PREVALENCE OF *TAENIA SOLIUM* AND SOIL TRANSMITTED HELMINTHS IN RURAL COMMUNITIES OF MONZE DISTRICT OF SOUTHERN ZAMBIA

BY

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A thesis submitted to the University of Zambia in fulfillment of the award of the degree of Master of Science in Veterinary Parasitology.

THE UNIVERSITY OF ZAMBIA

SCHOOL OF VETERINARY MEDICINE

LUSAKA

DECLARATION

I, **AMOS CHOTA** do hereby declare that this thesis represents my own work and that it has never been submitted before for the award of a degree or any other qualification at this university or any other university.

Signature: ……..Date: ……

DEDICATION

This thesis is dedicated to my late parents, Mr. and Mrs. Lengwe for having given me a strong academic foundation on which my dear elder brother, Mr. Robert Mwansa built. I also dedicate it to my beloved wife Juliet, my five children, Patience, Pevious, Brendah, Lillian and Brian.

APPROVAL

This thesis of Mr. Amos Chota is approved as fulfilling the requirements for the award of Master of Science in Veterinary Parasitology of the University of Zambia.

Supervisor……..Date:…..……

Examiner……Date…….....

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ABSTRACT

To determine the prevalence of *Taenia solium* and soil-transmitted helminth (STHs) in rural communities of Monze district of Southern Province of Zambia, a cross-sectional study was carried out in 11 villages in pigs and in five villages in humans. To detect *T. solium* infections in pigs, tongue examination of live pigs and assessment of the presence of circulating cysticerci antigen by B158/B60 monoclonal antibody-based enzyme-linked-immunosorbent assay (Ag-ELISA) in serum were used. Copro-microscopy and polyclonal antibody-based copro-antigen enzyme-linked-immunosorbent assay (Co-Ag-ELISA) were used to detect taeniosis in stool samples while cysticercosis in human sera was assessed using the Ag-ELISA. Copro-microscopy (McMaster method) was used to diagnose STHs in human stool.

Of the 275 pigs sampled, 6 (2.2%) were positive on tongue examination and 32 (11.6%) were positive on Ag-ELISA. Significant differences ($?^2$ = 36.08, $p < 0.05$) in the prevalence of porcine cysticercosis by Ag-ELISA were observed among the villages sampled but not on tongue examination. The overall prevalence of cysticercosis among the 163 humans sampled was 14.7%. There was no significant difference ($?^2$ = 4.84, p = 0.290) in the prevalence of human cysticercosis on Ag-ELISA among the villages.

Only one sample (0.8%) out of the total 133 human stool samples examined by microscopy (formol-ether concentration method) was positive for *Taenia* species eggs. Of the total 131 stool samples examined by copro-antigen Co-Ag-ELISA, 9.9% were positive for taeniosis. There were no significant differences ($?^2$ = 5.06, p = 0.247) in the prevalence of taeniosis by Co-Ag-ELISA among the five villages sampled. The overall prevalence of STHs among the 133 individuals examined by copromicroscopic examination was 16.5% comprising *Ancylostoma* spp. (15.04%) and *Trichuris* spp. (1.5%).

The results of this study confirm the co-endemicity of *T. solium* infections both in the intermediate host (pig/human) and the final host (human), implying that the factors that maintain the life cycle of the parasite are present in this study area. The study further revealed that STH infections, predominantly comprising hookworms, are endemic in the study area.

Since both *T. solium* and STH infections are associated with poor sanitation, success in controlling these parasites lies in synergized control strategies among medical health workers, veterinarians, community workers, policy makers and the community itself. Programmes that are community centered and driven like the on-going Community Led Total Sanitation (CLTS) should be embraced and scaled up to combat not only *T. solium* infections both in humans and pigs but STH infections as well. Education of villagers at schools, village meetings and on individual basis about the parasite life cycle and the connection between infected pigs and themselves and others getting cysticercosis, should be done with coordinated efforts between medical personnel and veterinarians. Further monitoring, research employing more sensitive diagnostic techniques and surveillance in both humans and pigs are recommended. Members of the community should also be educated on the life cycles of STHs for them to learn how to prevent infections instead of concentrating solely on mass deworming.

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for that I greatly thank them. I am most grateful to the village headmen and indeed the participants who volunteered to participate in this study and the pig farmers for allowing us to sample blood from their pigs.

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ABBREVIATIONS AND SYMBOLS

%	Percentage
=	Equal to
>	Greater than
=	Greater than or equal to
<	Less than
=	Less than or equal to
$?^2$	Chi-square
°C	Degrees Celsius
µg	Microgram
µl	Microlitre
µm	Micrometre
+ve	Positive
Ab	Antibody
Ab-ELISA	Antibody enzyme-linked immunosorbent assay
Ag	Antigen
Ag-ELISA	Antigen enzyme-linked immunosorbent assay
CLTS	Community-led total sanitation
Co	Copro
Co-Ag-ELISA	Coproantigen enzyme-linked immunosorbent assay
CSF	Cerebral spinal fluid
CT	Computerized tomography
DNA	Deoxyribonucleic acid
EITB	Enzyme-linked immunoelectrotransfer blot assay

ELISA	Enzyme-linked immunosorbent assay
EPG	Eggs per gram
FMD	Food and mouth disease
H_2SO_4	Sulphuric acid
ICONZ	Integrated control of neglected zoonoses
IgG	Immunoglobulin G
L1	Larval stage 1
L2	Larval stage 2
L3	Larval stage 3
M	Molarity
ml	Millilitre
mm	Millimetre
MoAb	Monoclonal antibody
N	Normality
n	Sample size
NaCl	Sodium chloride
NBCS	New born calf serum
NCC	Neurocysticercosis
No.	Number
OD	Open defecation
ODF	Open defecation free
OPD	Orthophenylene diamine
P	Probability
PBS	Phosphate buffered saline
PCR	Polymerase Chain Reaction

rDNA	Ribosomal Deoxyribonucleic acid
RFLP	Restriction Fragment Length Polymorphism
rpm	Revolutions per minute
SPSS	Statistical package for social scientists
STH	Soil-transmitted helminths
T20	Tween 20
TCA	Trichloroacetic acid
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

Taenia solium infections and those caused by soil-transmitted helminths (STHs) are of public health concern and are among the most important afflictions of humans who live in areas of poverty in the developing world and least developed countries (Crompton, 1999; Flisser *et al.*, 2003). These infections are to a great extent perpetuated by open defecation (OD). *Taenia solium*, a zoonotic tapeworm, transmitted between pigs and humans and among humans, is common in developing countries of Latin America, Asia and Africa (de Silva *et al.*, 2003). On the other hand, STHs, a group of parasitic nematode helminths causing infection through contact with parasite eggs or larvae that thrive in the warm and moist soil, are common in America, China and East Asia and Sub-Saharan Africa (de Silva *et al.*, 2003).

Taenia solium (the pork tapeworm) infections in humans include the infection by the adult tapeworm (taeniosis) and the infection by the metacestodes or larval stages (cysticercosis). Taeniosis may also be due to infection by the adult tapeworm of *Taenia saginata* (the beef tapeworm). While *T. saginata* causes bovine cysticercosis and human taeniosis, *T. solium* causes porcine cysticercosis and human taeniosis/cysticercosis (World Health Organisation, 1983; Yamazaki *et al.*, 2002). Man is, thus, not only infected by the adult *T. solium* tapeworm but also by the larval stage (cysticerci). Cysticerci of *T. saginata*, in contrast, are found exclusively in cattle and do not develop in humans (Cruz *et al.*, 1999; Garcia *et al.*, 2003).

Cysticercosis has the potential to cause neurocysticercosis (NCC), the infection of the central nervous system by the larval stages of the tapeworm (White, 1997) and this is the major cause of most morbidity and mortality leading to epilepsy, chronic headaches, seizures, hydrocephalus and other neurological manifestations (Garcia-Garcia *et al.*, 1999). White (1997) reported that cysticerci may also develop in the eyes with the consequent loss of vision.

The three main STHs that infect humans are the roundworms (*Ascaris lumbricoides*), whipworms (*Trichuris trichiura*) and hookworms (*Necator americanus* or *Ancylostoma duodenale*). Practically speaking, inhabitants of thousands of rural, impoverished villages throughout the tropics and subtropics are often chronically infected with several different species of parasitic worms, that is, they are polyparasitized (Hotez *et al.*, 2006). Although STH infections rarely cause death, hookworms have long been recognized as an important cause of intestinal blood loss leading to iron deficiency and protein malnutrition (Hotez *et al.*, 2004). School-aged children (including adolescents) and pre-school children tend to harbour the greatest number of intestinal worms and as a result experience growth stunting and diminished physical fitness as well as impaired memory and cognition (Crompton *et al.*, 2002). These adverse health consequences combine to impair childhood educational performance and reduced school attendance (Miguel *et al.*, 2003) with the consequent reduced future good wage-earning capacity. It is estimated that between a quarter and a third of pregnant women in sub-Saharan Africa are infected with hookworms and are at risk of preventable hookworm-related anaemia (Brooker *et al.*, 2008). Like *T. solium* infections, STHs are intimately

associated with poverty, poor sanitation, especially inappropriate and inadequate disposal of human excrement and lack of clean water.

Monze district is one of the rural places in Zambia where scavenging pig populations are high. Earlier studies in some districts of Eastern and Southern provinces have reported a high prevalence of *T. solium* infections in pigs (Phiri *et al.,* 2002; Sikasunge *et al.*, 2008a). Abattoir surveys of pigs at a Chibolya slaughter slab in Lusaka, all of which were from Southern province, showed that 10.0% and 20.6% were positive by lingual examination and meat inspection, respectively (Phiri *et al.*, 2002). Since man is the only final host for the tapeworm, the high prevalence of porcine cysticercosis implies that for the life cycle of this tapeworm to be complete, there must be the involvement of the human host. It thus, means that human *T. solium* infections must be present in these areas. However, previous research on *T. solium* infections in Zambia have focused on determining the rates of infections in pigs and not much work has been carried out on the rates of infections in man, the definitive host of tapeworm. Mwape *et al.* (2012) and Mwape *et al*. (2013) reported taeniosis prevalence rates of 6.3% and 11.9% respectively, both based on Co-Ag-ELISA, in the Eastern province of Zambia. This study simultaneously sought to provide vital information on the situation of the *T. solium* infections in humans and in pigs. This information is very useful in understanding the epidemiology of *T. solium* and thus helps in the structuring of the integrated control and prevention measures to reduce the risk and prevalence of this neglected zoonosis.

Understanding the population at risk of human STH infections is fundamental for appropriate resource allocation and cost-effective control. In particular, it allows for

reliable estimation of the overall drug needs and efficient targeting of control programs (Brooker and Michael, 2000). Generally, children are at a higher risk of both harbouring higher levels of infection (thus greater levels of morbidity) and becoming re-infected more quickly. This impacts negatively on the children's intellectual and physical development (Benthony *et al.*, 2006). Africa's and indeed Zambia's future depends on this young human resource and thus the information obtained from this study will greatly contribute to the knowledge and targeted allocation of resources in the fight against STH infections when conducting mass drug administration.

The general objective of this study was, therefore, to establish the prevalence of *T. solium* and STHs in rural communities of Monze district of southern Zambia. The specific objectives were:

(a) To determine the prevalence of porcine cysticercosis in the study area.

(b) To determine the prevalence of *T. solium* infections (cysticercosis and taeniosis) in humans in the study area.

