	Number of infections	IPs (p-value)	STH (p-value)	Intestinal protozoa parasite (p-value)
Age				
8 & 9	10/50(20%)	0.407	0.569	0.737
10 & 11	24/167(14.4%)	0.282	0.208	0.269
12 & 13	6/31(19.4%)	0.603	0.247	0.218
Gender				
Male	17/107(15.9%)	0.928	0.41	0.728
Female	23/141((16.3%)			
Room number				
1-3 rooms	39/219(17.8%)	0.061	0.214	0.032
4-6 rooms	0/21(0%)	**0.035**	0.596	0.082
> 6 rooms	1/5(20%)	0.978	0.0001	0.231
Water source				
Piped water indoors	6/43(14%)	0.671	0.427	0.923
Piped water outdoor	27/139(19.4%)	0.112	0.427	0.126
Well	0/5(0%)	0.324	0.804	0.231
Water vendors	5/22(22.7%)	0.38	0.135	0.622
Rain	2/34(5.9%)	0.081	0.322	0.052
Floor type				
Cemented	34/217(15.7%)	0.961	0.296	0.755
Tiles	4/20(20%)	0.604	0.605	0.596
Earthen	1/11(9.1%)	0.463	**0.022**	0.785
Source of food				
School prepared	21/169(12.4%)	**0.016**	0.185	**0.015**
Home prepared	19/77(24.7%)			
Parent occupation				
Unemployed	9/42(21.4%)	0.43	1	0.279
Farmer	0/7(0%)	1	1	1
Formal employment	12/72(16.7%)	0.535	0.865	0.393
Businesspersons	13/72(18.1%)	0.502	0.865	0.381
Informal employment	5/40(12.5%)	0.645	0.761	0.604
Animal rearing				
Cat rearing	7/55(12.7%)	0.439	0.062	0.888
Goat rearing	1/15(6.7%)	0.762	**0.046**	0.318

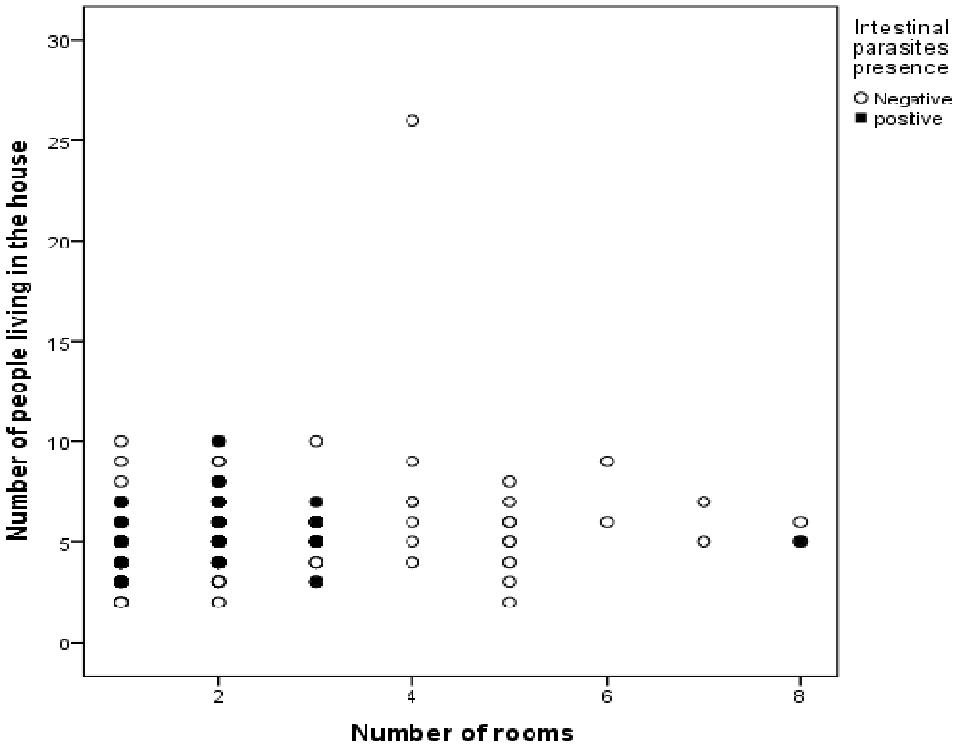

Figure 99: Distribution of intestinal parasitic infections by number of people living in the household stratified by number of rooms. Most infections were observed in overcrowded household i.e., smaller households with high population density. The black dots represent children positive for intestinal parasites and clear dots represent children negative for intestinal parasites. Both X and Y-axis represent counts.

Table 8: Risk factors associated with specific intestinal parasites (statistically significant variables are bolded)

Risk factor	*Entamoeba dispar* *p-value*	*Giardia intestinalis* *p-value*	*Hookworm* *p-value*	*Ascaris lumbricoides* *p-value*	*Trichuris trichiura* *p-value*
Rearing goats	**0.001**	0.636	0.8	0.8	**0.046**
Sex	0.533	**0.042**	0.252	0.252	0.41
Means of going home	0.234	**0.04**	0.747	0.747	0.575
Age	0.231	0.267	**0.008**	**0.008**	0.481
Types of shoes worn outside the house	0.883	0.404	**0.038**	**0.038**	**0.029**
Number of people living in the house	0.262	0.732	**0.025**	**0.025**	0.247
Number of rooms	0.774	0.889	0.916	0.916	**0.006**

Stepwise linear multiple regression revealed a reduction in infections caused by intestinal protozoa with an increase in parent employment (p=0.012), less congested households (p=0.014) and eating food prepared in school (p=0.023). Food source was shown to exert a significant influence (p=0.021) on both STH and intestinal protozoan parasites (Table 9) suggesting that parents should consider allowing their children to eat food prepared in schools. The number of rooms, as a major risk factor to intestinal parasitic infections, was significantly influenced by the type of household floor (p=0.002), the number of people living in the household (p=0.004), and the occupation of the parents (p=0.024) (Table 9).

Table 9: Stepwise linear regression analysis of risk factors associated with intestinal parasitic infections, their significance and 95% confidence interval.

