THE PREVALENCE OF INTESTINAL PARASITES EGGS AND PATHOGENIC *Escherichia coli* ON THE HANDS OF SCHOOL CHILDREN IN THE VHEMBE DISTRICT OF THE LIMPOPO PROVINCE OF SOUTH AFRICA

BY

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ABSTRACT

Introduction: Intestinal infections caused by soil transmitted helminth and diarhoegenic *Escherichia coli* (*E. coli)* are a major threat to the health and socio-economic wellbeing of children in developing countries. Soil-transmitted helminthes (STH), *Ascaris lumbricoides (A. lumbricoides)*, *Trichuris tricuria (T. trichuris),* Hookworms and diarhogenic *E coli* are transmitted through the faecal-oral route and enter the body through the ingestion of eggs (STH) or *E. coli* pathogens following contact with contaminated hands, food, soil or the deliberate act of eating contaminated soil.

Aim: This study aim to determine the prevalence of intestinal parasitic infection and diarhoegenic *E. coli* on the hands of school children in the Vhembe district of South Africa.

Methods: The study was conducted among school children aged 5 to15 years, attending grades 0(R) to 8 at the primary and secondary school levels in the Vhembe district region of the Limpopo province. A total of 358 hand washing samples was collected from the hands of school children using hand anionic (7X 1% quadrafos, glycol ether and dioctyl sulfoccinate sodium salt) soap solution. The Microscopic McMaster slide technique was used for the identification of intestinal parasitic eggs and the Colilert Quanti-Tray®/2000 technique was used for the enumeration of *E. coli*. A standardised Multiplex PCR protocol was utilized to characterize the positive pathogenic *E. coli* strains obtained from the Colilert Quanti-Tray®/2000. A structural questionnaire was used to associate the positive results with selected socio-demographic variables. The raw data was organized and analysed by the use of SPSS version 24 software.

Results: A prevalence of 2.6% intestinal parasite was found among the study population with hookworm and *Enterobius vermicularis* having detection rate of 0.6% and 2.0% respectively. However there were no *Ascaris lumbricoides* and *Trichuris trichiura* detected in the study population. A prevalence of 13.4% of the samples was positive for *E. coli* and 4.7% were identified as pathogenic *E. coli* strains: Enteroaggregative *Escherichia coli* (EAEC), Atypical Enteropathogenic *Escherichia coli* (APEC), Typical Enteropathogenic *Escherichia Coli* (TPEC) and Enterotoxigenic *Escherichia coli* (ETEC) distributed with prevalence percentage of 2%, 0.3%, 1.1% and 0.3% respectively. The study also revealed a significant association between hand child hygiene with the prevalence of *E. coli.*

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Conclusion: Environmental sanitation conditions like type of toilets and lack of safe drinking water is closely associated with the prevalence of *E. coli* among the school going children.

Key words: *E. coli*, hygiene, parasite eggs, prevalence, soil-transmitted helminthes

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DECLARATION

I, Sammy Mathebula (Student no. 11540952), declare that this thesis submitted to the University of Venda for a Master's degree in Microbiology under the department of Microbiology in the School of Mathematical and Natural Sciences is my original work with exception of assistance which is acknowledged and this thesis has not been submitted to any other university or institution in partial or entirely for the award of any degree.

Signed (student): …….. Date: …….

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DEDICATION

I dedicate this thesis to God Almighty and to the entire Mathebula family

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LIST OF ABBREVIATIONS

A/E	:	attaching and effacing
bfp	:	Bundle forming pilus
DEC	:	Diarrheagenic *Escherichia coli*
DNA	:	Deoxyribonucleic acid
E. coli	:	*Escherichia coli*
Eae	:	*E.coli* attaching and effacing gene
EAEC	:	Enteroaggregative *Escherichia coli*
EHEC	:	Enterohaemorrhagic *Escherichia coli*
EIEC	:	Enteroinvasive *Escherichia coli*
EPEC	:	Enteropathogenic *Escherichia coli*
ETEC	:	Enterotoxigenic *Escherichia coli*
ELISA	:	Enzyme linked immunosorbent assay
HeLa	:	Human cervical cancer cells (HeLa)
HUS	:	Haemorrhagic uremic syndrome
LT	:	Labile toxin
PCR	:	Polymerase chain reaction
ST	:	Heat stable toxin
STH	:	Soil transmitted helminth
stx	:	Shiga toxin gene
STEC	:	Shiga toxin-producing *E. coli*
WHO	:	World Health Organisation

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CHAPTER 1

INTRODUCTION

1.1 Study rationale

Hand hygiene is an important public health issue and of great importance to the wellbeing of humans. Microbial contamination of the hands has become a global health problem despite all the public campaign that promotes washing of hands (Chinakwe *et al*., 2012). The unhygienic habits of most people lead to the various infection via hands and fingernails and also play a major role in the faecal-oral transmission of diseases (Tambekar and Shirsat, 2013). Human faecal material is generally considered to be a great risk to human health as it is more likely to contain human enteric pathogens. In poor countries, 3 million children die of enteric diseases annually and even more suffer from debilitating diseases due to intestinal parasites (Kolsky and Blumenhal, 1995). In developing countries, 80% of the diseases are associated with poor domestic and personal hygiene and about 2.2 million people (mostly children and school students) die annually due to diarrhoea (WHO, 2006). Infectious diseases that are commonly spread through hand to hand contact include gastrointestinal disorders such as diarrhoea (Timbekar and Shirsat, 2009).

Intestinal parasitic infections caused by soil transmitted helminthes are among the most prevalent human infections affecting approximately one quarter of the world's population mainly of school age children due to their poor sanitation coupled with their eating habbits (Murray and Lopez, 1994). Intestinal nematodes which cause parasitic infections include the common roundworm *Ascaris lumbricoides (A. lumbricoides)*, the whipworm *Trichuris trichiura (T. trichiura)*, and the hookworms, *Necator americanus* (*N. americanus*) and *Ancylostoma duedenale* (*A. duedenale*) from which *Acaras lumbricoides*, the whipworm *Trichuris trichiura* are the most pathogenic (WHO, 2012). The prevalence of these intestinal parasites is determined by the socio-economic and health conditions, education as well as the presence of domestic animals in the home and contamination of water and food (Amere *et al*., 2013). The infections caused by intestinal parasites are responsible for high levels of mortality and morbidity, including iron-deficiency anaemia, seizures, portal hypertension and chronic

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diarrhoea (Stansfield *et al*., 2002). Negative effects of helminth infection include diminished physical fitness, growth retardation, delayed intellectual development and cognition (Hotez *et al*., 2008). Helminthiasis occurs usually asymptomatically or produces only mild symptoms which are often neglected until serious complications or chronic clinical picture appear (Xulong *et al*., 1995). Infection persists in communities with poor housing, unclean water, and poor sanitation, poor access to health care, poor education and low income (Crompton, 1999).

The high incidence of diarrhoeal diseases and other communicable diseases among school children may be due to poor knowledge and practice of personal and environmental hygiene (Hoque, 2003). Poor knowledge, practice and attitudes to personal hygiene, such as hand washing, has negative consequences for a child's long term overall development (Globa Hand Washing Day 1, 2008). Transmission of bacterial enteric infection via hands has an important consequence on school children such as diarrhoea caused by pathogenic forms of *E. coli* due to genes which are generally not present in other *Escherichia coli* (*E. coli*) (Lau *et al*., 2012). The classification of diarrhoeagenic *E. coli* strains is based on their virulence properties and comprises of six groups which include enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), enterohemorrhagic *E. coli* (EHEC or STEC), diffuse adhering *E. coli* (DAEC) (Servin, 2005) .

Children present a potent source of transmission to the larger community through sharing of common equipment in school, playing with one another and outright auto-inoculation by means of finger biting and sucking common among children of such age (Alo *et al*., 2013). Therefore this study seeks to find the prevalence of the pathogenic intestinal parasite eggs and *E. coli* on the hands of schools children in Vhembe district of the Limpopo province, South Africa.

1.2 Problem statement

Schools are an ideal environment for the spread of infection and infectious diseases. Schools, particularly those in rural areas, often completely lack safe drinking water and sanitation facilities or have facilities that are inadequate in both quality and quantity. At highest risk of morbidity are pre-school and school aged children (Matthys *et al*., 2011). Factors like poor

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developments of hygienic habits, immune system and over-crowding contribute to infection (Jarabo *et al*., 1995). Schools with poor water, sanitation, hygiene conditions and intense levels of person to person contact are high-risk environments for children and may increase children's susceptibility to environmental health hazards (WHO, 2009). Soil-transmitted helminthes *Ascaris lumbricoides*, *Trichuris trichiura* and hookworms (*Ancylostoma duodenale* and *Nector americanus*) and pathogenic *E. coli* are the most common infection worldwide and appear to flourish in communities where the socio-economic status is dominated by poverty, poor housing, poor sanitation and a need for health education (WHO 2006, Crompton 1988). World Health Organisation (WHO) estimates that *Ascaris lumbricoides* infects over one billion people, *Trichuris. trichiura* 795 million and hookworm 740 million (WHO, 2013). According to WHO, roughly a third of all *Ascaris* and *Trichuris* infections are in children under the age of 15 years and estimates suggests that 270 million pre-schools and over 600 million school children in developing countries are living in areas where the parasite are extensively transferred (WHO, 2010). Children are the most affected by intestinal parasite due to the heavy infection they harbour and because of their vulnerability to nutritional deficiencies (Golia *et al*., 2014). School-age children typically have the highest intensity of worm infection of any age group (World Bank, 2003). Apart from causing mortality and morbidity (Legesse and Erko, 2004), the health effects such as anaemia, growth stunting, protein calorie malnutrition, fatigue and poor intellectual development tend to occur and persist in the population affected by soil-transmitted helminth. Worms are easily spread among groups of children because they play together, touch each other, visit the toilet and often do not wash hands with soap afterwards (WHO, 2012). The faecal-oral route is significant in the transmission of parasitic infection and other intestinal infection to human through poor personal hygiene (Nyarango *et al*., 2008).

When the soil becomes contaminated, the eggs can be transferred on to vegetables, door handles and then on to the hands from where it is transferred to the mouth (Alo *et al*., 2013). The adverse effects of intestinal parasites (STH) among children are diverse and alarming. Intestinal parasitic infections have detrimental effects on the survival, appetite, growth and physical fitness, school attendance and intellectual performance of school age children (Hadidjaja *et al*., 1998). Despite their educational, economic and public health importance, they remain largely neglected by the medical and international community (Bethony *et al*., 2006). The role of contaminated hands in the transmission of soil-transmitted helminth is under-researched with a limited number of studies having investigated the presence or the number of

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eggs on hand (Jeandron *et al*., 2014). Therefore, this study sought to determine the prevalence of intestinal parasite eggs and pathogenic *E. coli* on the hands of school children from six randomly selected schools which include four primary schools and two secondary schools in the Vhembe District of the Limpopo province.

OBJECTIVES OF THE STUDY

1.3.1 PRIMARY OBJECTIVE

- To determine the prevalence of intestinal parasite eggs and pathogenic *E. coli* on the hands of school children in the Vhembe district of Limpopo province

1.3.2 SPECIFIC OBJECTIVE(S)

(a) To examine the hand rinse sample for intestinal parasite eggs using the McMaster slides technique.
(b) To test hand rinse samples for the enumeration of pathogenic *E. coli* using the Colilert Quanti-tray®/2000 method from IDEXX
(c) To characterize the pathogenic strains of *E. coli* using the standardised m-PCR protocol
(d) To determine factors associated with soil-transmitted helminths and *E. coli* among children aged 5 to 16 years in selected schools in the Vhembe district

1.4 RESEARCH QUESTION(S)

(a) What is the prevalence of soil-transmitted helminths infection among children aged 5 to 16 years in selected primary and secondary schools in Mutale and Thulamela municipalities in the Vhembe district of the Limpopo province?
(b) Which pathogenic *E. coli* strains are common in the hands of school children aged 5-16 years in selected primary and secondary schools in Mutale and Thulamela municipalities in the Vhembe district of Limpopo province?
(c) What are the risk factors associated with the prevalence of soil-transmitted helminthes and pathogenic strain of *E. coli.*

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1.5 RESEARCH HYPOTHESIS

Intestinal parasite (soil-transmitted helminths) eggs and pathogenic *E. coli* are prevalent on the hands of school children in the rural areas of the Vhembe region of Limpopo province.