(c) To establish the prevalence of STHs in humans in the study area.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Background

While the scientific study of the taeniid tapeworms can be traced back to the late 17th century, helminths (the word derived from Greek meaning "worms") have plagued humans since before the era of our earliest recorded history. There are two major phyla of helminths. The nematodes (also known as roundworms because of their appearance in cross section) include the major intestinal worms (also known as soil-transmitted helminths (STHs) or geo-helminths and filarial worms that cause lymphatic filariasis and onchocerciasis, whereas the platyhelminths (also known as flatworms) include flukes (also known as trematodes), such as the schistosomes and the tapeworms (also known as cestodes), such as the pork tapeworm that causes cysticercosis. The eggs of intestinal helminths can be found in the mummified faeces of humans dating back thousands of years and we can recognize many of the characteristic clinical features of helminth infections from the ancient writings of Hippocrates, Egyptian medical papyri and the Bible (Cox, 2002).

According to Cox (2002), Edward Tyson was the first to recognize "the head" known as the scolex of a tapeworm and described the anatomy and physiology of the adult tapeworm. This discovery laid the foundation of the current knowledge on the biology of the taeniid tapeworms of humans. There are about 40 species of adult tapeworms and about 15 larval forms which can infect man, dogs and other accidental hosts (Ashford and Crewe, 1998; Cox, 2002). Although there are differences between the broad

tapeworm and the taeniid tapeworms that were identified, the distinction between *T. solium* and *T. saginata* were not yet clearly distinguished (Cox, 2002). Although Goeze in 1782 had suspected that *T. solium* and *T. saginata* were different species, it was not until the middle of the 19th century that Kuchenmeister confirmed the differences based on the morphology of the scolex (Cox, 2002). The first indication that intermediate hosts were involved in the life cycle of taeniid tapeworms emerged in 1784 from studies using the pork tapeworm. A German pastor, Johann August Ephraim Goeze observed that scolices of the tapeworm in humans resembled cysts in the muscles of pigs (Kean *et al.*, 1978). Some 70 years later, Kuchenmeister, in much criticized experiments, fed pig meat containing cysticerci of *T. solium* to criminals condemned to death and recovered the adult tapeworms from the intestines at postmortem (Cox, 2002). From 1868 to 1869, J.H. Oliver further observed that *T. saginata* tapeworm infections occurred in individuals who had eaten infected beef. This observation was confirmed by an Italian veterinarian, Edoardo Perroncito in 1887 (Cox, 2002).

In 1947, Norman Stoll published a landmark paper entitled "This wormy world," in which he set out to estimate the number of people infected with helminths worldwide (Stoll *et al.*, 1999). Over the last 60 years, several estimates have confirmed Stoll's initial observation that hundreds of millions of people harbour parasitic worms (de Silva *et al.*, 2003).

Broadly, the literature review will cover morphology, lifecycles, prevalence, diagnosis (parasitological, immunodiagnostic and molecular methods) and prevention and control of *T. solium* and STHs.

2.2 Morphology

2.2.1 Morphology of *Taenia solium*

Taenia solium is a flat tapeworm belonging to the phylum *Platyhelminthes*, in the class Cestoidea, order Cyclophylidea and family Taenidae (Soulsby, 1982).

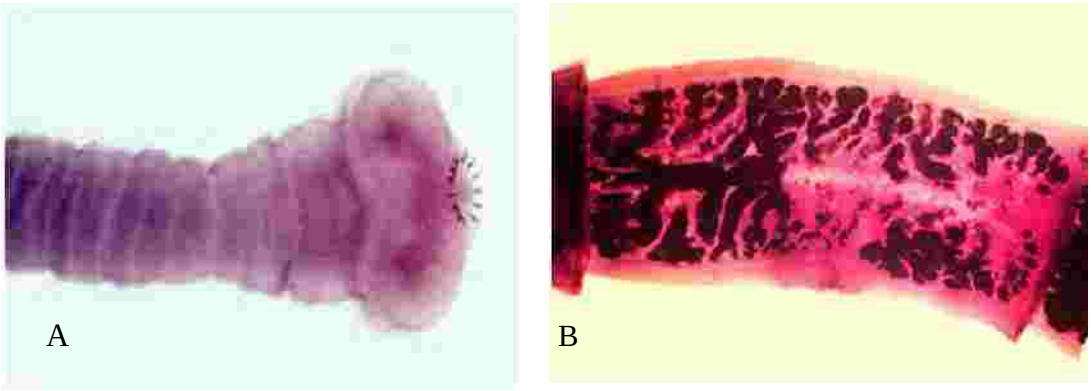

Figure 2.1: The scolex (A) and mature proglottid (B) of *T. solium*. (Source: http://www.dpd.cdc.gov/dpdx/HTML/ImageLibrary/Taeniasis_il.htm)

The tapeworm inhabits the upper part of the small intestine of man and measures 1.8-4.8m long with 800-900 proglottids (Gracey, 1986). The head of *T. solium* is globular and less than 1mm in diameter, while the rostellum is short and has a double crown of 26-28 hooks (Figure 2.1A) and the neck is long and slender. *T. solium* has no alimentary canal and its segmented body is called the strobila. Each segment (Figure 2.1B) is called a proglottid and is hermaphrodite, containing both male and female reproductive organs (Soulsby, 1982). The eggs of *T. solium* (26-34µm in diameter) are embyronated and can only be seen under the microscope as brown coloured and with a radiated appearance (Gracey, 1986).

Cysticercosis in pigs is caused by the presence of cysticercal larvae, *Cysticercus cellulosae,* the metacestode of *T. solium* (Shantz *et al.*, 1992). According to Gracey and Collins (1992), the *C. cellulosae* in pig muscles measure between 0.2cm when young and 2cm at full growth. Each cysticercus has the appearance of a transparent vesicle with the lateral invaginated scolex as a white spot. The scolex, similar to the adult tapeworm, possesses four suckers and a double row of 26-28 hooks (Gracey and Collins, 1992).

2.2.2 Morphology of selected/main soil transmitted helminths (STHs)

2.2.2.1 Morphology of *Ascaris lumbricoides*

Ascaris lumbricoides, a human small intestinal nematode, is characterized by its great size. The males measure 200 mm x 2 mm while females measure 300 mm x 6 mm; but may show great variation in size depending on the age (Muller, 2002; Crompton and Savioli, 2007). The males' posterior end is curved ventrally and has a bluntly pointed tail (Figure 2.2A). The mouth opens terminally and has three lips (Figure 2.2B), each with a pair of sensory papillae on the lateral margins and a row of teeth. The females have paired tortuous tubular ovaries, oviducts and seminal receptacles and uteri which lead into the vulva. The vulva opens on the ventral surface about one third of the body's length from the anterior end. The female lays about 200,000 eggs per day. Under the microscope, the eggs appear ovoid measuring about 45 – 75 µm x 35 – 50 µm. They have a thick shell which usually has a coarsely mammilated outer albuminous coat and stains light yellow (Muller, 2002; Crompton and Savioli, 2007).

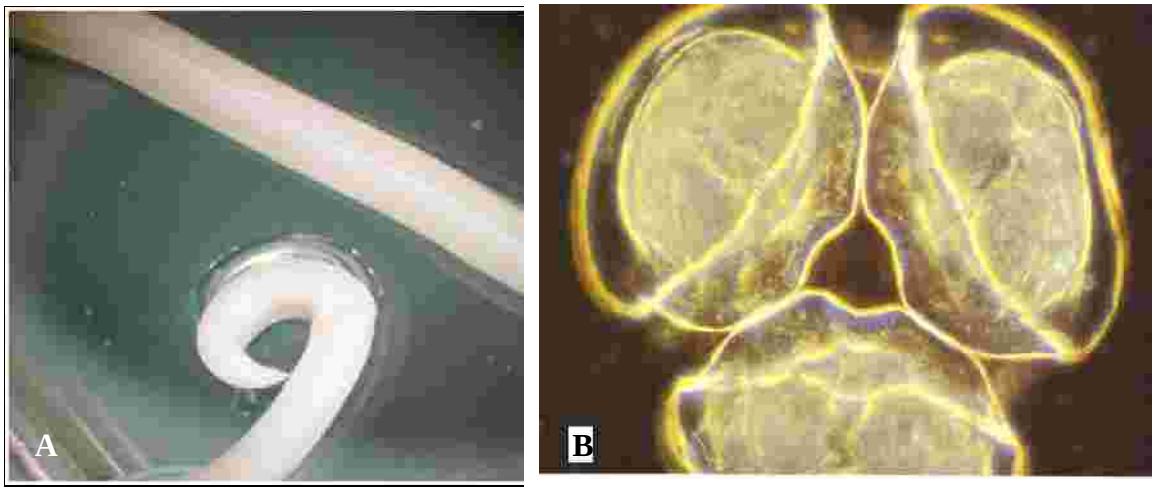

Figure 2.2: The posterior end (A) of a male *A. lumbricoides* and anterior end (B) of *A. lumbricoides* showing the three lips (Source: http://www.dpd.cdc.gov/ DPDX/images/ ParasiteImages/A F/Ascariasis/A_lumbricoides _adult_Orange County.jpg).

2.2.2.2 Morphology of hookworms

Hookworms are parasitic, mostly voracious blood sucking nematodes belonging to the order Strongylida, superfamily Ancylostomatoidea and family Ancylostomatidae (Muller, 2002). Like other nematodes, their bodies are cylindrical, elongate in shape and not segmented (Kassai, 1999). They have a subglobular buccal capsule and an oral opening unarmed or with teeth and cutting plates. Two subfamilies occur: Ancylostominae and Necatorinae (Soulsby, 1982). Two species commonly infect the small intestine of humans, *Ancylostoma duodenale* (Figure: 2.3A) and *Necator americanus* (Figure 2.3B). They are about 1cm long, white or light pinkish when living. The female is slightly larger than the male. The male posterior end is slightly expanded to form a copulatory bursa. Eggs measure 60 x 40µm; are oval, thin walled, colourless and contains 2-8 cells (Ball and Gilles, 1991).

According to Ball and Gilles (1991), the distinguishing features between *A. duodenale* and *N. americanus* include those highlighted in Table 2.1.