	Intestinal protozoa			
	P-value		**95% Confidence interval**	
Risk factors	**t**	**sig**	**Lower bound**	**upper bound**
Parent occupation	-2.591	**0.012**	-0.203	-0.026
Number of rooms	-2.547	**0.014**	-0.025	-0.255
Source of food	-2.329	**0.023**	-0.039	-0.268
Intestinal parasites				
Source of food	-2.333	**0.021**	-0.289	-0.024
Room number				
Type of floor	3.120	**0.002**	0.150	0.662
Number of people living in the house	2.876	**0.004**	0.037	0.200
Parent occupation	-2.272	**0.024**	-0.284	-0.020

CHAPTER FIVE
DISCUSSION

5.1 Prevalence of intestinal parasites

Reliable estimates and updates on the status of IPs in vulnerable regions are important to guide control. This study reports an overall intestinal parasitic infections prevalence of 17.3% among school-going children from informal settlements of Nakuru town. Higher prevalence of IPs has been reported from similar settings in Thika (>48.9%) (Ngonjo et al., 2012) and Nairobi (25.6%) (Mbae et al., 2013), Nigeria (86.2%) (Gyang et al., 2017) and Pakistan (52.8%) (Mehraj et al., 2008). The differences in prevalence could be attributed to the detection methods used, the socio-economic activities, ecological and environmental differences. In addition, improvements in sanitation, hygiene, and infrastructure supported by Government of Kenya and World Bank in regions of Kaptembwa and Rhonda, extending to London and Gioto, that were ongoing by the time of sample collection, and public health education by the department of health at the county (C. Gachahi., personal communication, June 4, 2018) could have contributed to the lower prevalence than expected. In addition, the ministry of health in the county has focused on training residents on health and hygiene education especially on food handling and maintaining sanitation systems which could have impacted on the overall intestinal parasite prevalence. The low prevalence of IPs however also suggests low environmental contamination with the infective pathogens. Regarding intestinal parasitic infections differences among the informal settlements, it is evident that IPs were able to infect the school-going children with the same intensity which could be because the three informal settlements were from the same region of the town, Nakuru town west, and all informal settlements were undergoing a similar treatment in terms of sanitations and hygiene improvements. The differences however were more evident in risk factors to infection which could be attributed to individuality differences influenced by hygiene, occupation, and literacy levels.

The prevalence of STH infections reported in urban informal settlements of Kenya ranged between 34%-40.7% (Odiere et al., 2011; see also Davis et al., 2014; Suchdev et al., 2014) which was incomparable to our study findings of 1.2% but comparable to what has been reported in another region of the world, Malaysia (Jamaiah & Rohela, 2014) and Columbia (Bouwmans et al., 2016). Prevalence of STH is always influenced by climate (humidity, temperature, rain) and soil factors; hookworm transmission for instance, peaks at temperatures around 40°C (Brooker et al., 2006). The low prevalence of STH could also be attributed to deworming programs facilitated by the county government in combination with

sanitation and hygiene improvements in schools. According to Nikolay and colleagues (2015), deworming and improved sanitation and hygiene have a synergistic effect in reduction of STHs. Among the STHs, as reported elsewhere (Odiere et al., 2011), *T. trichiura* (1.2%) was the most common STH diagnosed. This could be attributed to deworming programs which administer a single dose of albendazole-400mg and mebendazole-500g which has less efficacy on *T. Trichiura* (Silber et al., 2017; Matamoros et al., 2019) because it requires a 3-5day dosage to clear completely (Du et al., 2011). Otherwise in endemic regions, *A. lumbricoides* and *T. trichiura* are transmitted together since their infective stages are more resistant to the environment (Tilahun et al., 2014). The low prevalence of hookworm is consistent with a study conducted in Thailand (Polseela & Vitta, 2015). This low prevalence may be due to the fact that few children reported to walk barefoot as skin is the major portal of entry of hookworm into the body. In addition, hookworm has a slower rate of infection because its third-stage larvae have a shorter life expectancy (3–10 days) unlike *A. lumbricoides* eggs with several months infective period (Brooker et al., 2006) implying that the environment recovers from hookworm infection at a faster rate as compared to other STHs.

The overall intestinal protozoa parasite infections were (41.7%), *E. coli, E. dispar* and *G. intestinalis* (38.5%, 4.2% and 6.3% respectively). Predominance of *E. coli* and *E. dispar* is common in tropical regions and their implications in chronic and subclinical human disease mechanisms have not been well studied (Fotedar et al., 2007). Indeed *E. dispar* is often misdiagnosed microscopically (Leiva et al., 2006) and in immunodiagnostics for *E. histolytica* (Stauffer et al., 2003). *Entamoeba coli* has also been associated with abdominal fat deposition in children suggesting possible long-term implications (Zavala et al., 2015). However, *E. coli* has been referred as a commensal parasite that indicates faecal contaminated environment that is potentially related to poor hygiene and sanitation habits and inadequate sewerage systems (Hernández et al., 2019). Prevalence of *E. histolytica* in this study is consistent with what is reported in other regions of Kenya (<1%) (Sakari et al., 2017), Indonesia (0%) (Matsumura et al., 2019), and Peru (0%) (Cooper et al., 2017) done on asymptomatic children. The prevalence of *G. intestinalis* reported here was consistent with an earlier study (Mbae et al., 2016) in informal settlements of Nairobi using similar diagnostic methods.

5.2 Co-infections among study participants

The study reports a low prevalence of multiple infections which is consistent with findings reported in similar settings of Douala urban settings in Cameroon (Kuété et al., 2015) and school-going children in Turkey (Okyay et al., 2004). The coinfection rates are however lower than what have been reported in endemic regions of Kenya like Busia (Brooker et al., 2000). Co-infections lead to multiple morbidities due to the cumulative effect of each parasite (Gyang et al., 2017) resulting to reduced cognitive performance (Jardim et al., 2008), poor academic performance (Kvalsvig et al., 1991), longer duration of diarrhoea and significant muscle wasting compared to those that have single parasite infections (Ajjampur et al., 2009). The very low multiple infections could therefore be explained by the fact that they rarely remain asymptomatic and chances of diagnosing them in this study's asymptomatic population is limited. In addition, the highest percentage of the coinfections detected from this study were between *E. coli* and *E. dispar* which pathogenicity status has not been conclusive. However, for the co-infections detected for the pathogenic species, the low infection intensities, infection duration, and the immune status of the child may influence the appearance of symptoms (Miller et al., 2003).

5.3 Risk factors associated with intestinal parasitic infections.

Similar to a study in Nairobi informal settlements (Mbae et al., 2013), our findings showed no infection differences by sex. This could be explained by the fact that both genders were evenly exposed to the infective parasites and gender role differences are narrowing with time. However, male children had a higher likelihood to *Giardia intestinalis* infection that their female counterparts. This could be attributed to outdoor play habits of male children like playing football in contaminated environments and in the process may drink/play with water oozing from leaking pipes or flowing off drainages which could predispose them to infection. Moreover, unlike other studies which relate decreased risk to infection with increase in age, our study reports that children between the age of 12 and 13 years had a higher risk to *Ascaris lumbricoides* and hookworm infection. Usually, children in this age group are knowledgeable about disease causing IPs and are more likely to maintain personal hygiene. However, these might not be a common case for all children in this age group. Their parents/guardians may still need to supervise the personal hygiene status of their children.