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CHAPTER 2

LITERATURE REVIEW

2. INTRODUCTION

2.1 Global and regional overview of STHs and *E. coli* as well as its impact

Soil-transmitted helminths (STHs) are a group of the intestinal parasite causing human infection through contact with parasite eggs or larvae that grow well in warm and moist soil (WHO, 2005). This group belongs to the class Nematoda, which includes roundworms (*Ascaris lumbricoides*), whipworms (*Trichuris trichiura*), and two hookworms (*Ancylostoma duodenale* and *Necator americanus*) (Centre for Disease Control and Prevention, 2013). About 2 billion people in the world are infected with at least one species of soil transmitted helminths with 1 billion infections due to *Ascaris lumbricoides*, *Trichuris trichiura,* 800 million and hookworm, 740 million and 4 billion are at risk of infection (WHO, 2012). *Ascaris lumbricoides* and *Trichuria trichiura* are primarily spread through faecal transmission usually through ingestion of parasite eggs in faeces (Abera *et al*., 2013). Warm climates and adequate moisture, lack of personal or environmental hygiene, sanitation, and education, walking barefoot and poor health or nutritional status could increase the risk of STH infections (Alemu *et al*., 2011). The burden of soil-transmitted helminths infections is being increasingly recognized as a significant public health problem, especially in poor populations in sub-Saharan Africa (Uneke, 2010). Soil-transmitted helminths aggravate malnutrition and retard children's physical development (Luong, 2003) and they also destroy the tissues and organs in which they live and cause abdominal pain, diarrhoea, intestinal obstruction, anaemia, ulcers and various health problems.

Although these infections are not among the biggest diseases killers, their chronic infections cause morbidity in children that affect cognitive development and growth (Friedman, 2012; Knopp *et al*, 2008). STH infections cause higher disability than mortality, therefore, their burden of disease is better measured in disability adjusted life years (DALYs), the number of years lost due to ill-health, disability or early death (WHO, 2012).

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Table 2.1: Global Prevalence and Distribution of Helminth Infections (de Silva *et al*., 2003)

Helminth infections	Total cases	Major geographic areas
STH infections	2 billion	
Ascariasis	1.221 billion	Sub-Saharan Africa, India, China and East Asia
Trichuriasis	795 million	Sub-Saharan Africa, India, China and East Asia
Hookworm	740 million	Sub-Saharan Africa, Americas, China and East Asia
Schistosomiasis	187 million	
Schistosomiasis haematobium	119 million	Sub-Saharan Africa
Schistosomiasis mansoni	67 million	Sub-Saharan Africa, Americas
Schistosomiasis japonicum	1 million	China and East Asia

2.1.1. *Ascaris lumbricoides (A. lumbricoides)*

The human roundworm *A. lumbricoides* is one of the most common parasites in the world, infecting 1.2 billion people globally (de Silver *et al*., 2003). Infections are most commonly documented in sub-Saharan Africa, the Americas, China and East Asia (Table 2.1) (WHO, 2006). *A. Lumbricoides* is a prominent parasite in both temperate countries and tropical zones but it is more common in warm temperate countries and more prevalent where sanitation is poor (Dangana *et al*., 2011). *A. lumbricoides* occurs at all ages, but it is more prevalent in the 5-9-year-old group of pre-school and young children who are frequently exposed to contaminated soil than the adults (Dangana *et a*l., 2011). Ascariasis is transmitted through the faecal-oral route, eggs are ingested following contact with contaminated hands, food, soil or the deliberate act of eating contaminated soil. Infective *A. lumbricoides* eggs can survive and remain infective for several months or even for years in soil (Jeandron *et al*., 2014). While the majority of infections are asymptomatic, an estimated 8-15% (120-220 million cases) of those infected with *A. lumbricoides* demonstrates associated morbidity (Chan, 1997). The manifestations of ascariasis can be broadly characterised into acute and chronic symptoms. Human hosts tend to experience acute lung inflammation in breathing and fever as a result of larval migration through the pulmonary tissue. Abdominal distension and pain, nausea and

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diarrhoea are also characteristic symptoms of adult worm infection and chronic Ascariasis (Crompton, 2001). Adult worms usually reside in the jejunum but can be found in the entire small intestine especially when they are present in large numbers (Bethony *et al*., 2006). These worms live freely in the intestinal lumen, not attached to the mucosa and absorb nutrients directly from the hosts' intestinal content through their buccal cavity which is oriented to the intestinal flow (Anderson, 1992). A large number of worms can result in worm masses that may eventually obstruct the intestinal tract and require surgical intervention. In addition, adult worms can penetrate the intestinal wall causing local haemorrhage, peritonitis, and appendicitis (Hotez *et al*., 2003).

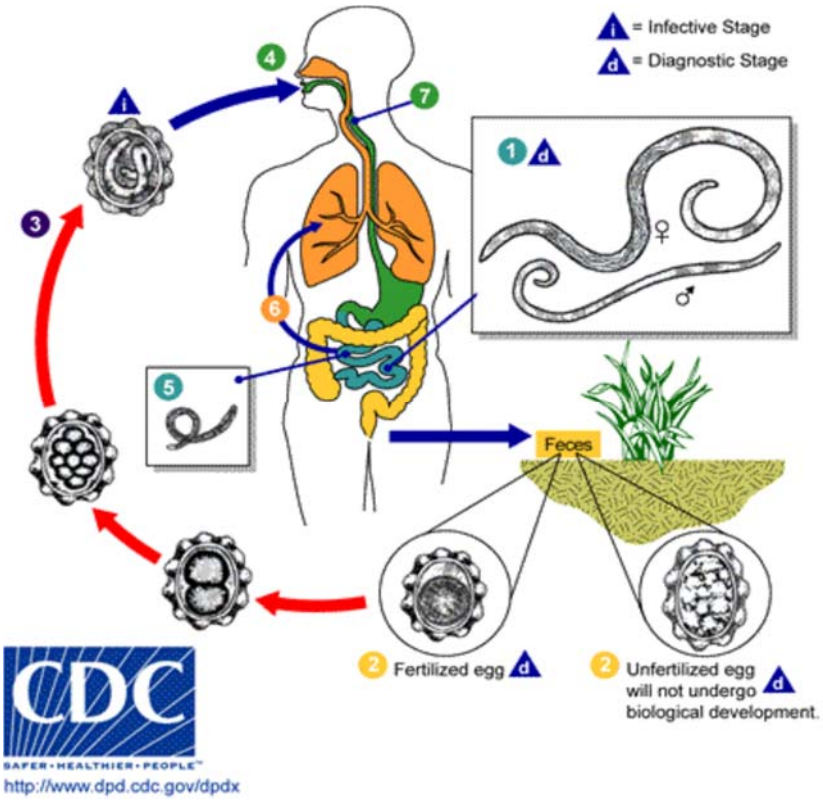

Figure 2.1 Life cycle of *Ascaris lumbricoides* (CDC, 2012)

The life cycle of *A. lumbricoides* is illustrated in Figure 2.1 Following the ingestion of infective eggs and when in contact with bile, L2 larvae hatch in the small intestine where they penetrate the mucosa and migrate to the lungs using the portal and systemic circulation. Once in the lungs, larvae grow and undergo two additional molts becoming fourth stage larvae (L4) (Bethony *et al*., 2006). At this point, L4 larvae penetrate the alveolar space, ascend to 7 the pharynx, are coughed up and then swallowed, returning this way to the small intestine. Once

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in the intestine, L4 larvae grow and undergo their last molt developing into adult worms. It takes between 2 - 3 months from the ingestion of the infective eggs to oviposition by mature female worms (Bethony *et al*., 2006, Hotez *et al*., 2003).

2.1.2. *Trichuria trichiura* (T. trichiura)

Trichuriasis is transmitted through accidental ingestion of contaminated soil or unwashed vegetables fertilized with human faeces. In countries where Trichuriasis is prevalent, it poses a threat to the healthy growth and development of millions of school children (de Silva, 2003). Once inside the body, whipworm eggs migrate to the small intestine and hatch into adult worms which embed themselves in the lining of the large intestine and colon. Adult female worms oviposit into the cecum at a rate of 5000 to 7000 eggs per day and can do so for up to 5 years. *Trichuris* eggs have not started to develop and are not infectious when they are excreted. It may take 2 weeks or longer for these eggs to begin embryogenesis and thus become infectious. The eggs are passed out of the body via the stool of the human host; if inadequate sanitation exists, they will again be transmitted into the soil, beginning the cycle again (Chang *et al*., 2008).

Children are at high risk for whipworm because they often play outside in the dirt or soil and put their hands in their mouths without washing them. Although the majority of infected individuals remain asymptomatic, a significant number of Trichuriasis patients, especially children with long-standing massive infections, have dysenteric syndrome presenting with chronic mucous diarrhoea, rectal prolapse, anaemia from chronic blood loss and iron deficiency, protein-energy malnutrition, and growth retardation (Ramdath *et al*., 1995).

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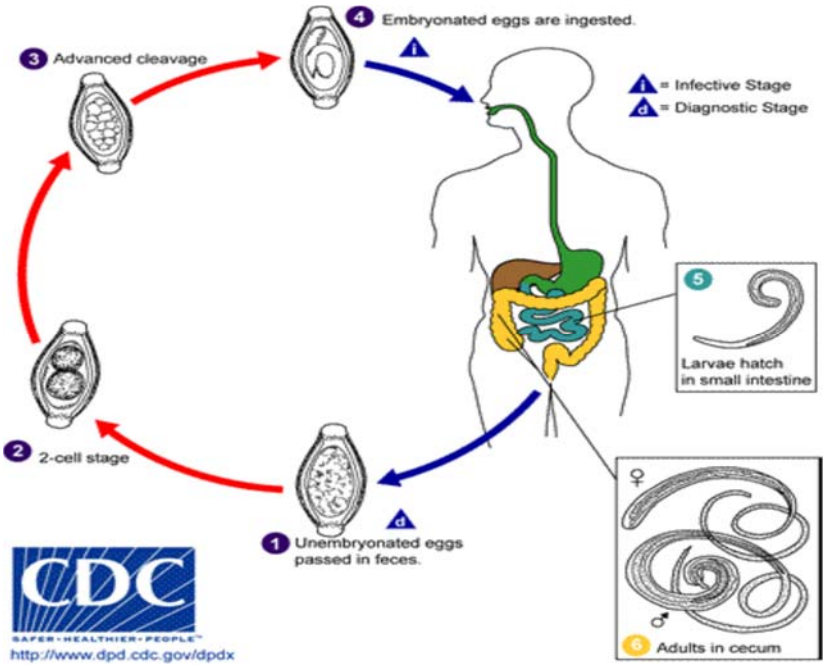

Figure 2.2: Life cycle of *Trichuris trichiura* (CDC, 2012)

Life Cycle of whipworms (*Trichuris trichiura*) as illustrated in Figure 2.2. The unembryonated eggs are passed with the stool. In the soil, the eggs develop into a 2-cell stage an advanced cleavage stage and then they embryonate, eggs become infective in 15 to 30 days. After ingestion (soil-contaminated hands or food), the eggs hatch in the small intestine and release larvae that mature and establish themselves as adults in the colon. The adult worms live in the cecum and ascending colon. The adult worms are fixed in that location, with the anterior portions threaded into the mucosa. The females begin to oviposit 60 to 70 days after infection. Female worms in the cecum shed between 3,000 and 20,000 eggs per day. The life span of the adults is about 1 year. (CDC, 2012)

2.1.3. Hookworms

Hookworm infection in humans is caused by an infection with *Necator americanus* and *Ancylostoma duodenale* and is transmitted through contact with contaminated soil. It is one of the most common chronic infections, with an estimated 740 million cases in areas of rural poverty in the tropics and subtropics (de Silva *et al*., 2003). Human beings are regarded as the only major definite host for these parasites, where the adult hookworms of the genera *Necator* and *Ancylostoma* parasitize the upper part of the host small intestine (Despommier *et al.*, 2005, Crompton, 2001). The major pathology of hookworm infection, however, results from

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intestinal blood loss as a result of adult parasite invasion and attachment to the mucosa and sub mucosa of the small intestine. Hookworms have long been recognized as an important cause of intestinal blood loss leading to iron deficiency and protein malnutrition. The iron deficiency anaemia that accompanies moderate and heavy hookworm burdens is sometimes referred to as hookworm disease (Hotez *et al*., 2004).