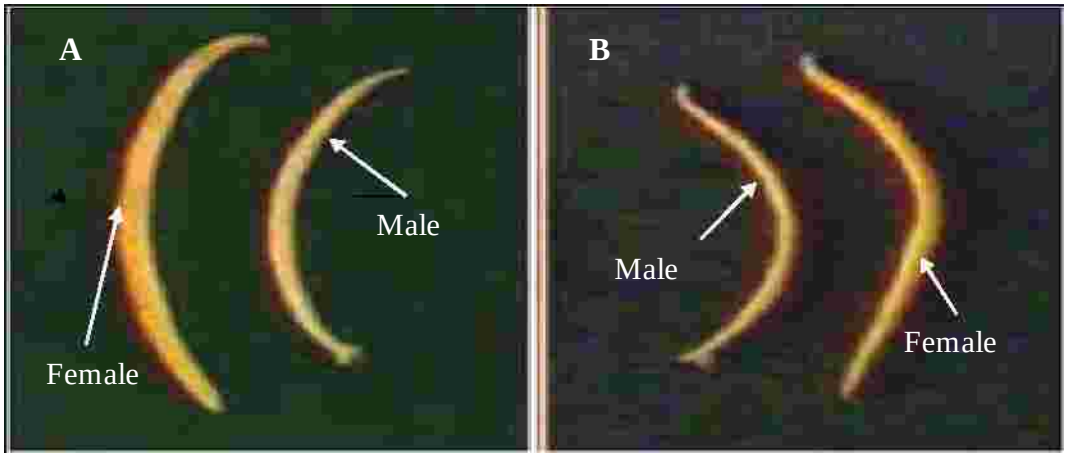

Figure 2.3: Female and male adults of *A. duodenale* (A) and *N. americanus* (B)

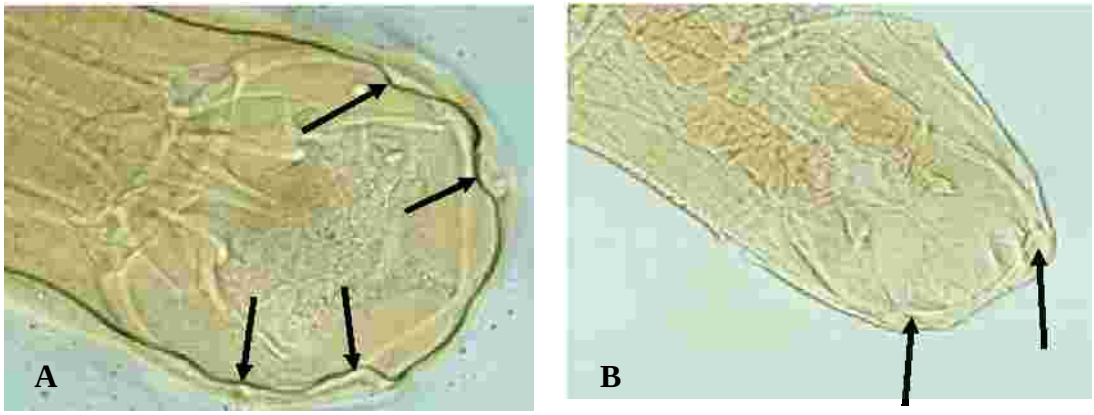

Figure 2.4: Anterior end of *A.duodenale* (A) showing mouth parts with four "teeth" (black arrows) and that of *N.americanus* (B) showing mouth parts with two cutting "teeth" (black arrows). (Source:http://www.dpd.cdc.gov).

Table 2.1: Table summarizing distinguishing features between *A. duodenale* and *N. americanus.*

Feature	*A. duodenale*	*N. americanus*
Size	Larger	Smaller
Shape	Head continuous in the same direction as the body giving it a C shape (Figure 2.3A)	Head curved in opposite direction to the body giving it an S appearance (Figure 2.3B)
Mouth	Two pairs of cutting "teeth" (Figure 2.4A)	One pair of ventral cutting "teeth"(Figure 2.4B)
Copulatory bursa	Circular in shape (a top view)	Oval in shape (a top view)
Copulatory spicule	One pair with separate endings	One pair which unite to form a terminal hooklet
Caudal spine	Present	Absent
Vulva position	Post-equatorial	Pre-equatorial
Portal of entry	Usually via ingestion rather than skin penetration	Usually via skin penetration rather than ingestion
Habitat	Small intestine (duodenum, jejunum)	Small intestine (jejunum, ileum)
Egg output per female worm per day	10,000-25,000	5,000-10,000

(Adapted from Ball and Gilles (1991)

2.2.2.3 Morphology of *Trichuris trichiura*

Trichuris trichiura which mainly infects man and simian primates belongs to the phylum Nematoda, class Adenophorea, order Trichurida, family Trichuridae and genus *Trichuris* (Soulsby, 1982). It is one of the worms that are referred to as "whip worms" due to their anterior long and slender oesophagus which constitutes the major portion of the length of the parasite's body (Figure 2.5).

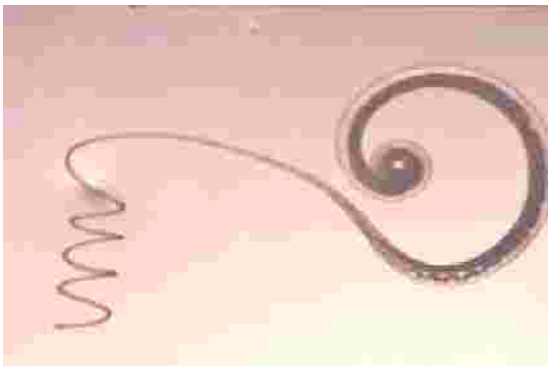

Figure 2.5: Gross morphology of male *Trichuris trichiura* showing the characteristic shape. (Source: http://www.google.co.za/search?q=Picture+of +Trichuris+trichiura)

It is the end that the worm threads into the mucosa of the host's colon and feeds on tissue secretions instead of blood. The posterior part is much thicker and shorter. The hind end of the male is curled (Figure 2.5) and there is one spicule surrounded by a protrusible sheath which is usually armed with fine cuticular spines. The female has a bluntly round posterior end with the vulva situated at the beginning of the wide part of the body. The females are larger than males; approximately 35-50 mm long compared to 30-45 mm (Stephenson *et al.,* 2000: Muller, 2002). The characteristic eggs are barrel shaped and brown and have bipolar transparent plugs.

2.3 Life cycles

2.3.1 Life cycle of *Taenia solium*

The life cycle of *T. solium*, as shown in Figure 2.6, involves the pig as the natural intermediate host of the larval vesicles or cysticerci and man as the sole definitive host of the adult form of the tapeworm. Humans get the infection (taeniosis) by ingestion of undercooked pork infected with *T. solium* cysticerci (Sciutto *et al.,* 2000). In the

stomach, the surrounding layer of the cysticerci larva is destroyed (Flisser *et al.*, 1990), so that when it reaches the small intestines the scolex evaginates and attaches to the intestinal wall by means of suckers and hooks (Muller, 1975). The adult tapeworm develops in the small intestine by forming proglottids which arise from the caudal end of the scolex (Soulsby, 1982; Flisser, 1994). About two months after infection, gravid proglottids (each containing 50,000 to 60,000 fertilized eggs) begin to detach from the distal end and are excreted in the faeces; (Garcia *et al.*, 2003b).

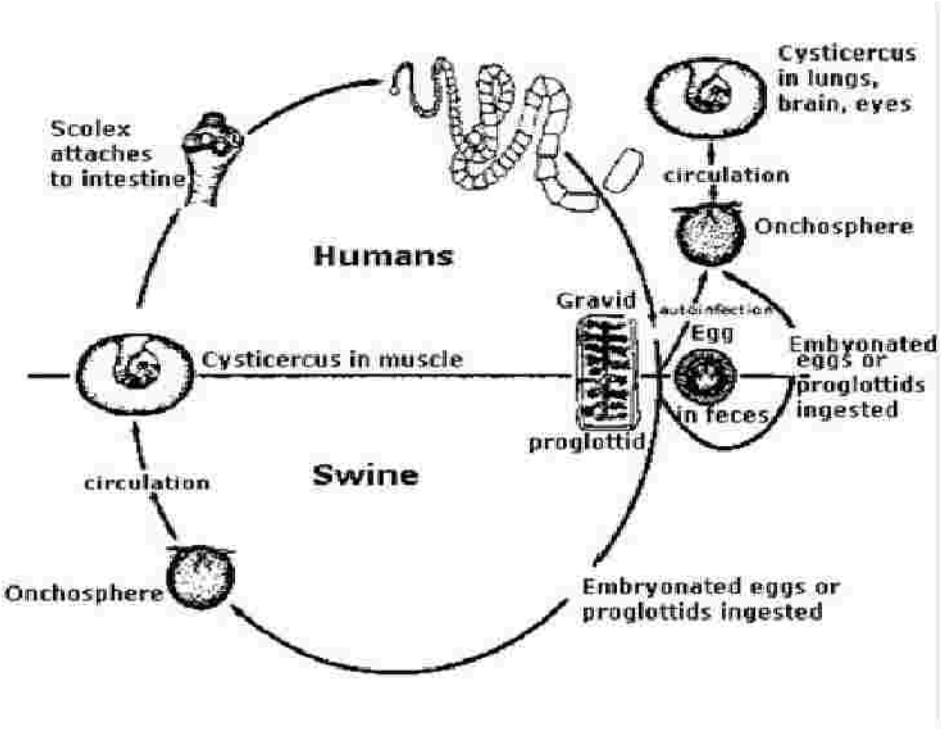

Figure 2.6: The life cycle of *Taenia solium*, showing the infective stages for both man and pig. (Source: www.nlc.net.au/...../taeniasislifecycle.htm)

In areas where pig husbandry practices (free-range pig farming) allow pigs free access to human faeces, pigs end up ingesting the parasite eggs or proglottids (White, 1997). In the pig's stomach the egg hatch and in the small intestine, they are activated, penetrate

the intestinal wall and through blood and lymph vessels, lodge in muscles, eyes and the central nervous system where they transform into cysticerci. This condition is called porcine cysticercosis.

Man may act as an intermediate host and become infected with cysticerci of *T. solium* by ingesting eggs emanating from either himself as a tapeworm carrier (autoinfection) or from others in his close environment through contaminated food or from dirty hands. Similar to the pathogenesis in pigs, cysticerci settle in the muscles, subcutaneously and have a tendency to lodge in the central nervous system, a condition called neurocysticercosis (NCC) (Flisser, 1994; Soulsby, 1982). Other routes of infection to humans include contaminated soil, water and vegetation (Schantz *et al.*, 1992).

2.3.2 Life cycles of soil transmitted helminths (STHs)

The lifecycles of STHs are direct; with no intermediate host involved. The adult stages reproduce sexually in their respective predilection sites and produce eggs, which are passed in human faeces (Soulsby, 1982). In areas where there are no latrines and faecal deposition is by open defecation (OD), the soil and water around the village or community becomes contaminated with faeces containing worm eggs. In the soil, under adequate conditions of temperature and moisture, eggs mature – a process that takes between two to four weeks depending on the type of worm (about two weeks for roundworms and hookworms and about three weeks for whipworm) (Anon, 2010). Some eggs stick to the vegetation around the area and if these are vegetables and if not carefully cooked, peeled or washed, the eggs may be accidentally ingested and the human, thus, gets infected. The eggs may also be ingested from water sources, which

have become contaminated. Therefore, for STHs there is no direct person-to-person transmission or infection from fresh faeces because eggs passed in faeces need about two to three weeks in the soil before they become infective (Anon, 2010).

2.3.2.1 Life cycle of *Ascaris lumbricoides*

The life cycle of *A. lumbricoides* (Figure 2.7) is such that infection occurs when infective embyronated eggs are accidentally ingested. In the duodenal region of the small intestine the eggs hatch and the resultant larvae develop up to L3 stage within the duodenum. The L3 penetrate the wall of the duodenum and enter the blood stream. Through the hepatic portal, they are carried to the liver and heart. They are then carried through the pulmonary circulation to break free in the alveoli where they grow and molt. In three weeks the larvae migrate up the trachea and are swallowed after induced coughing. They, thus return to the small intestine where they mature into adult worms. Fertilization can now occur and the female produces as many as 200,000 eggs per day. These fertilized eggs become infectious after 2 weeks in soil; they can persist in soil for 10 years or more (http: //www.comed.uobaghdad.edu.). They are resistant to many adverse conditions such as temperature, noxious chemicals, a number of detergents and humidity. The eggs are disseminated by rain, wind, insects (flies), birds and other animals. The life span of *A. lumbricoides* in humans is 1 to 2 years (Bentony, 2006; Crompton and Savioli, 2007).