Food and food handler's hygiene is an important factor in the transmission of IPs (Kamau et al., 2012). In other studies, schools with feeding programs have been reported to be areas of high risk of food security and vulnerability to intestinal parasitic infections (Hailu

& Lindtjørn, 2019). However, in this study, taking lunch prepared in school minimized intestinal parasitic infections. Teachers and matrons in schools are the caregivers who ensure that children observe basic hygiene practices. Feeding of freshly prepared food has been linked to a reduced risk of infection. Storage of cooked food for longer provides adequate time for bacterial and other pathogens proliferation. The home-packed lunch is subject to contamination also because the food containers may not be properly cleaned and sealed. Moreover, they may pack leftovers and food prepared along the streets or unwashed fruits which may be unhygienic (Houmsou et al., 2010). Food displayed along the streets attracts flies that transfer cysts, eggs, and larvae of IPs contaminating the food and posing a serious threat to children (Amuta et al., 2010). Children who live far and walk alone from school to home reported a high predisposition to intestinal parasitic infections. The children are often tired, exhausted and hungry by the time they leave school for home and their chances of feeding on unwashed fruits and contaminated street food which some maybe leftovers is high increasing their risk of infections. As personal hygiene is being emphasized in school-going children therefore, there is also need to overemphasize food safety and hygiene in the entire community. In addition, playing along their way home in contaminated environments may increase their likelihood to infections.

Limited water sources, socio-economic status and environmental conditions of most informal settlements inhabitants' increases the possibility of cross-contamination of water supplies. Cross-contamination of water has been reported to occur in local water sources such as streams and wells or leaking pipes (Eu et al., 2010). Also, water sources that are uncontaminated may be polluted during inappropriate transportation and storage in households leading to contaminated water ingestion (Singh et al., 2009). Rainwater for instance, has been shown to increase the likelihood of intestinal protozoa infections in this study. Its contamination may occur along the household roofs, collection pipes, in storage tanks, from animal droppings as they are reservoirs of intestinal protozoa parasites (Leelayoova et al., 2008) and in the process of transportation to the households from collection tanks. Those that rely on water vendors are mostly unaware of the sources of water. However, it is observed that water vendors do not always obtain water from taps as they claim but sometimes from broken pipes in gutters and running surface water. In the occasion the source of water is clean, the "jerry-cans" used for fetching water are mostly dirty and contaminated (Eu et al., 2010). Besides, *G. intestinalis*, a majorly water-borne protozoa, can survive very low temperatures like of collection tanks and is often resistant to water treatment procedures like chlorination (Osman et al., 2016).

Low socioeconomic factors are a known risk factor for intestinal parasitic infection (Forson et al., 2018). Household overcrowding as observed in our findings increases IPs and has been previously reported in children between 0-16years (Baker et al., 2013a). Programs based on reducing household overcrowding have evidently minimized infections (Baker et al, 2013b). This is consistent with our present study as a significant reduction of intestinal parasitic infections was reported in less congested households (between 4-6 rooms) and in households where parents were employed. Overcrowding in houses increases the potential spread of IPs from an infected to a healthy person by increasing transmission per contact (Forson et al., 2018). Smaller overcrowded households are associated with a strain in basic amenities such as water (Tilahun et al., 2014) suggesting that such chores are rather underdone or foregone. Unemployed parents may experience a strain in their budget and therefore prioritize other basic necessities at the expense of observing hygiene. It is also possible that unemployed individuals were likely to be illiterate which has been associated with increased intestinal parasitic infections (Forson et al., 2018). Earthen floor in overcrowded households also increased the risk of IPs infection, which suggests a possible soil-related transmission of intestinal parasites.

Intestinal parasites parasitize a wide range of mammalian hosts through zoonotic and anthroponomical means. Majority of IPs host range from human non-human hosts and although they may be isolated in all host their capacity to cause infection is host specific. Human pathogens such as *Entamoeba spp.* and *Giardia intestinalis* has been isolated in animal host and similarly animal pathogens have been isolated in human hosts (Heyworth, 2016). *Giardia intestinalis* assemblage E, a well-defined goats and sheep pathogen has been isolated in human, rabbits and non-human primate, mainly acquired through animal-animal and animal-human interactions (Foronda et al., 2008). Although this might not cause disease in a different host, they could act as important reservoirs to infection and maintain the transmission cycle in the environment. The association between goats and *Trichuris spp*. observed has not being reported before and therefore could be explained by a misdiagnosis of *Trichuris ovis* isolated in goat (Gul et al., 2016) to *T. trichiura* because they are similar morphologically although the ova is slightly larger in diameter. Further studies however could be done to investigate a possible link between STH and goat rearing. However, it is important to control IPs in animals as a focused strategy of managing human infections.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The prevalence observed from this study was low among school-going children from informal urban setting of Nakuru town. The current WHO recommendation for national deworming programs in schools is prevalence above 20% as they are at risk of infection. Based on results from this study, Nakuru town informal settlements need not to be included in the national deworming program. Although low coinfections were reported, the study confirms that individuals hosting multiple IPs can remain asymptomatic and become disease transmission reservoirs to healthy individuals. Important risk factors such as food and water hygiene, contact with domestic animals, earthen floors, long distances of home from school and high household population density were also identified in this study. The findings show that there is need to focus on how to mitigate these factors to avoid predisposition of intestinal parasitic infections. These could involve emphasizing on hygiene education for schoolchildren and food handlers, improved provision of water, regular deworming of domestic animals and supporting programs focused on improving housing and decongesting informal settlements household. In general, WASH practises need to be improved in informal settlements to maintain IPs infections low.

6.2 Recommendations

Although the study successfully evaluated the prevalence, risk factors and co-infection rates of IPs in three informal settlements of Nakuru, the following recommendations are necessary.

i. A proper assessment of the predisposing factors incorporating a larger sample size, which was not possible for this study, is required to give a conclusive report on the risk factors for infection.

ii. The low intestinal parasitic infections reported in this study, it is possible that informal settlements have been given a lot of focus especially towards improvements in sanitation and hygiene and deworming programs that target the low-income earners. Therefore, there is need to evaluate and compare the infection levels in urban cities with their corresponding informal settlements to confirm the extent of infections and affirm the risk factors.

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APPENDICES

Appendix I: Study approval from the Kenya Medical Research Institute

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel: (254) (020) 2722541, 2713349, 0722-205901, 0733-400003, Fax: (254) (020) 2720030
E-mail: director@kemri.org, info@kemri.org, Website. www.kemri.org

KEMRI/RES/7/3/1 **July 30, 2018**

TO: **NAOMI MUMBI CHEGE,**
PRINCIPAL INVESTIGATOR.

THROUGH: **THE DIRECTOR, CMR,**
NAIROBI.