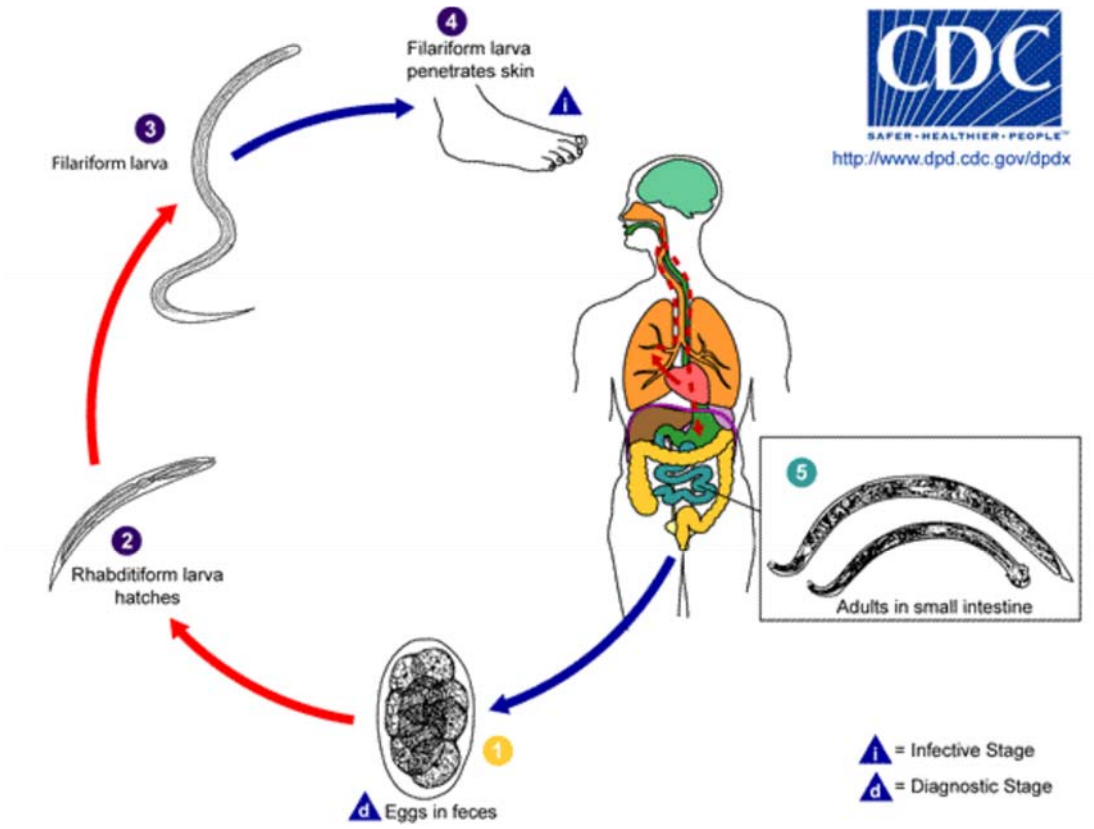

Figure 2.3: Life cycle of Hookworms (CDC, 2012)

Life cycle of hookworms as illustrated in Figure 2.3. Eggs are passed in the stool (1), and under favorable conditions (moisture, warmth, shade), larvae hatch in 1 to 2 days. The released rhabditiform larvae grow in the feces and/or the soil (2), and after 5 to 10 days (and two molts) they become filariform (third-stage) larvae that are infective (3). These infective larvae can survive 3 to 4 weeks in favorable environmental conditions. On contact with the human host, the larvae penetrate the skin and are carried through the blood vessels to the heart and then to the lungs. They penetrate into the pulmonary alveoli, ascend the bronchial tree to the pharynx, and are swallowed (4). The larvae reach the small intestine, where they reside and mature into adults. Adult worms live in the lumen of the small intestine, where they attach to the intestinal

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wall with resultant blood loss by the host (5). Most adult worms are eliminated in 1 to 2 years, but the longevity may reach several years.

2.1.4 Epidemiology of Soil-transmitted helminths

Soil-transmitted helminths infection are widely distributed throughout the tropical and subtropical (WHO, 2010). Climate is an important determinant of transmission of these infections with adequate moisture and warm temperature essential for larval development in the soil (Bethony *et al*., 2006). Equally important determinants are poverty and inadequate water supplies and sanitation (Brooker *et al*., 2006). Because STHs are transmitted through poor sanitation and hygiene, school aged children are typically at increased risk resulting in high prevalence and intensity of infection due to a high level of exposure (Montresor *et al*., 1998; WHO, 2002). The prevalence of Soil-transmitted helmith is higher in the tropical central highlands than in drier areas. However, the prevalence of soil-transmitted helminths differs in each school due to different environmental conditions such as climate, humidity, ecology, and soil type. Thus a survey of intestinal helminths and risk factors in the primary school population provides epidemiological information to design effective control programmes (Montresor *et al*., 2002). There is continual need to call the attention of the world to the burden of STH infections, some of which are among the neglected tropical diseases. The total burden of diseases due to STH infections and its consequences could be prevented in high-prevalence communities by massive de-worming of school age children (WHO, 2013). Knowledge of the distribution and extent of intestinal helminth infections in rural communities is thus essential for planning for the control and for effective treatment of soil transmitted helminth (Babatunde *et al*., 2013).

Estimates worldwide indicates that 4.5 billion people are at risk of STH infection and the global estimates of number of cases of *Ascaris lumbricoides* is 1.221 billion people, *Trichuris triciura* 795 million and hookworms 740 million, resulting in up to 135,000 deaths annually (Bethony *et al*., 2006; Hotez, 2009). The greatest numbers of STH infections occur in sub-Saharan Africa, China, India, East Asia and South America (Brooker *et al*., 2006). In most poor population of sub-Saharan Africa, the burden of soil-transmitted helminths is being increasingly recognised as a major public health problem, especially as a result of inadequate sanitation, lack of clean drinking water, poor hygiene, poverty and malnutrition (Phiri *et al*., 2000). Studies conducted on soil-transmitted helminths across Africa have shown wide

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distribution. A study conducted in Ozubulu, Anambra State, Nigeria by Ezeagwuna *et al*., (2009) to determine the prevalence of STH infection among the pupil from 4 schools found that the overall prevalence was 48.08% with *Ascaris lumbricoides* being 15.38%, *Trichuris Trichiura* and hookworm accounting for 25.38%. The females had the higher prevalence rate 55.47% than the males with rate of 39.84 (Ezeagwuna *et al*., 2009). A study done in Ethiopia, Butajira by Belyhum *et al*., (2010) on the prevalence and risk factors for soil-transmitted helminth infection in mothers and infants found that the hookworm was the most common intestinal helminth infection, detected in 36.1% of mothers and in 2.3% infants followed by *Ascaris lumbricoides* detected with prevalence of 8.8% in mother and 1.5% in children and the prevalence *Trichuris trichiura* was 3.2% in mothers and 1.1% in children. In South Africa, a study conducted in Mthatha, Eastern Cape Province to determine the prevalence of intestinal parasite in primary school children showed that *Ascaris lumbricoides* was the most common pathogen observed with 29.0% and *Trichuris trichiura* 3.7%. The findings showed no significant different in parasitic infection between urban and rural learners, gender and the age of the learners (Nxasana *et al*., 2013). Another study done by Muller *et al*., (2016) on intestinal parasite among schoolchildren in Port Elizabeth, South Africa found that *Ascaris lumbricoides* was the predominant parasite with the infection rate of 26% and *Trichuris trichiura* was found to be 22%, however hookworm were not observed on this study. Different studies revealed that poor sanitary condition such as open defecation and contamination of water bodies by faecal matter are the most important factors leading to the prevalence of intestinal parasite (Brooker *et al*., 2008)

2.1.5 Diagnosis of Soil transmitted Helminths

Soil-transmitted helminthes infections are typically diagnosed by identifying the parasites' eggs in a microscopic examination of stool specimens. With the proper training, species can be easily identified based on their eggs' morphological features (Bogitsh et al., 2012). Laboratory diagnosis of soil-transmitted helminth includes several egg concentration techniques e.g formalin ethyl acetate sedimentation which can detect even light infections. The Kato-Katz faecal-thick smear and the McMaster method are used as diagnostic tools to measure the intensity of infection by estimating the number of egg counts per gram of faeces (Santos *et al*., 2005). Other diagnosis for STHs may be the use of clinical symptoms which may include loss of appetite, reduced absorption of food intake, Vitamin A deficiency, anaemia, malnutrition, intestinal obstruction and abdominal pain result in cases of heavy

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infection with worms (Edelduok *et al.,* 2013). Levels of endemicity of STHs and degrees of intensity of infection were categorized according to 2011 WHO recommendations, i.e. *A. lumbricoides* infections: 1-4 999 epg; 5 000-49 999 epg and >50 000 epg for low, moderate and high intensity respectively, *T. trichiura* infections: 1-999 epg; 1 000-9 999 epg and >10 000 epg for low, moderate and high intensity respectively, Hookworm infections with 1-1 999 epg; 2 000-3 999 epg and >4 000 epg counts for low, moderate and high intensity respectively (WHO, 2011).

2.1.6 Risk factors associated with the prevalence of STH infection

(a) Behaviour, Household Clustering and Occupation

Specific occupations, household clustering and behaviours influence the prevalence and intensity of soil-transmitted helminth (STHs) infections (Bethony *et al*., 2006). STHs infections are more prevalent among the vulnerable segment of the population and are associated with poverty, limited access to clean water, poor personal and environmental sanitation, overcrowding, tropical climate and low altitude (WHO, 1987). Good hygiene behaviour and the effectiveness of hygiene promotion in schools are severely limited where water supply and sanitation facilities are inadequate or non-existent (WHO, 2009). Because of the high rates of hookworm infection among adults, occupation probably has a greater influence on hookworm epidemiology. Engagement in agricultural activities remains a common denominator for soil-transmitted helminths in humans, particularly hookworm infection (Hotez, 2002).

(b) Poverty and Urbanization

STHs are closely associated with poverty, poor sanitation and lack of clean water and depend for transmission on environments contaminated with egg carrying faeces. The establishment of safe water and improved sanitation are essential for the control of helminth infection. Even though STH infections are neglected diseases that occur predominantly in rural areas, social and environmental conditions in the unplanned slums of developing countries are ideal for the persistence of *A. lumbricoides* and *T. trichiura*. (Crompton and Savioli, 1993). *Ascaris* and *Trichuris* commonly occur both in urban environments, more especially urban slums and in rural areas and in some instances, the prevalence of *Ascaris* infection is actually greater in urban environments (Phiri *et al*., 2000). Many surveys have shown a high prevalence of these

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infections in children of slums, shanty towns and squatter settlements (Crompton and Savioli, 1993).