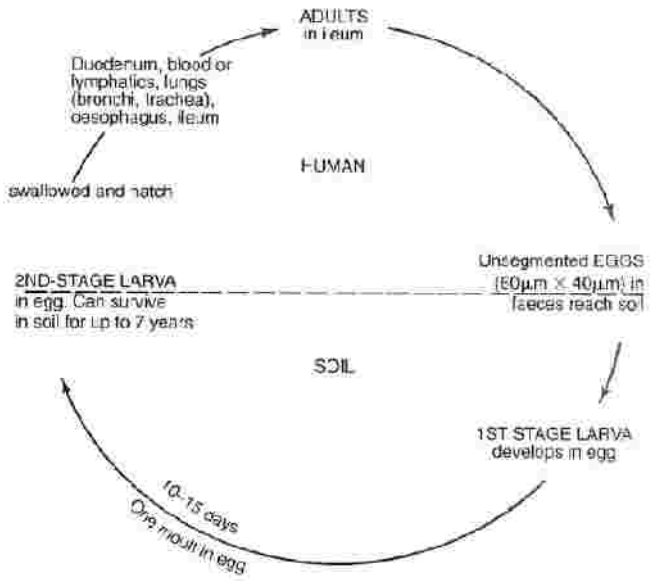

Figure 2.7: Life cycle of *A. lumbricoides* (Source: Muller, 2002).

2.3.2.2 Life cycles of hookworms

The lifecycles of hookworm species are similar (Figure 2.8).

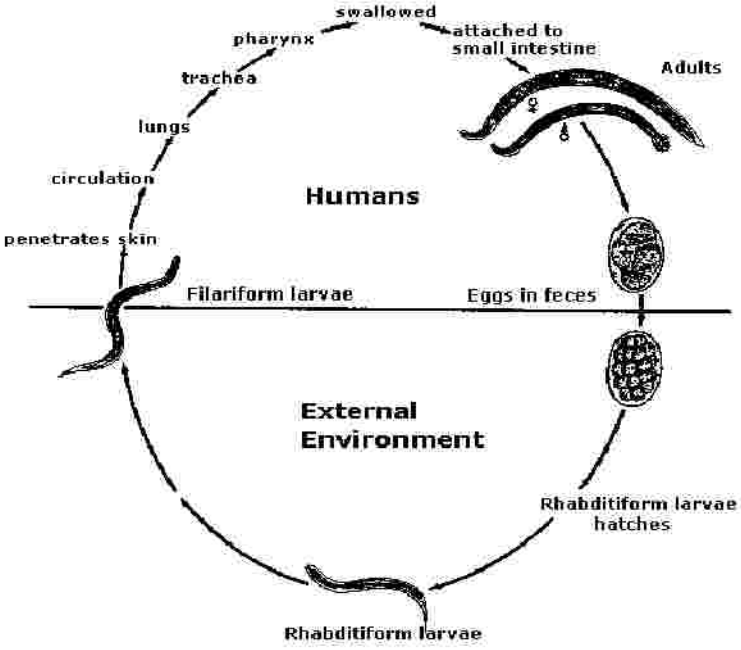

Figure 2.8: Life cycle of hookworms (Source: http://www.dpd.cdc.gov)

The parasites are dioecious, with male and female organs in separate individuals. They mate in host's small intestine and the females lay eggs. Usually the daily output of eggs for a single female is 10,000 and 30,000 (http://www.metapathogen.com). The eggs are passed to the environment with faeces. In about two days, in a warm and moist environment, rhabditiform larvae (L1) hatch from eggs and feed on bacteria and other microorganisms. By the third day, L1 molt into L2 rhabditiform larvae which further molt into filariform larvae (L3). This is the infectious non-feeding stage of the hookworm. The larvae migrate to grass blades ready to stick on the passing host; the larvae survive for several weeks without feeding until they exhaust their metabolic reserves; they adhere to the host on contact and penetrate the skin, usually between the toes, causing the so-called "ground itch". After entry, the L3 larvae are swept by the blood stream and in about 10 days after entry reach the heart and then the lungs, where they rupture capillaries and ascend the alveoli, bronchioles, bronchi and trachea; the host coughs up the larvae and swallow them. When the larvae reach the small intestine, they settle, start feeding and undergo two additional molts before maturing into adults and mating. Intestinal blood loss through the adult worms' voracious feeding habits begins just after egg production and continues for the life of the worm (on average from 1-3 years for *A. duodenale* and 3-10 years for *N. americanus*) (Hoagland, Schad, 1978).

2.3.2.3 Life cycle of *Trichuris trichiura*

Trichuris trichiura infects mainly humans although dogs are also a reservoir (Knopp *et al.*, 2010).

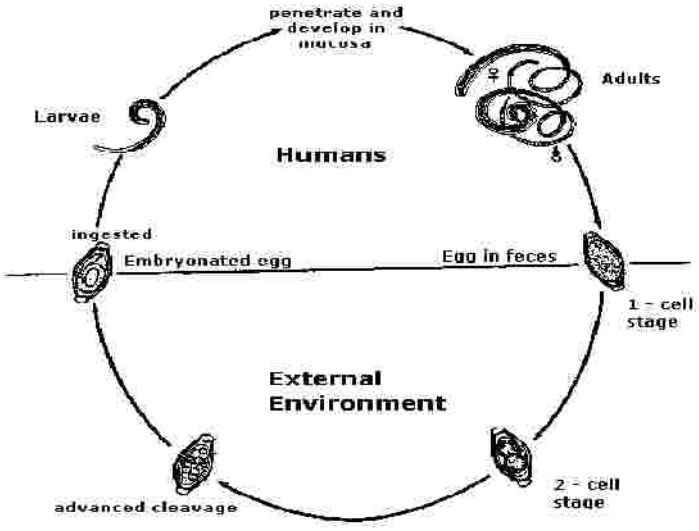

Figure 2.9: Life cycle of *T. trichiura* (Source: http//spojcts.mmi.mcgill.ca)

The life cycle (Figure 2.9) entails unembryonated eggs that are passed in the host's faeces to the soil. In the soil, the eggs develop into a 2-cell stage (segmented egg) and then into an advanced cleavage stage. At this stage, the egg becomes infective, a process that occurs in about 15 to 30 days. The infective eggs are ingested by way of soil-contaminated hands or food and hatch inside the small intestine, releasing larvae into the gastro-intestinal tract. The larvae burrow into the villi and develop into adults over two to three days. They then migrate into the cecum and ascending colon where they thread their anterior portion (whip-like end) into the tissue mucosa and reside permanently for their year-long life span. About 60 to 70 days after infection, female adults begin to release unembryonated eggs into the caecum at a rate of 3,000 to 20,000 eggs per day (Stephenson *et al.*, 2000).

2.4 Prevalence

2.4.1 Prevalence of *Taenia solium* cysticercosis/taeniosis

Approximately, 2.5 million people worldwide carry the *T. solium* tapeworm and not less than 20 million people are infected with cysticerci (Bern *et al.*, 1999). Cysticercosis affects thousands of individuals in less developed countries (Garcia *et al.*, 2000) and more developed countries with a high rate of immigration from endemic areas coupled with increased travel of people from non-endemic to endemic areas (Shantz *et al.*, 1998). It is emerging as a serious public health and agricultural problem in many poor countries of Latin America, Asia and Africa (Willingham III and Angels, 2006). Most of the studies conducted in Latin America and a few in Asia have involved sampling both in humans and pigs (Tables 2.2 and 2.3), respectively. In Africa, however, baseline data on the prevalence of *T. solium* has been collected on pigs but very little in humans.

In contrast to the high prevalence of cysticercosis in endemic areas, *T. solium* taeniosis seldom exceeds 4%; although in some countries prevalence of up to 7% have been reported (Cruz *et al.*, 1989). Mwape *et al.* (2012) found a taeniosis prevalence of 6.3% while the circulating *Cysticercus* antigen prevalence was 5.8% in a community-based study in a rural area in the Eastern Province of Zambia. Often *T. solium* cysticercosis situations are characterized by relatively high rates of human cysticercosis while the prevalence of intestinal *T. solium* in man is low, a phenomenon which has been nicknamed as the "*T. solium* cysticercosis/taeniosis paradox" (Joubert and Evans, 1997).

In Zambia, a survey by postmortem was conducted at an unofficial livestock market (Chibolya) in Lusaka, which indicated a prevalence of 20.6% to 56.6% (Phiri *et al.*, 2002). During this survey, it was found that all these pigs originated from Southern province. Phiri *et al.* (2002) suggested that since the pigs are subjected to ante-mortem tongue examination at several stages to determine infection status before being brought to urban centers (mostly Lusaka), it is highly possible that the prevalence after lingual examination is much higher in Southern province than found at the Lusaka slaughter slab survey.

Table 2.2: Prevalence of human cysticercosis and taeniosis in some selected countries of Latin America, Asia and Africa.

Country	Cysticercosis prevalence (%)	Taeniosis prevalence (%)	Reference
Mexico	12.0[a]	0.5[b]	Garcia-Garcia *et al.*, 1999
	10.8[a]	0.3[b]	Sarti *et al.*, 1992
Ecuador	5.0[a]	1.6[b]	Rodriguez *et al.*, 2003
Peru	21.0[a]	-	Garcia *et al.*, 1999
	8.0[a]	-	Diaz *et al.*, 1992
Bolivia	22.1[a]	-	Carrique-Mas *et al.*, 2001
Honduras	17.0[a]	2.5[b]	Sanchez *et al.*, 1999
	15.6[a]	0.6[b]	Sanchez *et al.*, 1998
China	3.0 – 4.0[a]	-	Rajshekhar *et al.*, *2003*
Vietnam	5.0 – 7.0[a]	-	Rajshekhar *et al.*, 2003
South Africa	7.4[a]	-	Sacks and Berkowitz (1990)
Nigeria	-	11.5[b]	Dada *et al.*, 1993
Benin	1.3[a]	-	Zoli *et al.*, 2003a
Burkina Faso	0.0 – 10.3[a]	-	Carabin *et al.*, 2009
Cameroon	4.5[a]	0.13[b]	Vondou *et al.*, 2002
Burundi	31.5[a]	-	Nsengiyunvia *et al.*, 2003
	4.6[a]	0.0 – 1.0[b]*	Newell *et al.*, 1997
Congo D.R.	21.6[a]	0.33[b]	Praet *et al.*, 2010
Gambia	1.7[a]**	-	Secka *et al.*, 2010
Ghana	CR	-	Zoli *et al.*, 2003b
Ivory Coast	CR	-	Zoli *et al.*, 2003b
Kenya	-	2.5[b]	Asaava *et al.*, 2009
	-	4 - 10[b]	Wohlgemut *et al.*, 2010
Mozambique	15.0-21.0[a]	-	Afonso *et al.*, 2011
Tanzania	16.7[a]	5.2[b]	Mwanjali et al., 2013
Rwanda	7.0[c]	-	Zoli *et al.*, 2003b
Senegal	11.9[a]	9.3[bd]	Secka *et al.*, 2011
Zimbabwe	12.0[a]**	-	Mason *et al.*, 1992
Zambia	5.8[a]	6.3[b]	Mwape *et al.*, 2012
	10.7[a]	3.1[b]	Mwape (2006)

a. Serological examination; b. Coprological examination; *. Study involving school children; **. Study involving epileptic patients; c. Autopsy d. Percentage of the cysticercosis positive individuals; CR case report

Table 2.3: Prevalence of porcine cysticercosis in some selected endemic countries of Latin America, Asia and Africa.