Dear Madam,

RE: **PROTOCOL NO. KEMRI/SERU/CMR/P00084/3689 *(RESUBMISSION OF INITIAL SUBMISSION)*: STATUS OF INTESTINAL HELMINTHES AND PROTOZOA PARASITES IN SCHOOL GOING CHILDREN FROM KIOTO INFORMAL SETTLEMENTS OF NAKURU TOWN, KENYA.**

Reference is made to your letter dated July 10, 2018. The KEMRI Scientific and Ethics Review Unit (SERU) acknowledges receipt of the following revised study documents on July 13, 2018;

1. Copy of the letter from SERU
2. Response page to the raised comments
3. Revised protocol (revised sections highlighted)
4. Approval documents from the county
5. CITI certificates of the P.I and the co-investigators
6. Curriculum vitae

This is to inform you that the issues raised during the 276th Ethics Review Committee (ERC) meeting of the KEMRI Scientific and Ethics Review Unit (SERU) held on **June 19, 2018** have been adequately addressed.

Consequently, the study is granted approval for implementation effective this day, **July 30, 2018** for a period of **one (1) year**. Please note that authorization to conduct this study will automatically expire on **July 29, 2019.** If you plan to continue with data collection or analysis beyond this date, please submit an application for continuation approval to SERU by **June 17, 2019**.

You are required to submit any proposed changes to this study to SERU for review and the changes should not be initiated until written approval from SERU is received. Please note that any unanticipated problems resulting from the implementation of this study should be brought to the attention of SERU and you should advise SERU when the study is completed or discontinued.

You may embark on the study.

Yours faithfully,

THE HEAD,
KEMRI SCIENTIFIC AND ETHICS REVIEW UNIT.

In Search of Better Health

Appendix II: Informed consent form for specimen collection and treatments (English format)

Study title: Prevalence, molecular characterization and risk factors associated with intestinal parasites among school going children in Gioto, London and Kaptembwa informal settlements of Nakuru town, Kenya.

Institution: Egerton University/Kenya Medical Research Institute (KEMRI)

Principal investigator: Naomi Mumbi Chege

Explanation of the purposes of the research

Your child is being asked to take part in a medical research study being performed by the Egerton University, Kenya Medical Research Institute (KEMRI) together with ministry of health services in Nakuru County. It is very important that you understand the following general principles that apply to all participants in our studies:

1) Participation is entirely voluntary.
2) Withdrawal from participation in this study is allowed at any time without penalty or any harm.
3) You are free to ask any question where u dint understand about the study for clarity.

What are intestinal parasites?

These are organisms that populate and survive from the gastro-intestinal tract in humans and other animals. They get transmitted when someone comes in contact with infected faeces (for example, through contaminated soil, food, or water). The two main types of IPs are helminths and protozoa. Helminths are worms; the most common include *roundworm*, *whipworm* and *hookworm* and *Entamoeba spp.,* which can multiply inside the human body causing diarrhoea, under-nutrition, iron deficiency anaemia and cognitive impairment in children.

Why conduct this study?

Although there is medicine available for the treatment of intestinal parasites, many infected people do not seek interventions mainly due to negligence. In Kenya, currently there is a national deworming program targeting all school-aged children in areas considered at risk of intestinal parasites. Scientists in Kenya do not know the status of these parasites among school-aged children in informal settlements of Nakuru Town. Therefore, the interest is to find out the prevalence and effect of these IPs in school-aged children in informal settlements of Nakuru Town. This will help scientists inform policy makers if there is need to include children form informal settlements of Nakuru in the National deworming program.

What is important for you to know?

To do this study, a stool sample from your child will be required. The stool will be taken to the laboratory for preparation and tested for IPs (roundworms, whipworms and hookworms, amoeba among others). Some of these tests may not be possible to perform immediately; the samples may need to be stored for a period not more than ten years. If the child is found to have any of the intestinal parasites, they will be offered treatment free of charge. Questions will be asked on aspects about life in this community. Pictures and notes may also be taken of activities in the community, which will help to explain our findings.

Your child will be assigned a study number, and the links between the name and number, and all data collected will be kept confidential. The information will be used only to find out about the parasitic infections status and how to manage them.

You and your family may not get any direct benefits from being in this study but the finding will help determine if children form informal settlements and other similar areas need to be included in the yearly national deworming program.

You can decide if you want to take part in this study. Taking part in this study will not cost you or your family anything. You may also leave the study at any time. You can leave for any reason without any problems.

Who can participate in the study?

The child will only be included in the study if you give consent to participate, and if your child agrees to participate. This work will include children between the ages of 8-13years

Risk involved

There are no risks or hazards involved in this study.

Questions about research

If you have any questions about this study, you may contact Naomi Chege the principal investigator Egerton University, Nakuru through Tel: +254716021405 during the study and in the future. If you have concerns about human rights, ethics and welfare issues you may contact the secretary KEMRI Scientific Ethics Review Unit, P.O Box 54840-00200, Nairobi; telephone 020-2722541,0722205901, 0733400003; email address: sure@kemri.org. Or Egerton university research ethic and integrity policy unit via Tel: 254-051-2217891/2 254-051-2217781, address P.O Box 536 Egerton Njoro.

Informed consent agreement

I, Mr/Mrs/Miss _____, being a person aged 18 years and over and being the lawful/legal guardian of: Mr/Miss (Child's name) ----------

---------- voluntarily agree that my child may be included in this study which I have read/has been read to me. I have been made to understand the implications and benefits of the study. I accept the tests to be carried out. I understand that I may withdraw him/her from the study at any time, for any reason, without any penalty or harm. All the above conditions have been explained to me in the _____ language in which I am fluent.

_____ Name of Child

_____ Age of child

_____ Parent's/Guardian's name

_____ Parent's/Guardian's signature

_____ Date

_____ Place

_____ Person Obtaining Consent

_____ Witness

Treatment Consent

If your child has intestinal parasites, he/she can be offered treatment. The treatments are free. Is it okay for your child to receive treatment if he/she has a worm infection?

_____ Yes _____ No

_____ Parent's/Guardian's signature

Assent for children:

Intestinal parasites live in the gut, feeding on the food that you have eaten and they affect your health. We are asking to test you for these intestinal parasites. To do this you are required to give us some fresh stool. We will treat you with standard drugs if we find you to be infected with any of these parasites.

You do not have to do this if you do not want to, but there is no harm if you do it. It might help you. Do you agree to participate?

_____ Yes _____ No

_____ Child's Name

_____ Name of person Obtaining Consent

_____ Signature

_____ Name of witness

_____ Signature

Appendix III: Informed consent form for specimen collection and treatments (Swahili version)

Fomu ya Idhini

Uchunguzi: Hali ya vimelea vya tumboni katika mitaa ya makazi yasiyo rasmi mjini Nakuru ya Gioto, London na Kaptembwa

Taasisi: Taasisi ya uchunguzi wa kiafya nchini Kenya (KEMRI).