(c) Climate, Water and Season

Adequate warmth and moisture are major features for the prevalence of STHs. *Ascaris* and *Trichuris* eggs have harder surfaces than hookworm and therefore are able to survive drier climates better, however, the rates of infection are low in arid climates (Brooker and Michael, 2000). It has been suggested that total rainfall in an area and its seasonal distribution may also help explain observed patterns of infection, wetter areas are usually associated with increased transmission of all three major STHs infections (Brooker and Michael, 2000).

(d) Sanitation and open defecation

The physical environment and cleanliness of a school facility can significantly affect the health and well-being of children. Human excreta are the biggest source of disease-producing organisms including parasites, bacteria and viruses (WHO, 1997). It is becoming increasingly difficult to ignore the serious consequences that open defecation has for school-aged children, such as high risk of hygiene-related morbidity and mortality. Factors such as poor maintenance, smelly and dirty latrines, lack of sanitation facilities, overcrowding and financial management play an important role in determining whether children will use the latrines or not (Ebong, 1994; Vernon *et al*., 2003, Lundblad, 2007). Therefore, success in eliminating faecal material from the school environment is dependent on, informed and responsible students, supervision of young children, toilets conveniently located, reliable, clean, odour-free, private, and well-maintained, a fence to stop animals from defecating in areas where children play (WHO, 1997). Younger children may require toilets of different dimensions than do older children and adults, and specific features need to be taken into account to make the toilets easy and comfortable to use (WHO, 2009). The disease spreads quickly in overcrowded spaces with limited ventilation, where hand-washing facilities or soap are not available and where toilets are in poor condition. Too often, schools are places where children become ill (WHO, 2009).

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2.2 *Escherichia coli* (*E. coli*)

Escherichia coli (*E. coli*) are Gram-negative bacilli and a member of the family *Enterobacteriaceae* encountered as a normal inhabitant of the human and other mammals intestines (Kaper *et al*., 2004). There are *E. coli* strains that are harmless commensals of the intestinal tract and others that are major pathogens of humans and animals (Sousa, 2006). Since *E. coli* is a part of the normal intestinal flora, the primary habitat of *E. coli* is the large intestine of warm blooded animals and its present in the environmental samples, food or water usually indicates recent faecal contamination or poor sanitary practices (Rompré *et al*., 2002) and therefore it is considered as an indicator of faecal contamination when it is present in water and food (Humbert *et al*., 2000). Infection due to pathogenic *E. coli* may be limited to colonisation of a mucosal surface or can disseminate throughout the body and have been implicated in urinary tract infection, sepsis or meningitis and gastrointestinal infection (Sousa, 2006). Pathogenic forms of *E. coli* causes a variety of diarrhoeal diseases in host due to the presence of specific colonisation factors, virulence factors and pathogenicity associated genes which are generally not present in other *E. coli* (O'Sullivan *et al*., 2006). The indices of pathogenicity among *E. coli* strains include pili, K- antigen, haemolysin, adhesive factor, enterotoxins, cytotoxins, effacement factors and cytotoxic necrotic factors (Galane and Le Roux, 2001). Diarrhoegenic *E. coli* (DEC) strains are one of the important causes of childhood diarrhoea around the world, especially in developing countries (Rodriguez, 2002). Animals, human beings and the environment serve as the natural habitats of strains of *E. coli*. Diarrhoegenic *E. coli* infection is always associated with poor sanitation and personal hygiene (Tumwine *et al*., 2002).

E. coli strains that causes diarrhoea can be classified into six categories based on their mechanism of pathogenesis and clinical diagnosis and these include: Enterotoxigenic *E. coli* (ETEC) which represents one of the most common cause of traveller's diarrhoea and causes acute watery diarrhoea (Gaastra and Svennerholm, 1999), Enteropathogenic *E. coli* (EPEC) which causes attaching and effacing lesion resulting in osmotic diarrhoea, Enterohaemorrhagic *E. coli* (EHEC) which cause haemorrhagic colitis or hemolytic-uremic syndrome (HUS), Enteroinvasive *E. coli* (EIEC) which causes *shigella*-like dysentery, Enteroaggregative *E. coli* (EAEC) which is primarily associated with persistent diarrhoea in children in developing

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countries and diffusely adherent *E. coli* (DAEC) which may induce inflammatory bowel diseases (Rodriguez, 2002).

2.2.1 Enterotoxigenic *Escherichia coli* (ETEC)

ETEC is an important cause of childhood diarrhoea in the developing countries where sanitation and clean supplies of drinking water are inadequate and it is a major cause of traveller's diarrhoea worldwide (Jafari *et al*., 2012). Enterotoxigenic *E. coli* (ETEC) are estimated to cause 600 million cases of human diarrhoea and 800,000 deaths worldwide mostly in children under the age of 5 (WHO, 1999). ETEC produce toxin which are heat-labile (LT) or heat-stable (STa and STb) that also causes diarrhoea (Kagambega *et al*., 2012). ETEC causes watery diarrhoea, which can vary from mild in nature, self-limiting to severe, cholera-like illness where rapid dehydration can be life-threatening (Sixma *et al*., 1991). Symptoms range from abdominal cramps, sometimes with nausea and headache (Dedeic-Ljubovic *et al*., 2009). It is primarily spread through food or water contaminated with human waste and the infectious dose is estimated to range from 10^6 to 10^{10} organisms. However immune compromised or vulnerable population such as children and the elderly may be susceptible to infection at lower doses (Gupta *et al*., 2007).

On infection, ETEC first establishes itself by adhering to the epithelium of the small intestines via one or more colonization factor antigen (Kaper *et al*., 2004). ETEC produces two toxin, heat-stable toxin (ST) and heat-labile toxin. These enterotoxins cause inhibition of sodium absorption and stimulation of secretion, which leads to watery diarrhoea (O'Sullivan *et al*., 2006).

2.2.2 Enteropathogenic *Escherichia coli* (EPEC)

EPEC are strains that has the ability to cause diarrhoea and that causes attaching and effacing (A/E) lesions which adhere to epithelial cells in distinctive pattern called localized adherence (Nataro and Kaper, 1998). Transmission of EPEC is via faecal-oral route with contaminated hands, contaminated surfaces and weaning foods or contaminated fomites serving as vehicles (Levine and Edelman, 1984). These organisms are a significant cause of infant diarrhoea in developing countries. Transmission occurs through contaminated hands and weaning foods

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with infective dose equal 10^{10}. Reservoirs of infection are both children with and without infection and adult carriers (Franzolin *et al*., 2005). Following ingestion, EPEC adheres to the epithelial cells of the intestine, causing either watery or bloody diarrhoea. Bloody diarrhoea is associated with attachment and an acute tissue-destruction process. Low grade fever and vomiting are also associated with infection (O'Sullivan *et al*., 2006). The pathogenesis of EPEC is unique for enteric bacteria pathogen since it is essentially non-invasive and produces no toxins.

The central mechanism of EPEC strain is the characteristic A/E lesion, which involve microvilli destruction, intimate adherence of bacteria to the intestinal epithelium, pedestal formation and aggregation of polarized actin and other elements of the cytoskeleton at sites of bacteria attachment (LEE). EPEC virulence genes are encoded on plasmid (bfp) and the chromosome (eae) (Trabulsi *et al*., 2002).

2.2.3 Enterohaemorrhagic *E. coli* (EHEC)

EHEC also known as Shiga-like toxin producing *E. coli* or Vero cytotoxin-producing *E. coli* characterised by its ability to produce a toxin that is cytotoxic to human cervical cancer cells (HeLa) and Vero cells (Ismaili *et al*., 1995). Shiga toxins produced by EHEC strains are among the potent of all bacterial toxins and constitute of two distinct types of toxin, Shiga toxin 1 (Stx1) and Shiga toxin 2 (Stx2) (Takeda, 1993). EHEC have the ability to form attaching and effacing lesions (A/E lesions) on epithelial cells of the host (O'Sullivan *et al*., 2006). The transmission of EHEC may occur by the ingestion of contaminated food, water or by person-to-person contact or by zoonotic transmission. Human infection with EHEC is associated with a range of symptoms from non-bloody diarrhoea, fever and vomiting through haemorrhagic colitis and haemolytic uramic syndrome (HUS). The presence of EHEC in the environment another course for concern because this pathogen can survive in the soil, manure, pasture and water which thus represent the significant vehicle of transmission (Lascowski *et al*., 2013). The infection dose is low around 10 to 100 organism which may facilitate spread from person to person at any age but usually affects the very young and the elderly and where hygiene conditions are poor (Vilchez et al., 2009).

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2.2.4 Enteroinvasive *E. coli* (EIEC)

EIEC are an important cause of diarrhoeal disease and they are responsible for a bacillary dysentery-like illness and are associated with *Shigella* dysenteriae (Hart *et al*., 1993). EIEC are a significant cause of morbidity and mortality in young children in developed countries and are more important in developing countries where sanitation and hygiene levels are of a poor standard (Hart *et al*., 1993). EIEC are transmitted through the faecal-oral route. Following the ingestion of EIEC the organisms invade the epithelial cells of the intestine resulting in a mild form of dysentery often mistaken for dysentery caused by *Shigella* species. The illness is characterized by the appearance of blood and mucus in the stools of infected individuals. Characteristic features of EIEC strains are their ability to induce their entry into epithelial cells and disseminate from cell to cell (Smith *et al*., 2004). A small number of bacteria need to be swallowed from 10-100 organisms as they are relatively resistant to gastric acid and bile and pass readily in to the large intestine where they multiply in the gut lumen. The infection is characterized by fever, severe abdominal cramps, malaise and watery diarrhea accompanied by toxaemia (James and James, 1998).

2.2.5 Enteroaggregative *E. coli* (EAEC)

EAEC are a significant cause of diarrhoea in developing countries and are epidemiologically associated with acute or persistent diarrhea (Nataro *et al*, 1998). EAEC strains are characterised by their ability to aggregatively adhere to tissue culture cells in a distinctive “stacked, brick-like” manner. Aggregative adherence in EAEC is mediated by either aggregative adherence fimbrae I (AAF/I) or AAF/II, which are encoded for by aggR genes. Malnourished hosts, especially children living in developing countries, may be unable to repair mucosal damage and thus may become prone to persistent or chronic diarrhoea (Jiang *et al*., 2003). Risk factors for EAEC include travel to developing countries, ingestion of contaminated food and water, poor hygiene, host susceptibility and possibly immunosuppressant HIV infection (Huang and DuPont, 2004). To initiate disease approximately 10^6 -10^8 of bacteria are required with appearing of different symptoms such as watery and mucoid diarrhea, vomiting, dehydration and occasionally abdominal pain and bloody stools (Hebbelstrup *et al*., 2014).

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2.2.6 Diffusely adherent *E. coli* (DEAC)

DAEC are comprised of heterogeneous groups of organisms with variable virulence. DAEC are divided into two classes, those which harbour afimbrial adhesins (Afa)/Drori antigen (Dr) adhesions and those that express an adhesin involved in diffuse adherence, which is a potential cause of infantile diarrhoea (Nataro and Kaper, 1998). DEAC uses adhesins to attach to the intestinal cells and are thus essential for the colonisation (Servin, 2005). Infection with DAEC causes diarrhea by binding to the plasma membrane of the intestinal cells. DAEC binds to the intestinal cells using a fimbrial adhesin (F1845) and the decay accelerating factor (DAF) and causes the F-actin on the surface of the intestinal cells to disassemble. The signalling pathway of tyrosine kinase Src-likefamily proteins is activated and causes further actin disassembly inside cells and all this result in the elongation of microvilli. The damage to the cells causes loss of electrolytes and apoptosis, leading to diarrhea (Fivaz, 2000). In developing and developed countries, DEAC has been associated with watery diarrhoea that can become persistent in young children as well as recurring urinary tract infection (Croxen, 2010; Servin, 2005).