Country	Porcine cysticercosis prevalence (%)	Reference
Mexico	4.0	Sarti *et al.*, 1992
Peru	61.0	Garcia *et al.*, 1999
	43.0	Diaz *et al.*, 1992
Bolivia	37.4	Carrique-Mas *et al.*, 2001
China	5.4 (0.8 40)	Rajshekhar *et al.*, 2003
Vietnam	0.04 – 0.9	Rajshekhar *et al.*, 2003
Cameroon	11.0	Pouedet *et al.*, 2002
Tanzania	0.04 – 4.9	Nsengwa (1995)
	3.2 – 46.7	Ngowi (1999)
Uganda	0 – 33.7	Kisakye and Masaba (2002)
Zambia	20.6 – 56.6	Phiri *et al.*, 2002
	8.2 – 20.8	Phiri *et al.*, 2002
	10.8 – 23.3	Sikasunge *et al.*, 2008a

2.4.2 Prevalence of soil transmitted helminth infections

According to de Silva *et al.* (2003), STH infections are the most prevalent infections of humans. They are said to infect nearly 2 billion people worldwide (Awasthi *et al.*, 2003). These infections are most prevalent in tropical and sub-tropical regions of the developing world where adequate water and sanitation are lacking coupled with adequate moisture and warm temperatures which are essential for larval development in the soil (Brooker *et al.*, 2006). The *A. lumbricoides* has been estimated to infect 1.221 billion people worldwide while *T. trichiura* and hookworms infect 795 million and 740 million people respectively (de Silva *et al.*, 2003).

The greatest numbers of STH infections occur in sub-Saharan Africa, East Asia, India and South America. It is estimated that over 35.4 million African school-aged children are infected with *A. lumbricoides*, 40.1 million with *T. trichiura* and 41.1 million with

hookworms (Brooker *et al.*, 2006). Since many children have multiple infections, it is estimated that 89.9 million are infected with any STH species in Africa.

Zambia is one of the developing countries where most communities are poor and live in unsanitary conditions and thus, STHs are potentially prevalent. Very few prevalence studies have been conducted, but some studies have reported considerably high prevalences of geohelminths including *A. lumbricoides* and hookworms. In a study carried out to investigate the effect of iron supplementation on geophagy in Zambian school children, prevalence of *A. lumbricoides* in children aged 7 to 15 years was 44.8% (Nchito *et al.*, 2004). This was higher in geophagous children than in non-geophagous children (39.6%). Chintu *et al.* (1995) also determined the frequency of parasitic infections in hospitalized children and reported *A. lumbricoides* to be one of the commonest parasites isolated. Helminth infections have also been reported in adults. In a study that assessed the prevalence of helminth infections in HIV-infected adults, 24.9% were infected with at least one of the STHs, with *A. lumbricoides* being the most common followed by hookworm (Modjarrad *et al.*, 2005). Wenlock (1997), in his research on hookworm and *Schistosoma haematobium* infection rates in 7 provinces of Zambia reported that 48.6% of the sampled individuals were positive for hookworm and further stated that regional figures ranged between 11.4% and 77.1%. In Kafue and Luangwa districts, hookworm infection prevalence of 11% and 4.3% for the plateau and valley regions respectively were reported (Simoonga, 2006). This was in school children aged 5 to 20 years. A study in Mazabuka district, Zambia found prevalence of *A. lumbricoides* and hookworm of 16.4% and 7.0% respectively in children aged 12 to 59 months (Halwindi *et al.*, 2011). In a more recent study, Siwila *et al.* (2010), in a pre-

school based study to determine intestinal helminth and protozoan infections in Kafue District of the Lusaka Province of Zambia, reported an overall intestinal helminth prevalence of 17.9% comprising *A. lumbricoides* (12.0%), *hookworm (8.3%)*, *Taenia* spp. (0.9%), *Hymenolepis nana* (0.6%) and *Schistosoma mansoni* (0.3%).

2.5 Diagnosis

2.5.1 Diagnosis of *Taenia solium* infections

Diagnostic methods for cysticercosis/taeniosis can be grouped as parasitological, immunological and molecular approaches.

2.5.1.1 Parasitological methods

Parasitological methods in pigs include tongue examination and meat inspection. However, in humans, parasitological methods include examination of faeces for detection of *Taenia* eggs (coproparasitology), morphological examination of adult intestinal taeniids and diagnosisis of subcutaneous cysticercosis by biopsy.

2.5.1.1.1 Parasitological methods in pigs

The most common method of diagnosing porcine cysticercosis *in vivo* at the village level is tongue examination. However, tongue examination; although highly specific, requires technical skills and has low sensitivity when applied on pigs with low cyst burden (Gonzalez *et al.*, 1990 Sciutto *et al.*, 1998a; Boa *et al.*, 2002). Dorny *et al.* (2004) found tongue examination to be 21.0% sensitive but 100% specific. The vesicular metacestodes can be palpated and are easily seen. However, fibrous or

calcified larvae (cysts) are more difficult to detect, as they tend to be quite small (Sciutto *et al.*, 1998b). This is in contrast with the calcified cysts of *Taenia saginata*, which are comparatively easy to identify at meat inspection because they often form white and fibrotic lesions (Onyango-Abuje *et al.*, 1996). Sciutto *et al.* (1998a) also estimated that more than 50% of pigs that harbour metacestodes show them in the tongue, and recommended the tongue as one of the sites for meat inspection in addition to the diaphragm or the shoulder muscles. They also reported that the maximal sensitivity obtained by tongue examination was 71% in experimentally infected pigs. Although detection of cysticercosis infection is routinely done at meat inspection, the technique is time consuming and infected carcasses are easily missed and passed on for human consumption (Gonzalez *et al.*, 1990). Another disadvantage of the current meat inspection procedures is that infection is detected after death of an animal, which is too late to make any decision over treatment (Onyango-Abuje *et al.*, 1996). In developing countries, meat inspection is lacking as most pigs are slaughtered locally in backyards without any inspection (Sakai *et al.*, 1998).

2.5.1.1.2 Parasitological methods in human

2.5.1.1.2.1 Coproparasitological examination

Coproparasitological examination allows detection of *Taenia* eggs from stool samples. However, the techniques employed, for example formol-ether concentration technique, are known to have both low sensitivity and specificity due to the intermittent nature of egg excretion, and non-uniform distribution of eggs in the faeces leading to underestimation of the prevalence of taeniosis (Allan *et al.*, 1997, Garcia *et al.*, 2003a). If destrobilation has led to a massive discharge of eggs these may be absent from the

faeces for up to several weeks thereafter (World Health Organization, 1983). Furthermore, *T. saginata* and *T. solium* eggs are identical under the light microscope leading to problems with diagnostic specificity. This is particularly important given the risks associated with *T. solium* infection (Allan *et al.*, 2003). The formol-ether concentration technique is the technique that is widely used for the detection of *Taenia* eggs in faeces.

2.5.1.1.2.2 Morphological examination

Identification of human adult intestinal taeniids to species level classically relies on the recovery of mature proglottids or scoleces. This recovery has, however, proven difficult due to the disintegration of the proximal end of the worm when modern cestoidal drugs are used (World Health Organisation, 1983). Jeri *et al.* (2004) improved the treatment method to obtain a recognizable tapeworm, making differentiation between *T. saginata* and *T. solium* easier. Proglottids can be stained with the Semichon's acetocarmine stain method to enable morphological differentiation. The differentiation of the two human *Taenia* species is based on the number of uterine branches present in well-preserved gravid proglottids or on the absence or presence of hooks on the scolex of the tapeworm (Mayta *et al.*, 2000). According to Harrison and Bogitsh (1991), *T. solium* gravid proglottid has 7 to 11 while that of *T. saginata* has 12 to 32 uterine branches. Faust *et al.* (1970), however, reported that the proglottids of *T. solium* have 15 or less uterine branches compared to those of *T. saginata*. Therefore, there is a possibility of an overlap making differentiation based on number of uterine branches not totally reliable.

2.5.1.1.2.3 Diagnosis of subcutaneous cysticercosis by biopsy

In some parts of Asia, especially, where subcutaneous cysticercosis is rather frequent (Rajshekkar *et al.*, 2003), it is easy to obtain biopsy material for further histological confirmation. The diagnosis of *T. solium* cysticercosis is made parasitologically by demonstrating the scolex with hooks or fragments of the bladder wall in biopsy or autopsy material. With less invasive techniques, such as fine needle cytology, the diagnosis of cysticercosis can often be made (Arora *et al.*, 1994).

2.5.1.2 Immunodiagnostic methods in pigs and humans.

The development of improved immunodiagnostic tools has contributed to our knowledge on cysticercosis/taeniosis. Serological tests have been developed for the detection of specific antibodies or for circulating parasite antigens in serum or body fluids such as cerebrospinal fluid (CSF) and more recently urine (Geerts *et al.*, 1981; Harrison *et al.*, 1989; Dorny *et al.*, 2003; Mwape *et al.*, 2011). Since pigs are the primary intermediate hosts, prevalence of porcine cysticercosis is a reliable indicator of active transmission zones (Sanchez *et al.*, 1997; Garcia-Garcia *et al.*, 1999). In epidemiological studies, serological tools can be applied to diagnose human and pig cysticercosis. Diaz *et al.* (1992) recommended serological studies in both humans and pigs as being useful for determining areas where the disease is endemic and defining and targeting high-risk families to *T. solium* antigen contact as well as for monitoring the success of control programmes by determining the incidence of new cysticercosis infections. Flisser (2002) reported that since there are no clinical features specific for cysticercosis, even asymptomatic brain lesions not uncommon, imaging methods

unavailable for epidemiological studies; the definition of cases is based solely on immunodiagnostic methods.

Garcia *et al.* (2001) and Gonzalez *et al.* (1999) noted that antibody detection has an important drawback of failing to distinguish between exposure to infection and an established infection. The occurrence of transient antibody response in *T. solium* infection both in humans and in pigs in field conditions was found to be a major contributor to the over estimation of cysticercosis prevalence in endemic areas of Peru and Columbia. Data from serological surveys in these areas demonstrated that about 40% of seropositive people were seronegative when re-sampled after one year (Garcia *et al.,* 2001). Further, for porcine cysticercosis, cross-reactions with *Cysticercus tenuicollis* are rather the rule than the exception in most antibody and mainly in antigen detecting tests (Dorny *et al.*, 2003). Secondly, antibodies may persist long after the parasite has been eliminated by immune mechanisms and/or drug therapy (Harrison *et al.*, 1989; Garcia *et al.,* 1997). Maternal antibodies transferred by colostrum from a seropositive sow to its piglets may persist for up to 7 weeks (Sikasunge *et al.*, 2010). The occurrence of cross-reactions with other diseases such as hydatidosis and ascariosis has been observed with *T. solium* antigen (Pinto *et al.*, 2000).

It has been observed that collection of cerebrospinal fluid, blood or serum is an invasive procedure that requires technical expertise and the use of disposable syringes. If the method is not carried out under stringent aseptic conditions there is the risk of acquiring blood-borne infections such as hepatitis B virus and human immunodeficiency virus (Parija *et al.*, 2004). Body fluids, including urine, saliva, and tear drops, collected using non-invasive methods could therefore be of immense value in the diagnosis and in

epidemiological studies of parasitic diseases. Of these, urine is increasingly used as a specimen alternate to blood for the diagnosis of many parasite infections (Parija, 1998; Mwape *et al.*, 2011). However, the major drawback of urine under field conditions is its loss of specificity (Mwape *et al.*, 2011).