Mchunguzi: Naomi Chege

Maelezo kuhusu madhumuni ya uchunguzi

Mtoto wako anaulizwa kushiriki katika uchunguzi wa kiafya unaoendeshwa na Taasisi ya uchunguzi wa kiafya nchini Kenya (KEMRI) wakishirikiana na wizara ya afya Nakuru. Ni muhimu uelewe kanuni zitakazotumika katika uchunguzi huu.

1) Ushiriki wako na mtoto wako ni kwa hiari yenu

2) Waweza wacha kushiriki katika uchunguzi huu wakati wowote bila kupata madhara, hasara wala kutozwa faini yoyote.

3) Ukisha soma kuhusu uchunguzi huu, tafadhali uliza maswali yatakayo kusaidia kuelewa uchunguzi huu zaidi.

Vimelea vya tumboni ni nini? Hawa ni wadudu wanoishi kwenye njia ya chakula ya binadamu na wanyama. Wanaenezwa mtu akipatana na chakula, maji, au mchanga uliona na mayai au wadudu hawa. Kuna aina mbili ya wadudu hawa, minyoo na protozoa. Minyoo ni wakubwa na mfano ni kama Minyoo, Tegu, Safura, Kichocho. Wadudu hawa husababisha kuhara, ukosefu wa damu na hata kutokuwa na kuelewa vyema.

Kwa nini tufanye uchunguzi huu?

Ingawa madawa ya kutibu vimelea hivi vya tumboni yako, waadhiriwa wengi wamekosa kuyatafuta. Nchini Kenya, kuna mpango wa kutibu vimelea vya tumboni unaolenga watoto wote wenye umri wa shule katika maeneo yaliyotajwa kuwa katika hatari ya vimelea vya tumboni. Wanasayansi wanadokeza kwamba, watoto kutoka maeneo ya makazi yasiyo rasmi katika miji mkuu kama vile Nakuru wako kwenye hatari kubwa ya vimelea vya tumboni pia. Nia yetu kwa hivyo ni kujua jinsi vimelea vya tumboni vilivyo sambaa na athari zake katika makazi yasiyo rasmi mjini Nakuru. Uchunguzi huu utasaidia kuwajulisha watunga sera kama kuna haja ya kujumulisha watoto kutoka maeneo yasiyo na makazi rasmi mjini Nakuru na maeneo mengine sawa katika mpango wa kitaifa wa kudhibiti minyoo

Nini muhimu kujua?

Kufanya uchunguzi huu, tutahitaji kinyesi cha mtoto wako. Kinyesi kitapelekwa kwenye maabara yetu na kupimwa wadudu wa tumboni (Minyoo, Tegu, Safura, Kichocho, amoeba namengine). Baadhi ya uchunguzi hayawezifanyika mara moja; twaweza kuhifadhi sampuli kwa muda usiozidi miaka kumi. Mtoto wako akipatikana na wadudu hawa watumboni atatibiwa.

Tutauliza maswali kuhusu jamii hii na twaweza chukua picha na vidokezo ambazo zaweza tusaidia kueleza matokeo ya uchunguzi huu. Twaweza tembelea nyumba tofauti na kuongea na akina mama, wazee na pia watoto katika vikundi au kwa binafsi. Mazungumuzo yetu yaweza rekodiwa ili tusikose jambo lolote muhimu.

Mtoto wako atapewa nambari kwa ajili ya uchunguzi huu, jina la motto halitafichuliwa. Tutatumia habari hii kujua juu ya wadudu wa tumboni na jinsi ya kuwadhibiti tu.

Hakuna faida kamili kwako au familia yako utakaposhiriki katika uchunguzi huu ila matokeo ya uchunguzi huu yatasaidia kudhibiti magonjwa yanayoletwa na wadudu watumboni na kujulisha serikali ikiwa kuna haja ya kuhusisha watoto kutoka makazi yasiyo rasmi jijini Nakuru na eneo zingine sawa na haya kwenye matibabu ya taifa ya wadudu wa tumboni. Ingawa utapata matibabu, haya matibabu yanapatikana pia kwenye hospitali za serikali.

Unaweza amua kama utashiriki kwenye uchunguzi huu. Kushiriki kwako hakutakugharimu wewe au familia yako chochote nawawezawacha kushiriki wakati wowote.

Nani waweza shiriki kwenye uchunguzi huu?

Twawezashirikisha mototo wako kwenye uchunguzi huu ikiwa utatoa idhinisho na kama mtoto atakubali kushiriki hasa walio umri wa miaka nane hadi kumi na mitatu.

Jasisi husika

Hakuna madhari au hatari yoyote kutokana na uchunguzi huu.

Maswali kuhusu uchunguzi

Ukiwa na swali lolote kuhusu uchunguzi huu, unawaeza kuwasiliana na Naomi Chege ambaye ni msmamizi mkuu wa uchunguzi huu kupitia nambari za simu; +254716021405 kutoka chuo kikuu cha Egerton Ikiwa una swali kuhusu haki za binadamu, maadili au shauri za maslahi, tafadhali wasiliana na afisi ya kamati ya marekebisho ya maadili kule KEMRI kupitia sanduku la posta 54840-00200, Nairobi; nambari ya simu; 020-2722541,0722205901, 0733400003; barua pepe ERCadmin@kemri.org. ama chuo kikuu cha Egerton kitengo cha maadili na marekebisho kupitia nambari za simu 254-051-2217891/2 254-051-2217781 sanduku la posta 536 Egerton Njoro

Idhinisho

Mimi Bwana/Bi_____ nikiwa na miaka 18 au zaidi na nikiwa mzazi au mlezi halali wa (jina la mtoto) _____ nakubali kwa hiari ya motto wangu ajumuishwe kwenye uchunguzi huu ambao nimesoma au nimesomewa. Nimeeelewa kiini na manufaa ya uchunguzi huu na ninakubali uchunguzi huu uendelee. Naelewa kuwa ninaweza kumuondoa mototo wangu kwenye uchunguzi huu wakati wowote bila faini au madhara yoyote. Nimeelezewa kanuni hizi zote kwenye lugha ninayoelewa vizuri

_____ Jina la mtoto

_____ Miaka ya mtoto

_____ Jina la mzazi/mlezi

_____ Sahihi la mzazi/mlezi

_____ Tarehe

_____ Mahali

_____ Jina la anayepokea Idhini

_____ Jina la shahidi

Idhinisho la kutibiwa

Ikiwa motto wako atapatikana na wadudu tumboni, anawezatibiwa. Matibabu ni ya bure. Unakubali motto wako apewe matibabu ikiwa atapatikana na wadudu wa tumboni?