2.3. Summary of literature review

Soil-trasmitted helminthes (STHs) infections and Diarrheagenic *Escherichia coli* (DEC) are among the most common cause of chronic infections affecting humans in developing countries (WHO, 2012). Infection thrives and persists in communities in need of better housing, clean water, appropriate sanitation, better access to health care, education and increased personal earnings (Crompton 1999).The unhygienic habits of most of the people lead to the various infections via hands and fingernails (Tambekar and Shirsat, 2013). Contaminated hands play a major role in faecal-oral transmission of diseases (Ray *et al*, 2011). Transmission of bacterial enteric infections via hands has important consequences on students, as they are more likely to take meal and water without washing hands therefore they are posed to risk of infection (Lau *et al*, 2012). Approximately 2 billion people are reported to be parasitized with helminthic worms, majority of them living in poor-resource settings of sub-Saharan Africa (Bethoney *et al*., 2006). Soil transmitted helminth infection constitute a major public health challenges among school-age children in Sub-Saharan Africa (Uneke, 2010). Studies indicates that most young children affected by intestinal helminths and pathogenic *E. coli* are from poor background, citing poor sanitary conditions as a major reason for the increase in the spread of

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these infections. The unhygienic and common practice of people defecating indiscriminately or dumping excrement nearby bushes, underneath bridges, along bush tracks, river banks, on open fields and on water bodies are reported to be the factors that exacerbate the prevalence of these infections (Xuan *et al*., 2012). STHs and DEC are transmitted through poor sanitation, poor hygiene and by contact with infected freshwater streams or lakes and school-aged children are usually at high risk due to high level of exposure resulting in high prevalence and intensity of infection (WHO, 2012). Helminths infection results in consequences such as decreased body resistance, retardation of physical and mental development of children, indigestion, diarrhoea, anorexia and lack of memory, increased morbidity rate, greater incidence of abortion, sterility, stillbirth and anaemia leading to school absenteeism by children (WHO, 2002). The link between contaminated hands and infectious disease transmission is one of the best-documented phenomena in clinical science but there is great need to focus on school community (Tambekar and Shirsat, 2013).

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CHAPTER 3

MATERIALS AND METHODS

3.1 Ethical Approval

Ethical clearance was obtained from the University of Venda's Higher Degree committee and the Vhembe district's Department of Education. Approval for access to schools were obtained in consultation with school principals and grade teachers and since the study involved children, both parent/ guardian consent and children's approval were required prior to inclusion in this study. Informed consent forms were provided and signed by the school principals and parent/ guardian of the children who took part in this study. Children, whose parents consented, were invited to participate in the study and all details of the study were properly explained, including their voluntary participation and right to withdraw at any time of the study.

3.2 Study area and communities

This study was conducted in the rural communities of the Mutale and Thulamela municipalities of the Vhembe District. Vhembe district Municipality is located in the Northern part of Limpopo Province and shares boarders with Capricorn, Mopani District municipalities in the eastern, and western, directions respectively. The District covers 21 407 square km^2 of land with a total population of approximately 1.240 035 million people (Stats SA, 2007) of which 1.1% of the people stay in urban area. The population mainly comprised of 54.4% women and 45.5% men, with 51.3% of the population being under the age of 20 years. The district settlement pattern is largely rural with approximately 774 dispersed villages and 287 190 households. Vhembe climate is typically subtropical, with mild, moist winters and wet, warm summers characterised by Lowveld (Arid and Semi-Arid). The area experiences annual rainfall of approximately 500 mm per annum of which approximately 87.1% rain falls between October and March. The land is very fertile and good for agriculture. The rendering of quality education in the district is negatively affected by dilapidated and shortage of classrooms and administration blocks, lack of electricity and also dilapidated and shortage of toilets. The streams closer to some of the primary schools are used as sources of drinking water, domestic activities and as refuse dumps for the community.

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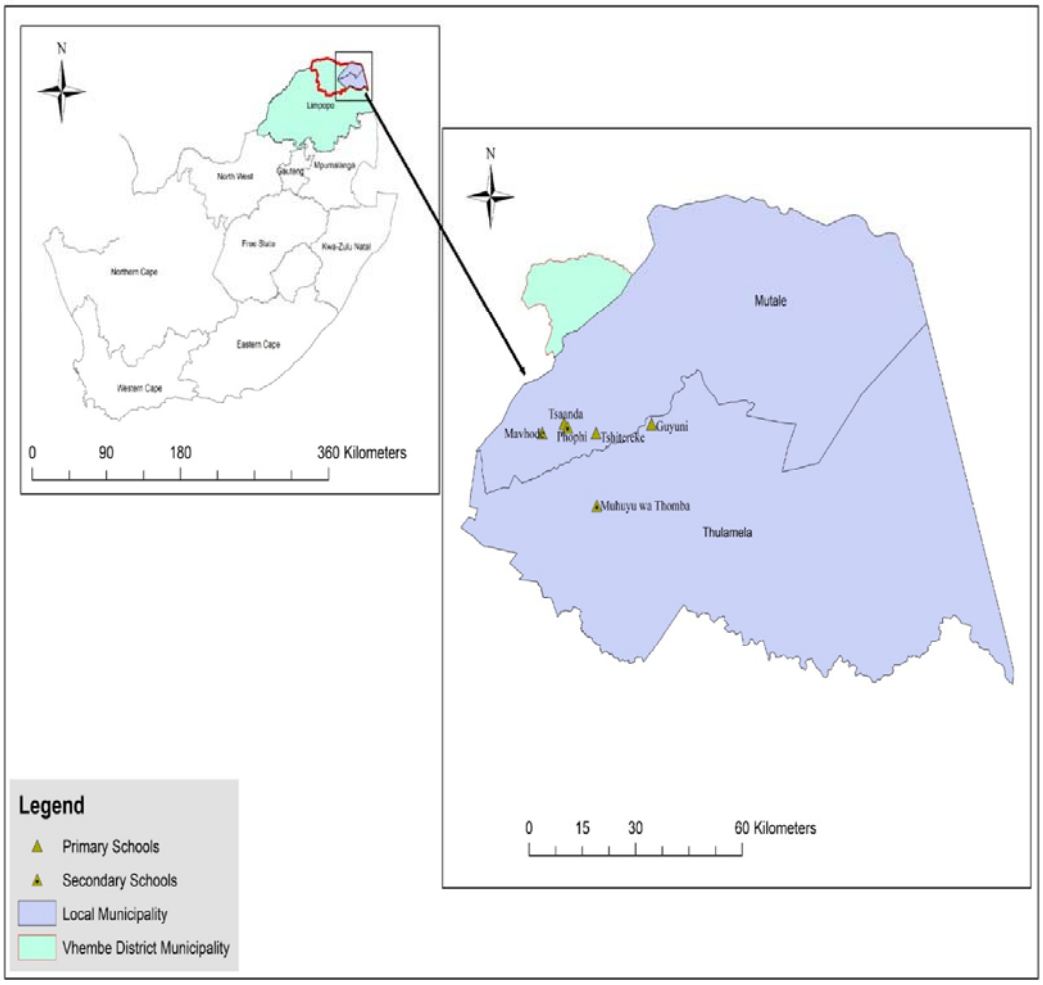

Figure 3.1: Map of the Study Area

3.3 Study participants and inclusion criteria

The target participant for the study were children in grade 0 to 8 aged from 5 to 16 years and attending primary and secondary schools in the Mutale and Thulamela municipalities of the Vhembe District. A total of six schools were included in this study namely; Tshaanda primary, Mavhode primary, Guyuni primary, Tshitereke primary, Phophi secondary and Muhuyu wa Thomba secondary and a simple random sampling was employed in recruiting participants for this study. Grade 0 to 8 children were chosen because of their age since soil-transmitted helminths (STHs) reach its maximum intensity at the age of 5-16 years. School-age children are considered at high-risk of STHs infections and major targets for systematic regular treatment at this age. These children are old enough to provide reliable information when interviewed for collecting basic demographical and epidemiological data (WHO, 2002). All

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primary school children who were studying in the study schools and whose parents or guardians signed a written consent form and willing to participate in the study were included in the study.

3.4 Sample collection

Structural questionnaires were used to obtain socio-demographic data like age, sex, size of the family, job occupation of parent or guardian, types of animals owned by the family, source of drinking water, hand washing preference and hand washing importance (Apendix 1 example of the questionnaire). Hand washing method was employed for collection of sample from the hands. Ziploc bag (26.8cm × 24.3 cm) was filled will 90 ml of 1% 7X quadrafos, glycol ether and dioctyl sulfoccinate sodium salt soap solution. Hands of the volunteer were placed into the bag and closed with a rubber band on the wrist. Volunteer's hands were massaged or washed inside the bag for 30 seconds each, ensuring as much dirt as possible was removed. The bag was opened and volunteer's hands were rinsed by applying 5 ml of sterile distilled water from a squeeze bottle ensuring that this water was reserved within the Ziploc bag. The content of the bag was transferred into two separate universal 50 ml Falcon tubes by cutting a lower corner of the bag. The sides of the bag was rinsed using 5 ml of sterile deionized water to remove excess microbial pathogens attached to the bags and to make up to a volume of 100 ml. All samples were be placed in a cooler bag at 4°C, transported to the laboratory and processed within approximately six hours after collection.

3.5 Microbiological analysis of hand washing samples

3.5.1 Enumeration of pathogenic *E. coli* using Colilert® Quanti-Tray®/2000

Upon arrival of the sample at the laboratory, 50 ml of the hand rinse sample was dispatched into a 100 ml vessel and 50 ml of the sterile distilled water was added to make a volume of 100 ml. The Colilert® Quanti-Tray®/2000 system was used for the enumeration of viable pathogenic *E. coli* from the sample according to the manufacturer's instruction (IDDEX). The reagent Colilert®-18 was added to the sample, dissolved before the mixture was poured into a Quanti-Tray®/2000. The Quanti-Tray®/2000 was sealed using a quanti-tray sealer and incubated at 35°C for 18 to 24 hours. The positive results indicated by yellow for total coliforms and fluorescing wells for dirrhoeagenic *E. coli* were counted and converted into the most

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probable number (MPN) of total coliforms (TC) and *E. coli* present in samples, using tables provided by the manufacturer. A total of 2 ml of the overnight culture media were removed from up to ten positive *E. coli* wells of the Colilert®-Trays®/2000 with sterile 1 ml Neomedic disposable syringes with mounted needle (Kendon Medical Suppliers) and aliquoted into 2 ml sterile Eppendorf tubes.

3.5.2 DNA extraction from the Colilert®-Trays®/2000

The DNA extraction and PCR analysis was analyzed by the Water and Health Research Center (WHRC), the following method was used for DNA extraction analysis. The Eppendorf tubes were centrifuged for 5 min at 13,000 × g to pellet the cells and the supernatant was discarded. DNA was extracted from the collected bacterial cells using the reported method published by Omar *et al* (2010). DNA was eluted from the celite with 100 µl Qiagen elution buffer (Southern Cross Biotechnology). The extracted DNA will be used as template in all PCR reactions (Omar *et al*., 2010, Omar & Barnard, 2014).

3.5.3 Multiple Polymerase Chain Reactions (m-PCR)

All m-PCR reactions were performed in a Biorad Mycycler™ Thermal cycler in a total volume of 20 µl. A multiplex m-PCR kit (Qiagen®) was used for the m-PCR protocol. Each reaction consisted of 1X Qiagen® PCR multiplex mix (containing HotstartTaq® DNA polymerase, multiplex PCR buffer and dNTP mix); 2 µl of the primer mixture (Table 3.1). 4 µl of sample DNA and 4 µl PCR grade water. The reactions were subjected to an initial activation step at 95°C for 15 min, followed by 35 cycles consisting of denaturing at 94°C for 45s, annealing at 55°C for 45s, extension at 68°C for 2 min and final elongation at 72°C for 5 min (Omar & Barnard, 2014). DNA was analyzed using a 2.5% (w/v) agarose gel in TAE buffer (40mmol^{-1} Tris acetate; 2 mmol^{-1} EDTA, pH 8.3) with 0.5 µgml^{-1} ethidium bromide. Electrophoresis were conducted for 1-2 h in electric field strength of 8 Vcm^{-1} gel and the DNA was visualized with UV-vis light (Gene Genius Bio Imaging system, Vacutec®). These procedures were followed for all the experiments. The relative sizes of the DNA fragments were be estimated by comparing their electrophoretic mobility with that of the standards run with the samples on each gel, either a 1kB or 100bp marker (Fermentas®) (Omar and Barnard, 2014).