2.5.1.2.1 Antibody Enzyme-Linked Immunosorbent Assay (Ab-ELISA)

Various techniques to detect antibodies to *T. solium* infections in man and pigs have been described such as the complement fixation test, hemaglutination, radioimmunoassay, enzyme linked immunosorbent assay (ELISA), dipstick ELISA, latex agglutination and immunoblot (Miller *et al.*, 1984; Tsang *et al.*, 1989; Ferreira *et al.*, 1997; Garcia and Sortelo, 1991; Ito *et al.*, 1998; Rocha *et al.*, 2002). Serodiagnosis of cysticercosis through detection of anti-parasite antibody has been widely evaluated using several target antigens, ranging from total *T. solium* extracts of the metacestodes (Flisser *et al.*, 1994) to selected preparations, such as cyst fluid, scolex or extracts of external membranes (Larralde *et al.*, 1986). The antigens used in immunoblot and ELISA for antibody detection have evolved, increasing both the sensitivity and the specificity of the tests (Dorny *et al.*, 2003). The possibility of using synthetic peptides based on identified antigenic epitopes has been explored (Gevorkian *et al.*, 1996). Pinto *et al.* (2000) conducted a study to evaluate antigens of *T. solium* and *T. crassiceps* cysticerci in the ELISA test for the diagnosis of porcine cysticercosis. Four antigens; (i) vesicular fluid, (ii) crude *T. crassiceps* antigens, (iii) scolex and (iv) crude *T. solium* antigen preparations were assayed. The results indicated that though all the antigens showed good performance, the vesicular fluid of *T. crassiceps* was the best followed by crude *T. crassiceps* antigen preparations. A separate study conducted by Nunes *et al.*

(2000) also found similar results. According to the study, the use of cyst fluid and crude antigens of *T. crassiceps* metacestodes obtained the best results of overall specificity and sensitivity of 100 and 96.4% respectively.

2.5.1.2.2 Antigen Enzyme-Linked Immunosorbent Assay (Ag-ELISA)

Due to the two drawbacks associated with antibody detection namely production of transient antibodies and persistence of antibody after infection, antigen detection has provided a suitable alternative (Dorny *et al.*, 2003). Several researchers (De Jonge *et al.*, 1987; Harrison *et al.*, 1989; Brandt *et al.*, 1992; Draelants *et al.*, 1995; Onyango-Abuje *et al.*, 1996 and Van Kerckhoven *et al.*, 1998) have contributed to the development of antigen detecting ELISAs. Harrison *et al.* (1989) developed an antigen detecting ELISA based on a mouse monoclonal antibody (MoAb) with a repetitive carbohydrate epitope found in lentil-lectin adherent glycoproteins present on the surface and in the secretions of *T. saginata* cysticerci. As the target glycoprotein contains multiple antigenic epitopes recognized by the MoAb, the same MoAb was used in the trapping and indicating layers of a double sandwich antigen ELISA (Ag-ELISA) that was designed to detect these glycoproteins in serum of *T. saginata* infected cattle. Another type of Ag-ELISA (the B158/B60 Ag-ELISA), which uses 2 different monoclonal antibodies, has been used in sero-epidemiological studies for *T. saginata* and *T. solium* cysticercosis in Zambia (Dorny *et al.*, 2002; Phiri *et al.*, 2002). The circulating antigen detecting technique offers the advantage over the Ab-ELISA of only demonstrating the presence of live cysts and is reported to give a better correlation between the actual presence of viable infective cysticerci and antigen positive cases (Harrison *et al.* 1989). It is also

reported to give fewer cross-reactions with other helminth infections (Dorny *et al.*, 2000).

Harrison *et al.* (1989) showed that when the drug praziquantel killed the cysticerci, the ELISA assay became negative, presumably because parasite products were no longer produced by the dead cysticerci.

Antigen detection may be done on serum as well as on CSF (Choromanski *et al.*, 1990; Garcia *et al.*, 1998, 2000). Antigen detection in CSF may be more appropriate for diagnosis than serum because of the localization of cysts in the brain; however, sampling CSF is more difficult than blood. Collection of other body fluids like urine, has offered an alternative to the more invasive procedure of collecting blood. Antigen detection in urine is being increasingly employed in the diagnosis of various parasitic infections such as schistosomosis (Kremsner *et al.*, 1993), Chaga's disease (Freilij *et al.*, 1987), leishmaniasis (Kohanteb *et al.*, 1987), malaria (Katzin *et al.*, 1991), filariasis (Zheng *et al.*, 1987), toxoplasmosis (Ayi *et al.*, 2005) cystic echinococcosis (Parija *et al.*, 1997; Ravinder *et al.*, 2000) and cysticercosis (Parija *et al.*, 2004; Mwape *et al.*, 2011).

The sensitivity and specificity of the antigen detecting ELISA are reported to be high. Praet *et al.* (2010) found a sensitivity of 90% and a specificity of 98%. However, in this same study, they found Ag-ELISA insensitive (sensitivity of 5%) to detect exposure to parasite because it only detects individuals with living cysts excreting antigens (Dorny *et al.*, 2003). Erhart *et al.* (2002) found a very good agreement between an ELISA for detecting circulating antigens, computerized tomography (CT) scanning and biopsy

examination of subcutaneous cysticerci. Remarkably low levels of cross-reactions have been observed in serum from a wide range of helminth and protozoan infections (Harrison *et al.*, 1989; Erhart *et al.*, 2002).

2.5.1.2.3 Enzyme-Linked Immunoelectrotransfer Blot (EITB) or Western Blot

The most specific and widely used test developed for the diagnosis of cysticercosis in human and pig serum samples is the EITB, an immunoblot of seven *Cysticercus* glycoproteins, purified by lentil lectin-purified chromatography, which gives close to 100% specificity and a sensitivity varying from 70 to 90% (Tsang *et al.*, 1989). However, a sensitivity of only 28% has been found in cases with single cysts in the brain (Wilson *et al.*, 1991). The antigen mixture used is not applicable for ELISA because of the presence of non-specific fractions (Dorny *et al.*, 2003). The EITB immunoblot has been applied in field studies to detect porcine cysticercosis in endemic areas of Peru, Guatemala and Mexico (Gonzalez *et al.*, 1990; Allan *et al.*, 1997; Sarti *et al.*, 1997). However, Sciutto *et al.* (1998b) found neither Ag-ELISA, Ab-ELISA nor EITB adequate for the diagnosis of porcine cysticercosis in lightly infected pigs (pigs with low cyst burdens) and such pigs may escape detection by meat inspection, thereby maintaining parasite transmission by allowing lightly infected carcasses to remain in the food chain. Furthermore, the major disadvantage of the test is the complicated nature of antigen preparation, the cost and instability of the reagents involved during the production (Rodriquez-Canul *et al.*, 1998). In addition, the equipment used is often unavailable in many laboratories in developing countries where cysticercosis is endemic (Rodriquez-Canul *et al.*, 1997). Furthermore, EITB commercial kits for cysticercosis

are difficult to obtain in endemic countries, so its use may be restricted to research studies (Pal *et al.*, 2000).

Wilkins *et al.* (1999) developed an immunoblot assay, to identify adult *T. solium* tapeworm carriers using excretory and secretory antigens collected from *in vivo* cultured *T. solium* tapeworms. The assay can be used to identify persons with current or recent *T. solium* tapeworm infections and provides a new important tool for epidemiological purposes, including control and prevention strategies.

2.5.1.2.4 Copro Ag-ELISA for taeniosis

The detection of parasite specific antigens in host faeces was first reported for canine *Echinococcus granulosus* by Babos and Nemeth (1962). Twenty years later the World Health Organization, in its guidelines on the diagnosis of echinococcosis (WHO, 1984), suggested that if it were possible to detect *Echinococcus* antigen in dog faeces then the same would be possible for *T. solium* in humans.

Parasite coproantingens constitute specific products in the faeces of the host that are amenable to immunological detection. If these products are associated with parasite metabolism they should be present independently of parasite reproductive material (i.e. taeniid eggs or proglottids) and should disappear from faeces shortly after removal of the intestinal infection (Allan *et al.*, 2003).

Coproantigen-based immunodiagnostic studies for *Taenia* in dogs and humans have all employed antigen capture ELISA assays using sera from rabbits hyperimmunised with either adult worm somatic or excretory-secretory products (Allan *et al.*, 2003). They have been used to detect antigen in sollubilised faecal samples. Allan *et al.* (2003)

further reported that antigen detection is genus specific with *T. saginata* and *T. solium* both reacting in the assays but with no cross reactions with faeces from other infections including *Hymenolepis* cestodes.

The levels of sensitivity of these assays are dependent on the assay format (both microplate and dipstick formats have been used to date) and the quality of the rabbit sera used in their production (high titre sera being better). In one field study, 98% of all diagnosed cases were diagnosed by the coproantigen ELISA test (55/56) in comparison to only 38% by microscopy (21/56)) (Allan *et al.*, 1996).

Whilst these assays have been applied successfully as part of field research programmes in endemic countries, issues such as cost and accessibility remain to be addressed if these tests are to be used routinely in endemic countries (Allan *et al.*, 2003). In developing countries ELISA is preferred because of its better availability, simplicity, and lower cost compared with immunoblot (Rosas *et al.*, 1986).

2.5.1.3 Molecular approaches

The Polymerase Chain Reaction (PCR) has nowadays not only become an important diagnostic tool but also a tool for the study of the phylogeny of infectious agents. This technology has been shown to differentiate parasites starting from small amounts of their DNA (Allan *et al.*, 2003).

Differentiation of human *Taenia spp*. by molecular assays is normally done on proglottids expelled from carriers after treatment (Eom *et al.*, 2002; Rodriguez-Hidalgo *et al.*, 2002; Gonzalez *et al.*, 2002). In recent years, PCR tests for species-specific confirmation of *Taenia spp*. have been developed based on the detection of the parasite

DNA in faecal samples (copro-DNA) (Yamasaki *et al.*, 2004), or on cysticercii (Yamasaki *et al.*, 2002; Yamasaki *et al.*, 2004) or eggs present in the faeces (Yamasaki *et al.*, 2004). However, the current DNA extraction methods are too expensive for use as a routine test (Nunes *et al.*, 2003).

Different methods and loci have been used for differentiating *Taenia spp*. Gonzalez *et al.* (2002) designated primers and used these in multiplex PCR giving specific detection of *T. saginata* and *T. solium*.

Mayta *et al.* (2000) used PCR-Restriction Fragment Length Polymorphism (PCR-RFLP) to differentiate *T. solium* and *T. saginata*. They amplified the 3' region of the 18S and the 5' region of the 28S ribosomal gene (spanning the 5.8S ribosomal gene) and used three restriction enzymes (AluI, D*de*I or *Mbo*I) for analysis of the PCR amplicons. Each enzyme gave a unique pattern for each species. In this assay, the primers amplified DNA from all cestodes, not only from *Taenia spp*.

Rodriguez-Hidalgo *et al.* (2002) also differentiated *Taenia spp* by PCR-RFLP using the 12S rDNA but developed new primers to reduce on the non-specific amplification experienced when using field samples. They, however, also used D*de*I as the restriction enzyme.

When Praet *et al.* (2013) compared coprology, Co-Ag-ELISA and copro-PCR using a Bayesian approach they found that the three tests had specificities of 99.9%, 92.0% and 99.0% respectively, and sensitivities of 52.5%, 84.5% and 82.7% respectively. Based on those results, they urged for additional studies exploring possible cross-reactions of the copro-AgELISA and for the use of more sensitive tests, such as copro-PCR, for the

detection of tapeworm carriers, which is a key factor in controlling the parasite in endemic areas.