_____ Ndio

_____ La

_____ Sahihi la mzazi/mlezi

Appendix IV. Data and confidentiality agreement

I,_____ in my role as a collaborator in the project **"Study title: Prevalence, molecular characterization and risk factors associated with intestinal parasites among school going children in Gioto, London and Kaptembwa informal settlements of Nakuru town, Kenya"** understands and agrees to comply with each of the following requirements:

- I will treat all information I obtain/collect from this study as confidential.
- I will not discuss the identity of each study participant or what was said by any individual participants with others, with the exception of those who are authorized to have access to the information.
- I will not use the collected information for any purposes other than this study.
- I will maintain all collected information, notes, and materials in my possession or in a secured location at all times until they are sent to the study director or research centre.
- If I use a computer to enter or store collected information, I will keep that information in password-protected electronic files only, and on a computer that has current virus protection software.
- I will report the loss of any collected information or materials or the corruption of any computer files containing collected information immediately to the study investigators.
- I will comply fully with any other data confidentiality procedures that I am instructed to follow for this study.

Your signature below indicates that you understand and accept the above requirements.

COLLABORATOR (print name)	
COLLABORATOR SIGNATURE	
DATE	
WITNESS (print name)	
WITNESS SIGNATURE	
DATE	

Appendix V: Questionnaire

Study title: Study title: Prevalence, molecular characterization and risk factors associated with intestinal parasites among school going children in Gioto, London and Kaptembwa informal settlements of Nakuru town, Kenya

1) Study No.
2) Sex: a) male b) female
3) Age:
4) Region of residence ----------
5) For how long have you lived in this residence? ---------
6) Distance of home from school a)>500m b)500M -1km c)>1km
7) Religion a) Christian b) Muslim c) other (specify)
8) Type of family a) Nuclear family b) Extended family
9) Type of shoes worn outside the house a) Closed shoes b) Slippers c) Barefoot/others
10) Type of house a) Brick/stone b) Wood only c) Iron sheet d) Mud e) Other (specify)
11) Type of floor a) Cemented b) Tiles c) Earthen d) Other(specify)
12) Parents occupation a) Formal b) Informal c) Businessperson d) Other(specify)
13) Disposal of faecal waste a) Private toilet b) Shared toilet c) Open defecation d) Cat method e) Other (specify)
14) Type of toilet facility
    a) Pit latrine b) Flush c) Non-functional flush toilet d) Others (please specify) ----------
15) Waste disposal a) Burn b) Abandoned c) Collected by garbage collectors d) Left to decompose
16) Where do you obtain the water you use for drinking?
    a) Tap water b) Borehole c) Rainwater d) Water vendors e) Others (mention)
17) Do you boil/treat the water before drinking?
    a) Yes b) No c) Use other commercial treating chemicals (mention which) ------
16) Do you have any domestic animal in your household?
    a) Dog b) Cat C) Pig D) Others(mention)
17) Do you get involved with the animal or their waste? a) Yes b) No
18) If yes how often? a) Three time a week b) Everyday c) Very rarely

Appendix vi: Key data analysis output

Bivariate Correlations																	
		Age of the child	Sex of the child	Distance from home	Eat where	Type of shoes outside house	Parent occupation	Type of house	Type of floor	Number of rooms	Number of people living in the house	Source of water	Cat presence	Goat present	Protozoa present	Helminth present	Intestinal parasites presence
Age of the child	Pearson Correlation	1	0.04	-0.08	.217**	0.052	-0.08	-0.08	-0.071	-0.049	0.095	-0.071	-0.003	-0.075	0.057	0.045	-0.028
	Sig. (2-tailed)		0.533	0.266	0.001	0.418	0.202	0.201	0.267	0.444	0.139	0.267	0.964	0.24	0.583	0.481	0.66
Sex of the child	Pearson Correlation	0.04	1	-0.03	-0.06	-.144*	.135*	.206**	0.055	-0.026	-0.09	0.074	0.064	0.086	-0.04	0.053	-0.006
	Sig. (2-tailed)	0.533		0.612	0.386	0.023	0.039	0.001	0.396	0.687	0.179	0.247	0.315	0.175	0.728	0.41	0.929
Distance from home	Pearson Correlation	-0.075	-0.034	1	0.049	-0.072	-0.08	-0.04	-0.057	0.113	0.036	0.102	-0.009	0.023	-0.02	0.071	0.037
	Sig. (2-tailed)	0.266	0.612		0.464	0.283	0.232	0.538	0.396	0.092	0.592	0.128	0.891	0.728	0.836	0.291	0.58
Eat where	Pearson Correlation	.217**	-0.056	0.049	1	0.002	-0.07	-0.04	-0.059	0.07	-0.07	0.072	-0.023	-0.099	-.248*	-0.09	-.154*
	Sig. (2-tailed)	0.001	0.386	0.464		0.971	0.265	0.529	0.359	0.277	0.264	0.259	0.717	0.121	0.015	0.185	0.016
Type of shoes outside house	Pearson Correlation	0.052	-.144*	-0.07	0.002	1	0.02	-0.06	-0.093	-0.083	0.027	-0.081	0.063	-0.068	0.17	-.139*	0.035
	Sig. (2-tailed)	0.418	0.023	0.283	0.971		0.758	0.327	0.147	0.195	0.678	0.202	0.32	0.283	0.099	0.029	0.585
Parent occupation	Pearson Correlation	-0.084	.135*	-0.08	-0.07	0.02	1	-0.05	-0.057	-.181**	-.142*	-0.03	0.074	0.096	-0.15	0.062	-0.053
	Sig. (2-tailed)	0.202	0.039	0.232	0.265	0.758		0.467	0.389	0.006	0.03	0.644	0.257	0.143	0.148	0.345	0.416
Type of floor	Pearson Correlation	-0.071	0.055	-0.06	-0.06	-0.093	-0.06	.321**	1	.200**	-0.05	.193**	0.063	0.014	0.001	0.125	-0.031
	Sig. (2-tailed)	0.267	0.396	0.396	0.359	0.147	0.389	0		0.002	0.453	0.002	0.325	0.825	0.994	0.05	0.628
Number of rooms	Pearson Correlation	-0.049	-0.026	0.113	0.07	-0.083	-.181**	-0.11	.200**	1	.173**	0.103	0.03	.230**	-.238*	.175**	-0.07
	Sig. (2-	0.444	0.687	0.092	0.277	0.195	0.006	0.075	0.002		0.007	0.108	0.637	0	0.02	0.006	0.273