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Table 3.1: Primers used in m-PCR reaction (Omar and Barnard, 2014)

Pathogen	Primer	Sequence (5'-3')	Size (bp)	Conc. (µM)	References
E. coli	*mdh* (F)	GGT ATG GAT CGT TCC GAC CT	304	0.1	Tarr *et al.* (2002)
	mdh (R)	GGC AGA ATG GTA ACA CCA GAG T			
EIEC	*ial* (F)	GGT ATG ATG ATG ATG AGT CCA	650	0.2	Lopez-Saucedo *et al.* (2003)
	ial (R)	GGA GGC CAA CAA TTA TTT CC			
EHEC/aEPEC	*eaeA* (F)	CTG AAC GGC GAT TAC GCG AA	917	0.3	Aranda *et al.* (2004)
	eaeA (R)	CCA GAC GAT ACG ATC CAG			
tEPEC	*bfpA*, (F)	AAT GGT GCT TGC GCT TGC TGC	410	0.3	Aranda *et al.* (2004)
	bfpM (R)	TAT TAA CAC CGT AGC CTT TCG CTG AAG TAC CT	410	0.3	
EAEC	*eagg (F)*	AGA CTC TGG CGA AAG ACT GTA TC	194	0.2	Pass *et al.* (2000)
	eagg (R)	ATG GCT GTC TGT AAT AGA TGA GAA C			
EHEC	*stx1* (F)	ACA CTG GAT GAT CTC AGT GG	779	0.3	Moses *et al.* (2006)
	stx1 (R)	CTG AAT CCC CCT CCA TTA TG			
	stx2 (F)	CCA TGA CAA CGG ACA GCA GTT			
	stx2 (R)	CCT GTC AAC TGA GCA CTT TG			
ETEC	*lt* (F)	GGC GAC AGA TTA TAC CGT GC	360	0.1	Pass *et al.* (2000)
	lt (R)	CGG TCT CTA TAT TCC CTG TT			
	st (F)	TTT CCC CTC TTT TAG TCA GTC AAC TG	160	0.5	
	st (R)	GGC AGG ATT ACA ACA AAG TTC ACA			
External control	*gapdh* (F)	GAG TCA ACG GAT TTG GTC GT	238	0.3	Mbene *et al.* (2009)
	gapdh (R)	TTG ATT TTG GAG GGA TCT CG			

F- Forward primer, R- Reverse primer

3.5.4 Microscopic analysis of intestinal parasite eggs

A volume of 50 ml of each sample was centrifuged using the 50 ml Falcon tube for 7 minutes at 2, 500 rpm in centrifuge. The supernatant was discarded leaving only the pellet and then 5 ml sugar-salt flotation solution was added and the pellet mixed. The homogenized pellet was transferred to McMaster slide. Direct microscopic examination of the sample for helminthes eggs was carried out using three McMaster slides per sample viewed at 10 x objectives (Jeandron *et al*., 2014)

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3.5.5 Data analysis

A structural questionnaire was used to associate the positive results with selected socio-demographic variables. The raw data was organized and analyzed by the SPSS version 24 software.

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CHAPTER 4

RESULTS

4.1 Selected socio-demographic characteristics of children

A total of 358 school going children aged 5-16 years from grade 0-8 were randomly recruited from primary and secondary schools in the Mutale and Thulamela municipalities of Vhembe District of the Limpopo province in South Africa.

4.1.1 Distribution of the study children among schools

A total of 6 schools were include in this study (Table 4.1), among all 358 school going children 22.4% came from Guyuni, 6% came from Phophi, 19.3% came from Tshaanda, 22.4% came from Tshitereke, 12% came from Muhuyu wa Thomba and 17.9% came from Mavhode (Table 4.1).

Table 4.1: Distribution of study population in the schools under study

School	Frequency	%	Valid %	Cumulative %
Guyuni	80	22.3	22.3	22.3
Mavhode	64	17.9	17.9	40.2
Muhuyu wa thomba	43	12.0	12.0	52.2
Phophi	22	6.1	6.1	58.4
Tshaanda	69	19.3	19.3	77.7
Tshitereke	80	22.3	22.3	100.0
Total	358	100.0	100.0	

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4.1.2 Gender and age of the children

Out of 358 children sampled, 48% were males and 51% were females (Table 4.2). The average age was 9.81 ± 2.932. The major category of children studied was 8-10 years old group (34.6%) and 0.3% were aged 16 years (Table 4.2).

Table 4.2: Gender and age distribution of study population

Gender		Frequency	%	Valid %	Cumulative %
Valid	Male	174	48.6	48.6	48.6
	Female	184	51.4	51.4	100.0
	Total	358	100.0	100.0	
Age					
Valid	5-7 years	94	26.3	26.3	26.3
	8-10 years	124	34.6	34.6	60.9
	11-13 years	85	23.7	23.7	84.6
	14-16 years	55	15.4	15.4	100.0
	Total	358	100.0	100.0	

4.1.3 Relationship between caregivers and children

Most of the caregivers to the children were mothers (40.8%), teachers comprised 21.8% and 2.2% were uncles, aunts and other family members (Table 4.3).

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Table 4.3: Caregiver relationship to the child

	Caregiver	Frequency	%	Valid %	Cumulative %
Valid	Brother	11	3.1	3.1	3.1
	Father	25	7.0	7.0	10.1
	Grandmother	32	8.9	8.9	19.0
	Mother	146	40.8	40.8	65.4
	Other	8	2.2	2.2	67.6
	Sister	38	10.6	10.6	78.2
	Teacher	78	21.8	21.8	100.0
	Total	358	100.0	100.0	

Key: Teacher, Other-Uncles, aunts and friends

4.1.4 Toilet system available for the children at home and at school

Most children had access to a private pit latrine at home (73.7%) and a shared flushing toilet at school (82.7%). A considerable number of children had access to a shared latrine at home (17%) and very few used a shared latrine at school (3.1%) (Table 4.4). A very low proportion of children used the open field or rivers as a toilet facility both at home 3.6% and at school 3.1% respectively

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Table 4.4: Toilet types accessible to the children

Type of toilet at home		Frequency	%	Valid %	Cumulative %
Valid	Private flushing toilet	6	1.7	1.7	1.7
	Shared flushing toilet	3	0.8	0.8	2.5
	Private latrine	264	73.7	73.7	76.3
	Shared latrine	61	17.0	17.0	93.3
	Open	13	3.6	3.6	96.9
	River	11	3.1	3.1	100.0
	Total	358	100.0	100.0	
Type of toilet at school					
Valid	Private flushing toilet	6	1.7	1.7	1.7
	Shared flushing	296	82.7	82.7	84.4
	Private latrine	24	6.7	6.7	91.1
	Shared latrine	11	3.1	3.1	94.1
	Open	9	2.5	2.5	96.6
	River	12	3.4	3.4	100.0
	Total	358	100.0	100.0	

4.1.5 Playing environment for the children at home and at school

As an environmental factor which may influence the prevalence of microbes under study, the playing environment of the children at home and at school were considered. Most children at home (79.3%) play outside while 10.6% of children play in more than one environment. A total of 1.7% of the children plays near rivers or lakes (Table 4.5). The highest percentage observed was for children who play outside (80.7%), followed by children who play in more than one environment (10.6%), and children who play outside near rivers and lakes (1.7%).

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Table 4.5: Playing environment of children

Playing site at home		Frequency	%	Valid %	Cumulative %
Valid	Outside ground	284	79.3	79.3	79.3
	Outside by river/lake	6	1.7	1.7	81.0
	Inside floor	30	8.4	8.4	89.4
	Other	38	10.6	10.6	100.0
	Total	358	100.0	100.0	

Playing site at school

	Outside ground	289	80.7	80.7	80.7
Valid	Outside by river/lake	6	1.7	1.7	80.7
	Inside floor	26	7.3	7.3	82.4
	Other	37	10.3	10.3	89.7
	Total	358	100.0	100.0	100.0

4.1.6 Contact of children with soil and animals

It was observed that most children (43%) were in contact with soil more than 2 hours before the survey was conducted and most children (99.2%) were in contact with animals more than 2 hours (a day prior the survey) before the survey was conducted.

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Table 4.6: Contact of children with soil and animals

Last soil contact		Frequency	%	Valid %	Cumulative %
Valid	0 (now)	1	0.3	0.3	0.3
	10 minutes ago	60	16.8	16.8	17.0
	30 minutes ago	60	16.8	16.8	33.8
	1 hour ago	80	22.3	22.3	56.1
	Over 2 hours ago	157	43.9	43.9	100.0
	Total	358	100.0	100.0	

Last animal contact

Valid	30 minutes ago	3	0.8	0.8	0.8
	Over 2 hours ago	355	99.2	99.2	100.0
	Total	358	100.0	100.0	

4.1.7 Hand hygiene for children

A large percentage of the children washed hands with soap before eating (36.8%), followed by washing their hands after visiting the toilet (25.7%) (Table 4.7). Some children would wash their hands on more than one occasion (20.4%). Children washed their hands after playing were 17.1%. Most of the children had not washed their hands in the last 2 hours or more from the time the survey was conducted (83.5%) (Table 4.7).

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Table 4.7: Hand hygiene of the children

Hand washing situation		Frequency	%	Valid %	Cumulative %
Valid	After playing	61	17	17.1	17.1
	Before eating	132	36.8	36.8	36.8
	After going to the toilet	92	25.7	25.7	53.8
	0ther	73	20.4	20.4	100
	Total	358	100.0	100.0	

Last hand wash

Valid	10 minutes ago	2	0.6	0.6	0.6
	30 minutes ago	4	1.1	1.1	1.7
	1 hour ago	53	14.8	14.8	16.5
	Over 2 hours ago	299	83.5	83.5	100.0
	Total	358	100.0	100.0	

4.2 Total coliform and *E. coli* determined by the Colilert® Quanti-Tray® method

A total number of 350 (97.2%) school children tested positive for Total coliforms and 48 (13.4%) tested positive for *E. coli*. As summarized in Table 4.8, 8 (2.2%) samples had no Total coliforms, 80 (22.3%) were found to have a count of Total coliforms between 0-100 cfu/100ml, 66 (18.4 %) samples had a count of 100-500 cfu/100ml, 20 (5.6%) samples had a count of 500-1000 cfu/100ml, 9 (2.5%) samples had a count of 1000-1500 cfu/100ml and 167 (46.6 %) samples had a count of Total coliforms greater than 2000 cfu/100ml. The microbial analysis for the presence of *E. coli* showed that 310 (86.6%) out of the 358 samples had a count of 0 cfu/100ml, 41 (11.4%) had a count of 0-100cfu/100ml, 2 (0.6%) had a count of 100-500 cfu/100ml and the remaining 5 (1.4%) had a count of over 200 cfu/100ml (Table 4.8).

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Table 4.8: Summary of Total Coliforms and *E. coli* counts per 100ml of solution from the hands of school going children

MPN (cfu/100ml)		Total coliforms frequency	Total coliforms %	*E. coli* frequency	*E. coli* %
Valid	0	8	2.2	310	86.6
	0-100	80	22.3	41	11.4
	100-500	66	18.4	2	0.6
	500-1000	20	5.6	0	0
	1000-1500	9	2.5	0	0
	1500-2000	8	2.2	0	0
	>2000	167	46.6	5	1.4
	Total	358	100.0	358	100

4.3 Prevalence of STHs among school going children

The soil-transmitted helminths (STH) under study were *Ascaris lumbricoides* (*A. lumbricoides)* and *Trichuris trichiura(T. trichiura)* and hookworms. The prevalence of both *A. lumbricoides* and *T. trichiura* was found to be 0% in the study population. However, there was a prevalence of 0.6% hookworms and 2% of *Enterobius vermicularis* (*E. vermicularis*) (Figure 4.1).