2.5.1.4 Neuro-imaging for human cysticercosis

Cysticercosis in humans may be diagnosed by computer axial tomography (CT) scan and magnetic resonance imaging (MRI) that visualize living and calcified cysticerci or the oedematous lesions they cause (Carpio *et al.*, 1998). Dumas *et al.* (1997) stated that only the presence of cystic lesions demonstrating the scolex should be considered pathognomic of neurocysticercosis. The scolex is visualized as a bright dot within the cyst. This produces the so-called 'hole-with-dot' image that may be seen in vesicular cysts located in the brain parenchyma, subarachnoid space or the ventricular system (Lozano-Elizondo, 1983), thereby providing evidence on the number and location intracranial cysticerci, their viability and the severity of the host inflammatory reaction against the parasite (Garcia *et al.*, 2003a).

It has been claimed that CT has sensitivity and specificity of 95% for the diagnosis of neurocysticercosis (Nash and Neva, 1984) although CT images are rarely pathognomonic for this disease and thus, should be used in conjunction with reliable serologic tests such as EITB (Garcia *et al.*, 1994).

Martinez *et al.* (1989) stated that MRI is the most accurate technique to assess the degree of infection, the location and the involuntary stage of the parasite. It visualizes well the perilesional oedema and the degenerative changes of the parasite, as well as small cysts or those located in the ventricles, brainstem, eyes and basal racemose vesicles. However, CT scan is more sensitive for the detection of calcification (Martinez

et al., 1989). The main disadvantages of neuroimaging techniques are their high cost and scarce availability (Garcia *et al.*, 1994).

2.5.2 Diagnosis of STH infections

Many STH infections present without specific signs and symptoms. In some cases, especially of hookworm infection, persistent eosinophilia during blood examinations is a common finding (Nutman *et al.*, 1987).

There are many methods that are used to examine for STH infections. They include, among others, coprological methods (direct microscopic examination, formalin-ether concentration, Kato-katz, McMaster), molecular methods (PCR) and imaging methods (ultrasonography, endoscopy, radiology and anoscopy).

2.5.2.1 Coproparasitological methods

Direct microscopic examination of faeces is adequate for detecting hookworm infection (Bentony *et al.*, 2006). Concentration techniques can also be used, for example, formalin-ether concentration technique can be used to detect light infections. Other methods that can be used are the Kato-Katz and McMaster methods, which apart from detecting the eggs, can also be used to measure the intensity of the infection by estimating the eggs per gram (EPG) of the faeces (Bentony *et al.*, 2006). To date, Kato-Katz is the diagnostic method recommended by the World Health Organization (WHO) for the quantification of STH eggs in human stool (WHO, 1991), because of its simple format and ease-of-use in the field. The chief limitation of the Kato-Katz method, however, arises when it is used with the objective of simultaneous assessment of STH in faecal samples from subjects with multiple species infections. This is because

helminth eggs of different species appear at different intervals (clearing times). In addition, hookworm eggs rapidly disappear in cleared slides, resulting in false negative results if the interval between preparation and examination of the slide is too long (> 30 minutes). These properties have impeded standardization of the Kato-Katz method in large scale studies at different study sites (Ramsan *et al.*, 1999; Speich *et al.*, 2010). Moreover, quantification of the intensity of egg excretion is based on a fixed volume of faeces, rather than the mass of the faeces examined. Its quantitative performance is, therefore, questionable, as the intensity of eggs excreted is expressed as the EPG (Engels *et al.*, 1997), and the density of the faeces can vary. The McMaster is an alternative method for monitoring large-scale treatment programs. It is a robust (accurate multiplication factor) and accurate (reliable efficacy results) method which can be easily standardized (Levecke *et al.*, 2011). However, both Kato-Katz and McMaster methods do not distinguish between the eggs of *Ancylostoma* and *Necator* and, therefore, recovery of adult worms through expulsion or preparation of feacal cultures to obtain the third stage larvae using the Harada-Mori *in vivo* culture method may be necessary (Pawlowski *et al.*, 1991).

A new technique, FLOTAC (trade name), which is mainly used in the veterinary medicine/cases, was suggested as suitable diagnostic tool particularly in situations of low parasite infection intensities (Crigoli, 2006). Recent studies found that a single FLOTAC examination was more sensitive than triplicate Kato-Katz thick smear for the diagnosis of low-intensity STH infections (Knopp *et al.*, 2009). In particular, the FLOTAC technique improves the ability to diagnose human hookworm infections accurately (Utzinger *et al.*, 2008), which is generally underestimated when using Kato-

Katz thick smear. FLOTAC was thus suggested as a suitable method for a rigorous surveillance of helminth control programs, monitoring of STH transmission and verification of local elimination (Knopp *et al.*, 2009).

The results of a comparative study of 4 techniques, i.e. ether-based concentration, Parasep, Solvent Free, McMaster and FLOTAC, showed that despite the fact that McMaster was less sensitive than FLOTAC, the former technique was the most feasible and easy to perform under field conditions (Levecke *et al.*, 2009).

2.5.2.2 Molecular methods

Polymerase chain reaction (PCR) is normally used for identification of species (Zhan *et al.*, 2001) and also to differentiate hookworm infections from other helminths like *Trichostrongylus spp.* whose eggs cannot easily be distinguished from those of hookworm (Yong *et al.*, 2007). Lele *et al.* (2009) developed a PCR diagnosis of *A. lumbricoides* from the morphologically identical *A. suum* which infects pigs.

2.5.2.3 Imaging methods

Ultrasonography and endoscopy are used for diagnostic imaging of the complications of *A. lumbricoides* like intestinal obstruction and hepatobiliary and pancreatic involvement (Khuroo *et al.*, 1990; Koumainodou *et al.*, 2004). Radiology can be used to detect adult worms after barium meal (Muller, 2002). In *Trichuris trichiura* infections, since adults may extend as far as the rectum in heavy infections, anoscopy can be used to diagnose infections (Gilman *et al.*, 1983).

2.6 Treatment, Prevention and control

2.6.1 Prevention and control of taeniosis/cysticercosis

Researchers have come up with various recommendations and control strategies for the prevention and control of the taeniosis/cysticercosis disease complex. Gonzalez *et al.* (2003) stated that the life cycle of *T. solium* is sustained because pigs have access to infected faeces and cysticercosis-infected pork is available for consumption. Therefore, eradication of cysticercosis is possible by removing the disease from either human or pig or both (Pal *et al.*, 1999).

From the human perspective, efforts to educate villagers at schools, village meetings and on an individual basis have been highly successful in terms of teaching villagers the parasite lifecycle and the connection between infected pigs and themselves or others getting cysticercosis (Keilbach *et al.*, 1989; Sarti *et al.*, 1997) including the importance of good personal hygiene and proper cooking of suspected infected pork. Garcia *et al.* (1999) suggested that the only proven way of eradicating cysticercosis is the improvement of sanitary conditions, portable water and sewerage connection as occurred in Europe in the early 1990s. They, however, noted that economical and geographical constraints make this impossible in the near future for most developing countries. The World Health Organization (1983) recommends the detection and treatment of tapeworm carriers or treatment of the whole population. In regions where taeniosis is only due to *T. saginata* and human cysticercosis does not occur, praziquantel may be used. Mass treatment of the human population may be performed where *T. solium* is endemic (Allan *et al.*, 1997). For treatment of human taeniosis in *T. solium* endemic areas, niclosamide is preferred because it is highly effective against the

intestinal stage of the parasite and has no effect on the cystic stage (WHO, 1983; Miyazaki, 1991; Allan *et al.*, 1997).

From the pig perspective, Sarti-G *et al.* (1992) recommended that effective and long-lasting control of transmission of *T. solium* from pigs to humans must include measures to deny pig's access to human faeces. Pal *et al.* (1999), however, noted that in developing countries, pigs are free-roaming and raised by subsistence farmers who cannot afford enclosed pens or proper animal feed. They further noted that meat inspection in developing countries is difficult because meat is sold off the abattoir system. The potential of a vaccine for controlling porcine cysticercosis has been described in the past and some promising results were also reported (Gonzalez *et al.*, 2003). A successful vaccine that has the potential of interrupting the cycle should decrease over time the number of infected pigs and humans. While it may be potentially possible to vaccinate the human population against *T. solium*, a less expensive option is the vaccination of pigs to prevent the disease transmission thereby indirectly reducing the number of new human cases of neurocysticercosis (Assana *et al.*, 2010). Nevertheless, the potential use of a vaccine will depend on its availability and cost (Gilman *et al.*, 1999). Sciutto *et al.* (1995) reported that vaccination of pigs against *T. solium* cysticercosis should be further investigated before being massively applied. Huerta *et al.* (2000) vaccinated pigs of mixed genetic make-up, and established that there was effective protection to experimental challenge against *T. solium* cysticercosis, since vaccination lowers the number of viable cysticerci capable of developing into tapeworms. They further noted that since the pig is an indispensable intermediate host, lowering the prevalence of pig cysticercosis through effective vaccination could reduce

transmission. Scuitto *et al.* (1995) obtained similar results when they found that immunized pigs harboured more damaged cysticerci than controls. Sciutto *et al.* (1995) concluded that immunization does induce some restrictions to parasite survival even if these were eventually overwhelmed by other parasite-promoting factors.

Lightowlers (2003) reported that recent vaccination trials have been able to produce a vaccine called TSOL18 against *T. solium* cysticercosis. This vaccine has been reported to offer 100% protection against *T. solium* cysticercosis in pigs. In a recent field evaluation of the TSOL18 vaccine in pigs against a natural exposure to the parasite acquired through the consumption of the faeces of humans infected with *T. solium* taeniosis in the northern region of Cameroon, a combined application of TSOL18 vaccination prevented any detectable infection in pigs raised in circumstances where there was a 20% infection in unvaccinated animals (Assana *et al.*, 2010).

Treatment of porcine cysticercosis is another option to be considered in the control of *T. solium* infections. Both praziquantel and albendazole given orally have been found effective for treating porcine cysticercosis but involve multiple dosing making them impractical for large-scale control programs (Flisser, *et al.*, 1990; Torres *et al.*, 1992; Gonzalez *et al.*, 1995;). Subcutaneous injection of inexpensive albendazole sulphoxide, 15 mg/kg daily for eight days has been found 100% effective in killing muscle cysts but less effective at killing brain cysts in pigs. It, however, also requires multiple doses (Peniche-Cardena *et al.*, 2002). In contrast, an inexpensive veterinary benzimidazole, oxfendazole, has been found to be more than 95% effective in killing cysts in pigs when given in a single dose of 30 mg/kg and pigs may remain resistant to re-infection for at least three months after treatment (Gonzalez *et al.*, 1997). Brain cysts have been found

to survive the single-dose therapy; however, this may be inconsequential since pig brains are usually cooked for consumption (Gonzalez *et al.*, 1997). Infested meat in oxfendazole-treated pigs need at least eight weeks for all the cysts to degenerate and up to 12 weeks to achieve a clear, acceptable appearance of the pork for human consumption thereby greatly increasing the meat's commercial value (Gonzalez *et al.*, 1998; Gonzalez, 2002). This was, however, disputed by Sikasunge *et al.* (2008b) when the results of their study showed that the clearance of the entire dead cysts takes longer time and that it might, among other factors, depend on the cyst intensity of the treated pig. The findings of Sikasunge *et al.* (2008b) were in agreement with the conclusions made by Peniche-Cardeña *et al.* (2002) that although albendazole sulphoxide was able to kill the cysts in the muscles, more time was needed for total disappearance of degenerated cysticerci from the meat.