	tailed)																
Number of people living in the house	Pearson Correlation	0.095	-0.086	0.036	-0.07	0.027	-.142*	-0.07	-0.048	.173**	1	-0.018	-0.084	-0.078	-0.08	0.074	0.047
	Sig. (2-tailed)	0.139	0.179	0.592	0.264	0.678	0.03	0.29	0.453	0.007		0.778	0.188	0.226	0.419	0.247	0.463
	N	246	246	222	244	246	233	244	243	245	246	246	246	246	96	246	246
Source of water	Pearson Correlation	-0.071	0.074	0.102	0.072	-0.081	-0.03	0.1	.193**	0.103	-0.02	1	0.049	0.042	-0.19	0.095	-0.085
	Sig. (2-tailed)	0.267	0.247	0.128	0.259	0.202	0.644	0.119	0.002	0.108	0.778		0.44	0.514	0.059	0.136	0.18
	N	248	248	224	246	248	235	246	245	246	246	248	248	248	96	248	248
Cat presence	Pearson Correlation	-0.003	0.064	-0.01	-0.02	0.063	0.074	0.111	0.063	0.03	-0.08	0.049	1	0.068	-0.02	0.119	-0.049
	Sig. (2-tailed)	0.964	0.315	0.891	0.717	0.32	0.257	0.083	0.325	0.637	0.188	0.44		0.285	0.888	0.062	0.439
Goat Rearing	Pearson Correlation	-0.075	0.086	0.023	-0.1	-0.068	0.096	0.005	0.014	.230**	-0.08	0.042	0.068	1	-0.1	.127*	-0.019
	Sig. (2-tailed)	0.24	0.175	0.728	0.121	0.283	0.143	0.936	0.825	0	0.226	0.514	0.285		0.318	0.046	0.762
Protozoa present	Pearson Correlation	0.057	-0.036	-0.02	-.248*	0.17	-0.15	0.139	0.001	-.238*	-0.08	-0.193	-0.015	-0.103	1	.c	.937**
	Sig. (2-tailed)	0.583	0.728	0.836	0.015	0.099	0.148	0.177	0.994	0.02	0.419	0.059	0.888	0.318		0	0
Helminth present	Pearson Correlation	0.045	0.053	0.071	-0.09	-.139*	0.062	-0.05	0.125	.175**	0.074	0.095	0.119	.127*	.c	1	.252**
	Sig. (2-tailed)	0.481	0.41	0.291	0.185	0.029	0.345	0.451	0.05	0.006	0.247	0.136	0.062	0.046	0		0
Intestinal parasites presence	Pearson Correlation	-0.028	-0.006	0.037	-.154*	0.035	-0.05	0.084	-0.031	-0.07	0.047	-0.085	-0.049	-0.019	.937**	.252**	1
	Sig. (2-tailed)	0.66	0.929	0.58	0.016	0.585	0.416	0.188	0.628	0.273	0.463	0.18	0.439	0.762	0	0	

** Correlation is significant at the 0.01 level (2-tailed).

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

c Cannot be computed because at least one of the variables is constant.

Univariate Correlations

Dependent Variable: Intestinal parasites presence

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval		Partial Eta Squared	Noncent. Parameter	Observed Power[b]
					Lower Bound	Upper Bound			
Intercept	-5.792	3.300	-1.755	.113	-13.257	1.674	.255	1.755	.348
[Goat_pres=N0]	-.329	.339	-.970	.357	-1.097	.438	.095	.970	.140
[Goat_pres=yes	0[a]								
[Cat_pres=N0]	.782	.194	4.030	.003	.343	1.221	.643	4.030	.947
[Cat_pres=Yes]	0[a]								
[sourceofwater=Piped indoor]	.701	.385	1.819	.102	-.171	1.572	.269	1.819	.370
[sourceofwater=Piped outdoor]	.315	.318	.991	.348	-.404	1.034	.098	.991	.144
[sourceofwater=Water vendor]	1.475	.761	1.938	.085	-.247	3.197	.294	1.938	.410
[sourceofwater=Rainwater]	.268	.292	.917	.383	-.393	.930	.085	.917	.130
[House_pple=2]	2.037	.623	3.270	.010	.628	3.447	.543	3.270	.828
[House_pple=3]	.430	.683	.629	.545	-1.115	1.974	.042	.629	.087
[House_pple=4]	1.125	.756	1.488	.171	-.585	2.836	.198	1.488	.266
[House_pple=5]	2.003	.666	3.007	.015	.496	3.509	.501	3.007	.763
[House_pple=6]	.879	.714	1.230	.250	-.737	2.495	.144	1.230	.197
[House_pple=7]	2.107	.776	2.714	.024	.351	3.863	.450	2.714	.677
[House_pple=8]	1.963	.692	2.836	.020	.397	3.529	.472	2.836	.714
[House_pple=9]	3.038	1.195	2.543	.032	.335	5.740	.418	2.543	.621
[House_pple=10]	2.105	.900	2.338	.044	.069	4.142	.378	2.338	.550
[no_rooms=1]	-.086	.704	-.122	.906	-1.677	1.506	.002	.122	.051

[number_rooms=2]	.026	.661	.039	.970	-1.470	1.522	.000	.039	.050
[[number_rooms=3]	.820	.791	1.038	.327	-.968	2.609	.107	1.038	.153
[[number_rooms=4]	1.718	.989	1.738	.116	-.518	3.955	.251	1.738	.343
[[number_rooms=5]	.572	.699	.818	.435	-1.010	2.153	.069	.818	.114
[number_rooms=6]	4.207	1.933	2.177	.057	-.165	8.579	.345	2.177	.493
[parent_occup=unemployed]	-1.415	1.348	-1.050	.321	-4.465	1.635	.109	1.050	.156
[parent_occup=unemployed]	-.261	1.320	-.198	.848	-3.247	2.725	.004	.198	.054
[parent_occup=farmer]	-1.284	1.323	-.971	.357	-4.276	1.708	.095	.971	.140
[parent_occup=busiessperson]	-.817	1.230	-.664	.523	-3.601	1.967	.047	.664	.092
[parent_occup=informal employment]	-1.142	1.323	-.864	.410	-4.134	1.850	.077	.864	.121
[typeofhouse=Stone/brick]	-.020	.265	-.077	.940	-.619	.578	.001	.077	.051
[typeofhouse=Wooden]	-1.012	.292	-3.470	.007	-1.671	-.352	.572	3.470	.869
[typeofhouse=ironsheet]	.203	.475	.426	.680	-.873	1.278	.020	.426	.067
[shoe_out=closed shoe]	1.162	.507	2.293	.048	.015	2.308	.369	2.293	.534
[shoe_out=slippers]	1.450	.525	2.764	.022	.263	2.638	.459	2.764	.692
[eat=home-school]	.522	.134	3.894	.004	.219	.825	.627	3.894	.932
[D_home=500m]	-.171	.253	-.675	.517	-.743	.401	.048	.675	.093
[D_home=500m-1km]	-.455	.219	-2.074	.068	-.951	.041	.323	2.074	.457
[Age=8]	-.318	.653	-.487	.638	-1.796	1.160	.026	.487	.072
[Age=9]	1.058	.393	2.690	.025	.168	1.948	.446	2.690	.669
[Age=10]	.084	.401	.210	.838	-.823	.992	.005	.210	.054
[Age=11]	.815	.415	1.963	.081	-.124	1.754	.300	1.963	.418
[Age=12]	1.062	.369	2.879	.018	.228	1.896	.479	2.879	.727