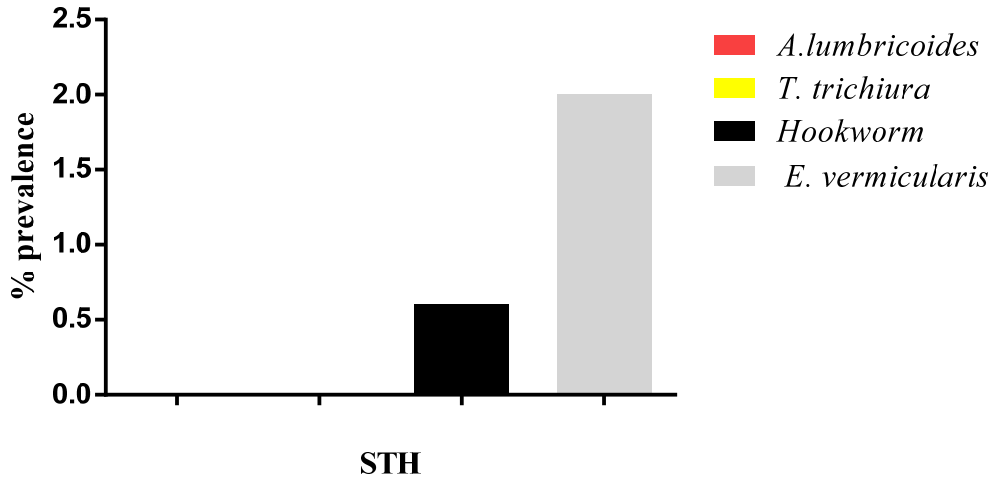

Figure 4.1: Prevalence of STH among children.

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4.4 Association of selected risk factors associated with the prevalence of STHs and *E. coli*

4.4.1 Socio demographic characteristics and prevalence of STHs and *E. coli*

Bivariate analysis using Pearson Chi square test on the socio-demographic characteristics of the children with regard to STHs and *E. coli* infection was analysed. There was no significance between genders of the children in association with the prevalence of STH. There was also no significant association between the gender of the children and prevalence of *E. coli* (Table 4.9; $\chi 2$ =3.17, df=4 *P*> 0.05). There was no significant association between age and the prevalence of *E. coli* ($\chi 2$ =3.17, df=4 P> 0.05). The $\chi 2$ represents the Chi squared value and P represents the significance level (Table 4.9).

Table 4.9: Relationship between Socio demographic factors and prevalence of *E. coli* among children

MPN (cfu/100ml)		0	0-100	100-500	>2000	Total	Total +ve samples (%)	Test statistics
Gender	Male	154	17	1	2	174	20 (11.5)	$\chi 2$ =3.17, df=4 P = 0.53
	Female	156	24	1	3	184	28 (15.2)	
Total		310	41	2	5	358	48 (13.4)	
Age grouped	5-7	79	14	1	0	94	15 (16)	
	8-10	107	15	0	2	124	17 (13.7)	$\chi 2$ =3.17, df=4 P= 0.53
	11-13	72	9	1	3	85	13 (15.3)	
	14-16	52	3	0	0	55	3 (5.6)	
Total		310	41	2	5	358	48 (13.4)	

4.4.2 Child hand hygiene practices

Bivariate data analysis by Pearson Chi squared test revealed that there was a significant association ($\chi 2$ =35.337, df = 24, P < 0.05) between hand washing situations and prevalence of the *E. coli*. There were more *E. coli* identified from the children whose hand rinsed sample

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were collected before eating at the school and those who were collected after going to the toilet. The occurrence of *E. coli* in the children who washed their hands after playing was very low compared to the prior mentioned categories. However, there was no significant association between the times hands were last washed with the occurrence of *E. coli* (Table 4.10).

Table 4.10: Relationship between child hand hygiene and occurrence of *E. coli*

MPN (cfu/100ml)		0	0-100	100-500	>2000	Total	Total +ve samples (%)	
Washing hands with soap	After playing	56	2	0	0	61	2 (3.3)	**Test statistic** $\chi 2 = 35.337$, df = 24, p =0.0453
	Before eating	117	15	0	0	132	15 (11.4)	
	After toilet	76	14	0	2	92	16 (17.4)	
	0ther	62	7	2	3	73	12 (16.4)	
	Total	310	41	2	5	358	48 (13.4)	
Last soap hand wash	10 minutes ago	2	0	0	0	2	0 (0)	$\chi 2 = 7.43$, df = 12, p =0.827
	30 minutes ago	4	0	0	0	4	0 (0)	
	1 hour ago	42	11	0	5	58	16 (27.6)	
	Over 2 hours	262	30	2	5	299	32 (12.4)	
Total		310	41	2		358	48 (13.4)	

4.4.3 Environmental sanitation

There was a significant association between the environmental sanitation aspects like the source of drinking water and type of toilet available to the study participants at home with the occurrence of *E. coli* (Table 4.11; P< 0.05). The highest percentage occurrence of *E. coli* was observed in bottled water (non-commercial storage containers) (25%) and in river water (17.5%). There were no *E. coli* detected from the samples isolated from participants who had access to water from the well or reservoir pumps. There was a significant association between the types of toilet accessible to the children. The highest occurrence of *E. coli* came from the group which used river site as a toilet (27.3%) and the group using the shared latrines (16.4%). No *E. coli* was isolated from the children using flushing system toilets.

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Table 4.11: Relationship of environmental sanitation with prevalence of *E. coli*

MPN (cfu/100ml)		0	0-100	100-500	>2000	Total	Total +ve (%)	
Water Source	Tap	248	34	2	2	286	38 (13.3)	**Test statistics**
	Bottled	9	3	0	0	12	3 (25)	
	Well	13	0	0	0	13	0 (0)	$\chi 2$ =41.673
	Pump/Resevoir	7	0	0	0	7	0 (0)	df=16
	River/stream/spring	33	4	0	3	40	7 (17.5)	P=0.0001
Total		310	41	2	5	358	48 (13.4)	
Type of toilet	Private flushing toilet	6	0	0	0	6	0 (0)	$\chi 2$ =41.673 df=20 P= 0.013
	Shared flushing toilet	3	0	0	0	3	0 (0)	
	Private latrine	230	27	2	5	264	34 (12.9)	
	Shared latrine	51	10	0	0	61	10 (16.4)	
	Open	12	1	0	0	13	1 (7.7)	
	River	8	3	0	0	11	3 (27.3)	
Total		310	41	2	5	358	48 (13.4)	

4.5 Identified *E. coli* strains by multiplex PCR (m-PCR)

The prevalence of *E. coli* was 13.4% as 48 out a total of 358 samples tested positive for *E. coli* (Table 4.8). Out of the 48 (13.4%) of the identified *E. coli*, 31 (8.7%) were non-pathogenic commensal *E. coli*. Therefore only 17 (4.7%) samples tested positive for pathogenic *E. coli*. Of the 4.7% pathogenic *E. coli* 7 (2%) were identified as EAEC, 1 (0.3%) were identified as atypical EPEC (*eaeA*), 4 (1.1%) were identified as typical EPEC (*bfp* and *eaeA*) and 1 (0.3%) were identified as ETEC. Some of the children's sample had mixed infection of *E. coli* strain, 1 (0.3%) sample had combination of atypical EPEC and EAEC infections and 1 sample (0.3%) also carried both typical EPEC and EAEC strains of *E. coli*. While 2 other samples (0.6%) carried the typical EPEC and the EHEC pathogenic strains.

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4.5.1 Distribution of pathogenic *E. coli* amongst school going children

From the total of 358 samples tested 17 (4.7%) was found to be pathogenic *E. coli*. The occurrence of the pathogenic *E. coli* was more frequent in Tshitereke primary school (58%) and no pathogenic *E. coli* was found in Muhuyu wa thoma primary school (Figure 4.2).

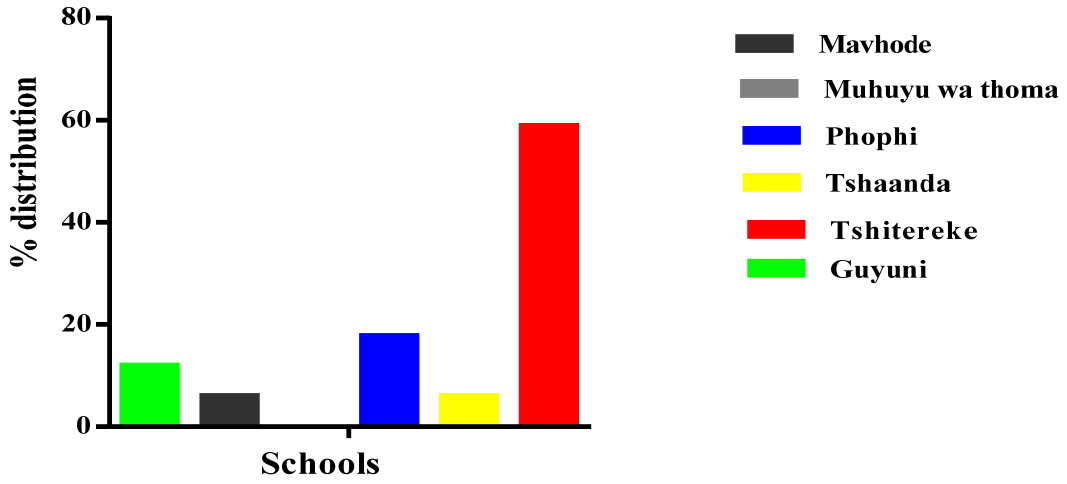

Figure 4.2: Distribution of the pathogenic *E. coli* across schools

Pathogenic *E. coli* was also found in Phophi high school at an incidence of 17.6%, followed by Guyuni primary with an incidence of 11.8%. In addition, Tshaanda primary school had a relative incidence of 5.9% and no pathogenic *E. coli* was isolated from Muhuyu wa thoma primary school.

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CHAPTER 5

DISCUSSION

Globally, soil-transmitted helminthes (STHs) infection has been identified as the main cause of illness and disease where more than two billion people are infected. Specifically, STHs infections during childhood remain a major public health challenge in developing countries where access to basic sanitary facilities may not be adequate **(**Tandukar *et al*., 2015**)**. Pathogenic *Escherichia coli* are also known to cause diarrhoea which has led to the loss of lives to hundreds of thousand people annually (Servin, 2005). The occurrence of intestinal infections due to STHs and *E. coli* in rural sub-Saharan Africa like other infectious diseases has been associated with poverty. Those affected are usually vulnerable due to access to environmental sanitation like proper toilets and drinking water sources **(**Adagdaba *et al*., 2012**)**. This study aimed at investigating the prevalence and factors associated with STHs infections and *E. coli* among children aged 5–16 years from selected schools in the Vhembe district of the Limpopo province.

5.1 Factors associated with soil-transmitted helminthes and *Escherichia coli* infection among school going children

The potential factors associated with STHs and *E. coli* infection were grouped into three categories; (1) selected socio-demographic characteristics of the children, (2) their caregivers' hygiene practices of the children and (3) environmental sanitation conditions of the children households.

5.1.1 Socio demographic factors of the children

Considering the socio demographic characteristics of the children, the findings of this study showed that there was no significant relationship between the gender of the children and infection by any of the STHs under study or *E. coli*. However, it was found that females were at higher risk of infection (15.2%) than males (11.5%) by any of the pathogenic *E. coli* (Table 4.9). These findings were in line with other studies that have shown no associations of gender differences with parasitic infections (Obala *et al*., 2013). Presently in the Vhembe region, children do similar chores regardless of gender at home, including farming which will expose

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both ganders equally so to the intestinal pathogens. The age of the child did not have any significant association with infection by any of the three STHs and *E. coli* (Table 4.8).

5.1.2 Hygiene practices of the school going children

There was a significant relationship between hand hygiene and prevalence of *E. coli*. There was a high occurrence of *E. coli* from the children who indicated that they wash their hands with soap after going to the toilet and before eating with 17.4% and 11.4% respectively. This might have resulted due to the method of hand washing employed after going to the toilet because the toilet facilities in the schools were in poor condition and the pit latrines for the schoolchildren were so dirty that the pupils preferred to defecate in vegetation surrounding the school compounds and there were no basin and soap for hand washing after using the toilet which put children in a high risk of contaminating their hands with faeces. This might also have resulted from the fact that younger children are not hygiene conscious and they are very playful where they interact with contaminated soil as compared to older children. The time frame from the last time the children washed their hands and prevalence of *E. coli* had no significant relationship after analysis by the Pearson Chi squared test.

5.1.3 Environmental sanitation

The current study found a significant relationship between household water source for drinking and the prevalence of *E. coli.* The occurrence of *E. coli* was highest (17.5%) in the population who use rivers as a source of drinking water. The lowest occurrence was from the population who used wells and reservoirs as a source of water (Table 4.10). There was a significant association between the type of toilet at households of the children and the prevalence of *E. coli*. The *E. coli* was found to occur in higher incidence (27.3%) in the population that used the open veld as a toilet facility. Furthermore, a high prevalence of 16.4 % occurred in the population which had access to shared pit latrines while 12.9 % occurred from the children who had private latrines at home. The difference between the two types of latrines mentioned above can be explained by the fact that one of the toilets is shared by families. Sharing toilets could encourage communicability of infections. These findings concur with previous studies that showed a significant association between the type of toilet latrine facility available and the prevalence of intestinal pathogen infection in children **(**Oloruntoba *et al*., 2014, Edelduok *et al*., 2013**)**..

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5.2 Prevalence of STHs among school going children

The findings of this study revealed that the overall prevalence of intestinal parasite obtained in the study population was 2.6% with 0.6% hookworm and other intestinal parasite *Enterobius vermicularis* (E. vermicularis) found to be 2% in the study population. However, *Ascaris. lumbricoides and Trichuris. trichiura* which formed the scope of this study were not obtained in the study population (Figure 4.1). The findings of this study are incomparable to previously reported studies, for example, a study conducted by Alo *et al*., (2013) which was investigating the prevalence of intestinal parasite from the fingers of school children in Ohaozara, Ebonyi State, Nigeria, found that the parasites isolated from the fingernails of the primary school children were *A. lumbricoides, E. vermicularis, T. trichiuria,* and *A. duodenale,*with prevalence rates of 20.0%, 17.8%, 12.9% and 6.5% respectively. Another study which was also conducted in Nigeria by Yahaya *et al*., (2015) on prevalence of intestinal parasite eggs in the fingernails of "*Almajiris*" in Birnin Kudu Local Government Area in Jigawa State, found that the prevalence of intestinal parasites among the overall population studied was 54.8%, which is higher compared to 2.6% found in this study. The parasites isolated from the fingernails of the qur'anic school children were, *A. lumbricoides,* Hookworm, *E. vermicularis* and *T. trichiura*, with prevalence rates of 29.5%, 24.3%, 19.0% and 8.1% respectively. In South Africa, a study in Cape Town found that the overall prevalence of Intestinal parasite was 55.8% with *A. lumbricoides* having the infection rate of 24.8%, *T. trichiura* 50.6%, hookworm 0.08% and this results were based on a study concerning paradoxical helminthiasis and giardiasis in Cape Town among children attending nine schools in low income community (Adams *et al*., 2005). *E. vermicularis* is a cosmopolitan intestinal parasite of people, especially children. However, Studies carried out in South Africa of gastro-intestinal parasite also attest to very low prevalence of *E. vermicularis* of below 5% (Masala and Appleton, 2003). There is less information on the prevalence of intestinal parasitic infection in the Limpopo province. A study done by Samie *et al*., (2009) which looked at the prevalence of intestinal parasite and bacterial pathogens in the stools of diarrhoeal and non-diarrhoeal patients from Vhembe district also attested to lower prevalence of STHs with *A. lumbricoides* having the prevalence of 7.8%, *T. trichiura* 2.3% and hookworm having prevalence of 7.7%. However, in the later mentioned studies, stool samples were used in contrast to the hand wash sample that was used in this current study. The rate of intestinal parasites found in the study population may be attributed to low socio-economic status and low level of personal hygiene.

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5.3 Prevalence of *E. coli* among school going children

The prevalence of *E. coli* was 13.4% in the study population. Only 17 (4.7%) samples tested positive for pathogenic *E. coli*. The highest incidence of the pathogenic *E. coli* was the *eagg* gene (EAEC) and the *bfpA* gene typical EPEC pathotype. These findings agree with a study done by Omar and Barnard, (2014) which revealed the highest prevalence of *E. coli* as the pathotypes EAEC (29.4%) and EPEC (27%). In another study, all five diarrhoeagenic *E. coli* were detected in environmental water, domestic storage containers water and stool samples (Omar and Barnard, 2010). The prevalence of EAEC was 17% followed by 10% prevalence of ETEC (Omar *et al*, 2010). The findings of this study also agree with the study done in the rural areas of the Vhembe district in the Limpopo province where the highest prevalence of *E. coli* pathotypes was EAEC (14%). None-the-less the prevalence of *E. coli* in this study was lower than in other studies (Omar & Barnard, 2010; Omar and Barnard, 2014). More over 1 (0.3%) *E. coli* was isolated in association as atypical EPEC and EAEC and 1 sample (0.3%) also carried both typical EPEC and EAEC strain. Furthermore, 2 samples (0.6%) carried the typical EPEC and the EHEC pathogenic strains.

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CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

The main aim of this study was to determine the prevalence of STHs and *E. coli* from the school going children between the ages of 5-16. The prevalence of hookworm detected in the study population was found to be 0.6% and *E. vermicularis* was 2%. *A. lumbricoides* and *T. trichiura* were not detected in this study. There was a prevalence of 13.4% of *E. coli* detected of which 4.7% were pathogenic *E. coli,* EAEC, atypical EPEC, typical EPEC and ETEC were distributed with prevalence percentage of 2%, 0.3%, 1.1% and 0.3% respectively. The study also revealed that there was no significant association between hands hygiene of children with the prevalence of STHs, however there was significant association between hand hygiene of children with the prevalence of *E. coli*. Environmental sanitation conditions like water sources for drinking and type of toilets used is closely associated with the prevalence of *E. coli* among the school going children. Therefore, for as long as poverty, poor sanitation, hygiene and lack of safe drinking water still persists in developing countries, STHs infection and pathogenic *E. coli* will remain a public-health threat.

6.2 Recommendations

6.2.1 The study highlights the occurrence of pathogenic *E. coli* in school going children. Therefore, good hand hygiene awareness campaigns and practices should be implemented and health education knowledge be imparted to children at the early stages of life in order to reduce hand occurrence of *E. coli* and other intestinal pathogens in children.

6.2.2 A significant association with the type of toilet and prevalence of *E. coli* has been attested in this study, hence flushing toilets as compared to latrines are recommended in schools.

6.2.3 Government and relevant stakeholders should bridge the gap between rural areas and urban areas to cover the environmental sanitation gap.

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6.2.4 Other interventions such as improving health education, hygiene, socio-economic development, use of sanitary latrines and wearing of shoes may also contribute to the reduction of intestinal parasitosis.

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APPENDIX 1: Questionnaire

Hand rinse questionnaire

Hand rinse ID		Date	
Location		Field workers	
School name		School year	

ID	variable name	QUESTION	ANSWER	code
1a	age	AGE	____YEARS	
1b	sex	SEX	☐ MALE ☑ FEMALE	
1c	hand	WHAT HAND DO YOU WRITE WITH/ USE MOST?	☐ RIGHT ☐ LEFT	1 2
2a	pHH	HOW MANY PEOPLE LIVE IN YOUR HOUSEHOLD?	____INDIVIDUALS	
2b	cHH	HOW MANY CHILDREN UNDER 12 YEARS LIVE IN YOUR HOUSEHOLD?	____CHILDREN UNDER 12	
2c	income	WHAT DO YOUR PARENTS DO?	☐ FARMING ☐ PUBLIC SECTOR (e.g. teacher, doctor) ☐ OWN BUSINESS OTHER_____	1 2 3 9
2d	own_animal	DOES YOUR FAMILY OWN ANY ANIMALS?	☐ CATTLE ☐ PIGS ☐ DOGS ☑ CHICKEN OTHER_____	1 2 3 4 9
2e	water_HH	WHERE DO YOU GET WATER FROM TO DRINK IN YOUR HOUSEHOLD?	☐ TAP ☐ BOTTLED ☑ WELL ☐ PUMP/RESERVOIR ☐ RIVER/STREAM/SPRING ☐ RAIN OTHER_____	1 2 3 4 5 6 9
2f	toilet_HH	WHEN YOU NEED TO USE THE TOILET AT HOME WHERE DO YOU GO?	☐ PRIVATE FLUSHING TOILET ☐ SHARED FLUSHING TOILET ☐ PRIVATE LATRINE ☑ SHARED LATRINE ☐ OPEN ☐ RIVER/STREAM OTHER_____	1 2 3 4 5 6 9
3a	water_sc	WHERE DO YOU GET WATER FROM TO DRINK IN SCHOOL?	☐ TAP ☐ BOTTLED ☐ WELL ☐ PUMP/RESERVOIR ☐ RIVER/STREAM ☐ RAIN OTHER_____	1 2 3 4 5 6 9
3b	toilet_sc	WHEN YOU NEED TO USE THE TOILET AT SCHOOL WHERE DO YOU GO?	☐ SHARED FLUSHING TOILET ☑ SHARED LATRINE ☐ OPEN ☐ RIVER/STREAM OTHER_____	1 2 3 4 9

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4a	*play_HH*	WHERE DO YOU PLAY AT HOME?	☐ OUTSIDE GROUND ☐ OUTSIDE BY RIVER/LAKE ☐ INSIDE FLOOR OTHER_____	*1* *2* *3* *4* *9*
4b	*play_sc*	WHERE DO YOU PLAY AT SCHOOL?	☐ OUTSIDE GROUND ☐ OUTSIDE BY RIVER/LAKE ☐ INSIDE FLOOR OTHER_____	*1* *2* *3* *9*
5a	*last_soap*	WHEN DID YOU LAST WASH YOUR HANDS TODAY WITH SOAP?	☐ 10 MINUTES AGO ☐ 30 MINUTES AGO ☐ 1 HOUR AGO ☐ 2+ HOURS AGO	*1* *2* *3* *4*
5b	*teach_soap*	HAVE YOU BEEN TAUGHT TO WASH YOUR HANDS WITH SOAP? BY WHOM?	☐ YES ☐ NO BY WHOM _____	*1* *2*
5c	*soap_activities*	WHEN DO YOU NEED TO WASH YOUR HANDS WITH SOAP? (tick all that apply)	☐ AFTER PLAYING ☐ BEFORE EATING ☐ AFTER GOING TO THE TOILET	*1* *2* *3*
6a	*last_soil*	WHEN DID YOU LAST TOUCH SOIL?	☐ 10 MINUTES AGO ☐ 30 MINUTES AGO ☐ 1 HOUR AGO ☐ 2+ HOURS AGO	*1* *2* *3* *4*
6b	*last_animal*	WHEN DID YOU LAST TOUCH AN ANIMAL?	☐ 10 MINUTES AGO ☐ 30 MINUTES AGO ☐ 1 HOUR AGO. ☐ 2+ HOURS AGO	*1* *2* *3* *4*

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