Stepwise linear logistic regression Coefficients

el	Unstandardized Coefficients		Standardized Coefficients			95.0% Confidence Interval for B		Correlations			Collinearity Statistics	
	B	Std. Error	Beta	t	Sig.	Lower Bound	Upper Bound	Zero-order	Partial	Part	Tolerance	V
(Constant)	.697	.127		5.490	.000	.443	.951					
Parent occupation	-.111	.047	-.291	-2.352	.022	-.206	-.017	-.291	-.291	-.291	1.000	1
(Constant)	.936	.159		5.871	.000	.617	1.255					
Parent occupation	-.120	.046	-.313	-2.618	.011	-.211	-.028	-.291	-.323	-.312	.994	1
Number of rooms	-.112	.048	-.280	-2.342	.023	-.208	-.016	-.255	-.292	-.279	.994	1
(Constant)	1.108	.171		6.495	.000	.767	1.450					
Parent occupation	-.114	.044	-.299	-2.591	.012	-.203	-.026	-.291	-.322	-.298	.991	1
Number of rooms	-.118	.046	-.294	-2.547	.014	-.211	-.025	-.255	-.317	-.293	.991	1
Eat where	-.275	.118	-.268	-2.329	.023	-.512	-.039	-.268	-.292	-.268	.994	1

ependent Variable: Protozoa present

Appendix VII: Publication

ORIGINAL RESEARCH

The prevalence of intestinal parasites and associated risk factors in school-going children from informal settlements in Nakuru town, Kenya

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Correspondance Naomi Chege; naomichege22@gmail.com

Abstract

Background

Intestinal parasites are a major public health problem in the developing world and have attracted increasing levels of interest from health researchers over the past decade. Epidemiology-based studies have shown that the prevalence of intestinal parasites is high and they frequently recur in regions with poor sanitation and inadequate sewerage facilities. In this study, we determined the prevalence of intestinal parasites, their egg intensities per sample, and associated risk factors in an informal settlement.

Methods

This was a cross-sectional study conducted in three randomly selected public primary schools located in the informal settlements of Nakuru town. A total of 248 stool samples were collected from asymptomatic pupils and screened, using the Kato Katz technique, for infections caused by soil-transmitted helminths (STH). A random subset of stool samples (n=96) was also screened by polymerase chain reaction (PCR) to detect intestinal protozoa. Socio-demographic variables were collected using a pre-tested structured questionnaire; these data were analysed to identify risk factors for infection.

Results

The overall prevalence of intestinal parasites was 17.3% (43/248 pupils). The overall prevalence of both STH and intestinal protozoan parasites was 1.2% and 41.7%, respectively. The most commonly diagnosed STH infection was *Trichuris trichiura* (1.2%), followed by hookworms (0.4%) and *Ascaris lumbricoides* (0.4%). The prevalence of intestinal protozoan parasites ranged from 0% to 38.5% and included *Entamoeba histolytica*, *Entamoeba hartmanni*, *Entamoeba dispar*, *Giardia intestinalis*, and *Entamoeba coli*. All infections were light, with an egg intensity <100 for each of the STH infections. The prevalence of multiple infections, including intestinal protozoan parasites, was 5.2% (n=5) and 0.4% (n=1) for STH in the subset samples. Finally, our analysis identified several significant risk factors for intestinal parasitic infections, including goat rearing (p=0.046), living in a home with an earthen floor (p=0.022), the number of rooms in the household (p=0.035), and the source of food (p=0.016).

Conclusion

The low prevalence of intestinal parasites in the informal settlements of Nakuru may be attributed to improvements in hygiene and sanitation, deworming, and general good health practices that are facilitated by the Department of Public Health.

Keywords: Prevalence, risk factors, intestinal parasites, informal settlements, school-children

Introduction

Intestinal parasitic infections are a serious public health

home for a substantial number of city residents[5]. Such affordable housing attracts low-income earners from the

Appendix VIII: Nakuru county health services permit

REPUBLIC OF KENYA
OFFICE OF THE GOVERNOR
NAKURU COUNTY

Telegrams "PROVMED"Nakuru
Tele: Nakuru 2216710 Fax 22
EMAIL: cohealth.nakuru@gmail.co
When replying please quote

CHIEF OFFICER, HEALTH SERVICES
NAKURU COUNTY
P.O. BOX 2060
NAKURU

Ref no. NCG/CDMS/GEN.VOL.1/255

9th February 2018

Dr. Elizabeth Matey
Principal Investigator
KEMRI - CMR

RE: LETTER OF AUTHORIZATION TO CONDUCT RESEARCH – HEZBON A. NYAMWENO

This letter serves as an authorization from the Department of Health Services Nakuru County Research steering committee for Dr. Elizabeth Matey to conduct research on "**Status of soil transmitted Helminthes (STH) and intestinal protozoa in informal settlements of Nakuru County".**
The County Research steering committee acknowledges receipt of Ethical clearance letter from KEMRI. The researcher by a copy of this letter is required to avail the research document (1 hard copy and a soft copy) and Research permit from NACOSTI. You are further required to present the preliminary findings to this committee and share the final publication.

E. Kiptoo
For County Director of Planning & Admin
NAKURU COUNTY

Appendix IX: Nakuru county Ministry of Education permit

MINISTRY OF EDUCATION
STATE DEPARTMENT OF EARLY LEARNING AND BASIC EDUCATION

Telegrams: "EDUCATION",
Telephone: 051-2216917
When replying please quote

Ref.CDE/NKU/GEN/4/1/21
VOL.VII/46

COUNTY DIRECTOR OF EDUCATION
NAKURU COUNTY
P. O. BOX 259,
NAKURU.

21st May, 2018

The Headteachers
Milimani Primary (London)
Prisons Primary
Kaptembwa Primary
NAKURU COUNTY

RE: RESEARCH ON STATUS OF SOIL TRANSMITTED HELMNTHES (STH) AND INTESTINAL IN SCHOOL GOING CHILDREN FROM INFORMAL SETTLEMENT OF NAKURU TOWN

Kenya Medical Research Institute will conduct the above research in the Month of June, 2018 in schools located/situated in informal settlements within Nakuru of which your school has been selected.

You are requested to ensure consent to carry out research on the pupils has been sought and granted from the parents by signing the consent letters given to their children by the researchers.

Kindly give the bearer, Dr. Elizabeth Martey, the Principal Investigator, KEMRI, any necessary assistance to spearhead this research.

Thank you.

AKOKO OKAYO
FOR: COUNTY DIRECTOR OF EDUCATION
NAKURU