Integrated approaches to control *T. solium* infections should be used. Lightowlers (2003) stated that the future control of *T. solium* infections lies in an integrated approach including chemotherapy, as a single control measure is unlikely to achieve effective and long lasting control. Willingham III and Engels (2006) stated that since cysticercosis is generally related to poverty and its associated manifestations, all strategies to control the disease must consider costs and locally available resources.

2.6.2 Prevention and control of STHs

Periodic deworming focused on school children has been adopted as the way of controlling morbidity due to infections with STHs (Albinico *et al.*, 1998). This helps to reduce and maintain the worm burden under the threshold associated with disease in school-age children (Savioli *et al.*, 2002). Since benzimidazoles can now be used in

children below 24 months and in pregnant women (WHO, 2002b; Montresor *et al.*, 2003), it helps to maintain infections at low levels in these individuals as well. The drugs are easily accessible now because they can be produced cheaply by generic manufactures and be delivered cheaply (WHO, 2005; Bentony *et al.,* 2006). The periodic deworming can be in form of universal treatment (everyone is treated irrespective of age, gender, occupation or infection status), targeted treatment (groups may be defined in terms of risk or social characteristics) or selective treatment (treatment after determination of infection status) (Crompton and Savioli, 2007).

Currently, the large-scale administration of benzimidazoles drugs (i.e. albendazole and mebendazole) is the most widely used method to control morbidity due to STH infections, and a scale-up of these large-scale treatment programs is underway in Africa, Asia and Latin America (Levecke *et al.*, 2011). However, due to the scarcity of alternative antihelmintics, it is imperative that monitoring systems are designed to detect any change in drug efficacy due to emerging resistance of the parasites against benzimidazoles (Humphries *et al.*, 2011).

Improved standards of sanitation can also help reduce infection. Thorough washing, cooking of vegetables and supervision of children's play areas helps to reduce infection. However, infection and re-infection in children is difficult to control and as long as poverty persists in developing countries, STHs will continue to be of public health concern worldwide. There is need for people to live in clean environments, have access to clean water and to be educated so that health education can easily be understood. The health education should aim at reducing contamination of the soil by promoting the use

of latrines and hygienic behaviors (WHO, 2002a). Furthermore, health education must be in tune with local perceptions and traditions (Crompton and Savioli, 2007).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area and animals

This study was conducted in *Taenia solium* endemic communities in the eastern part of Monze district (Figure 3.1). The district is located in the centre of the Southern province of Zambia. Monze district lies between latitudes 16° 00´S and longitudes 27° 15´E and 27° 25´E. Monze district was selected based on previous reports that indicated high prevalence of porcine cysticercosis, human cysticercosis/taeniosis and STHs (Phiri *et al.*, 2002; Mwape, 2006; Sikasunge *et al.*, 2008a; Halwindi *et al.*, 2011; Ngandu *et al.*, 1991; Wenlock, 1997). In the western part of Monze district, pigs are rarely reared for religious reasons. The study area is located on a plateau and the vegetation is predominantly *Brachystegia* "Miombo" and *Acacia* "Munga" woodland.

Most of the inhabitants of Monze district belong to the Tonga ethnic group which has long been associated with cattle rearing. However, the advent of cattle diseases such as theileriosis (corridor disease) compounded with poor rainfall amount and pattern and the resultant poor grazing area, has resulted in a serious reduction in cattle population. The farmers have, therefore, recognized a quicker and higher return on their investment when they rear pigs. Higher consumer demand for pork has also contributed to the increase in pig rearing (Phiri *et al.*, 2003). The selected study area is predominantly a rural setting with haphazardly built cluster houses whose immediate environments consist of grassy areas.

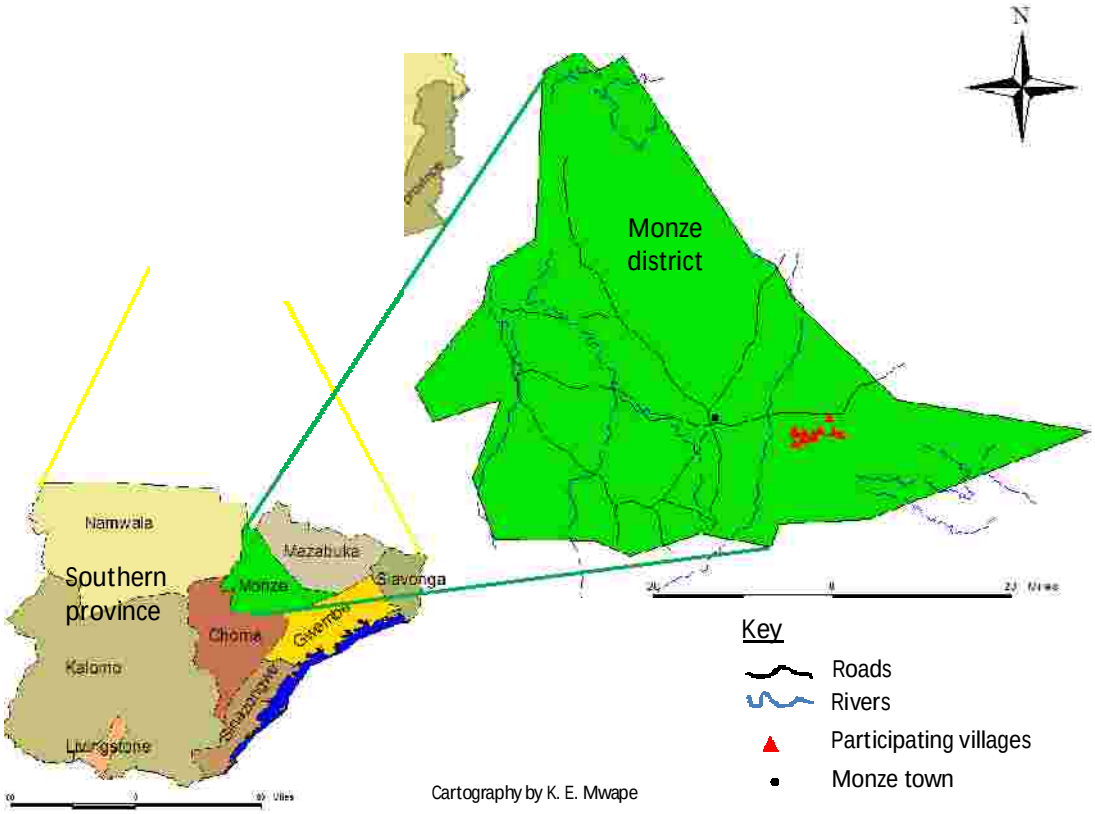

Figure 3.1: Map of Zambia showing the location of the participating villages in Monze district in Southern province

3.2 Ethical considerations

As the research involved human subjects, approval was obtained from the University of Zambia Research and Ethics Committee (Ref.: 037-12-10). In addition, further approval was sought from the Ministry of Health of Zambia and also from the local district health authorities. Community leaders (Village Headmen) were also asked for permission to conduct the study in their areas. Permission was sought from pig owners to sample their pigs. Finally permission was sought from the individual subjects to take part in the study after written informed consent. For individuals below the age of 16, permission was sought from their parents or guardians. Subjects were not forced to participate and

were free to drop out at any stage of the study. All participants found positive for taeniosis and STHs were provided with treatment, namely niclosamide and mebendazole respectively through the local Rural Health Centre. Those positive for cysticercosis were referred to the district hospital for follow-up and provision of standard of care.

Collection of human samples was done by qualified medical staff from a local clinic. Collection of samples from pigs was done by qualified University of Zambia technical staff with the assistance of local veterinary assistants.

3.3 Study design

This study was a cross-sectional study carried out in the framework of the Integrated Control of Neglected Zoonoses (ICONZ) project which sought to assess the impact of community-led total sanitation (CLTS) on *T. solium* and STHs in Monze district. The design of the ICONZ study was a community based randomized trial. The community was chosen as the unit of randomization because it was the natural foundation for implementing a sanitation trial and the transmission cycle was to have a community level dynamics. Specifically, this study aimed at collecting baseline data on prevalence of human cysticercosis/taeniosis, porcine cysticercosis and STHs in the study communities.

Eligible communities were identified during the pilot survey by conducting key informant interviews with chiefs, village headmen and rural health centre/health post workers during community, rural health centre/health post visits. The eligibility criteria for selection of communities included the following; willingness to collaborate, no

current promotion of water, sanitation or hygiene programs, rural setting, minimum of 10 pig keeping households and maximum of 50 households. A census was conducted to determine the number of inhabitants in each community, number of pig owners, number of children from 6 to 12 years old and number of those older than 12 years.

Two randomly selected persons from each household , both from pig keeping and non-pig keeping households, were sampled per household (one 6 = 12 years old and the other > 12 years old) and 50 households per community.

For pig sampling, qualified veterinary personnel from the University of Zambia were recruited for blood sampling and tongue palpation of pigs and additional veterinary assistance was sought from the local District Veterinary Office (DVO). All the pigs were sampled per household if the number was less than five but 50% of the pigs were sampled per household if the number was more than five. Sows with advanced pregnancy, those that recently farrowed and piglets less than three months old were excluded from the study. This was done to avoid stress in sows and the possibility of having false positives due to passive immunity in pigs less than three months old (Sikasunge *et al.*, 2010; Gonzalez *et al.*, 1999).

The sample sizes were calculated using the formula, $n = Z^2PQ/L^2$ where n is the required number of individuals to be examined, Z is the Z value for a given confidence level, P is a known or estimated prevalence, Q = (1-P) and L is the allowable error of estimation was used (Martin *et al.*, 1987). In this study, 95% confidence level with allowable error of estimation of 0.05 was used. To get the sample size for pigs, the prevalence (P) of 22.7% of porcine cysticercosis, as reported by Sikasunge *et al.*

(2008a) was used, whereas a human cysticercosis prevalence of 10.7% as reported by Mwape (2006) was used for calculating the sample size for human. Therefore, at least 270 pigs and 147 human beings were to be sampled from the study area to investigate for prevalence of *T. solium* infections in the study area. For STHs an estimated prevalence of 12% as reported by Wenlock *et al.*, 1977 was used. At least 162 humans had, thus to be sampled for STH prevalence investigation.

3.4 Community Sensitization

The communities were sensitized on the study through organized community sensitization meetings (Figure 3.2), during which the purpose of the study was explained to the community members.

Figure 3.2: A sensitization meeting explaining the purpose of the study to the community in the study area.

3.5 Data collection

3.5.1 Pigs

3.5.1.1 Pig selection

Pigs from willing owners were included in the study provided they were older than three months old. A pig was classified as young if it was less than 1 year and as an adult if it was 1 year or older (Pouedet *et al.*, 2002; Sikasunge *et al.*, 2008a). A local Veterinary Assistant was requested to assist in pig sampling with the help of the village headmen and/or their assistants. All the pigs in a household were sampled if the herd size was less than 5, whereas 50% was sampled for herd sizes of more than 5.

3.5.1.2 Tongue examination of pigs for cysticercosis

To examine pigs for the presence of cysticercosis, the pig to be examined was restrained in lateral recumbence; left recumbence if the examiner was right handed and right recumbence if the examiner was left handed. The pig was restrained with the help of three people. Of the three people, one firmly held the pig's head at the level of the ear, the second person held the hind legs while the third person held the forelegs. A hard wooden stick was used as a mouth gag to maintain the mouth open. Using a mutton cloth for grip, the tongue was pulled out, examined and palpated all along its ventral side for the presence of cysticerci (Figure 3.3).

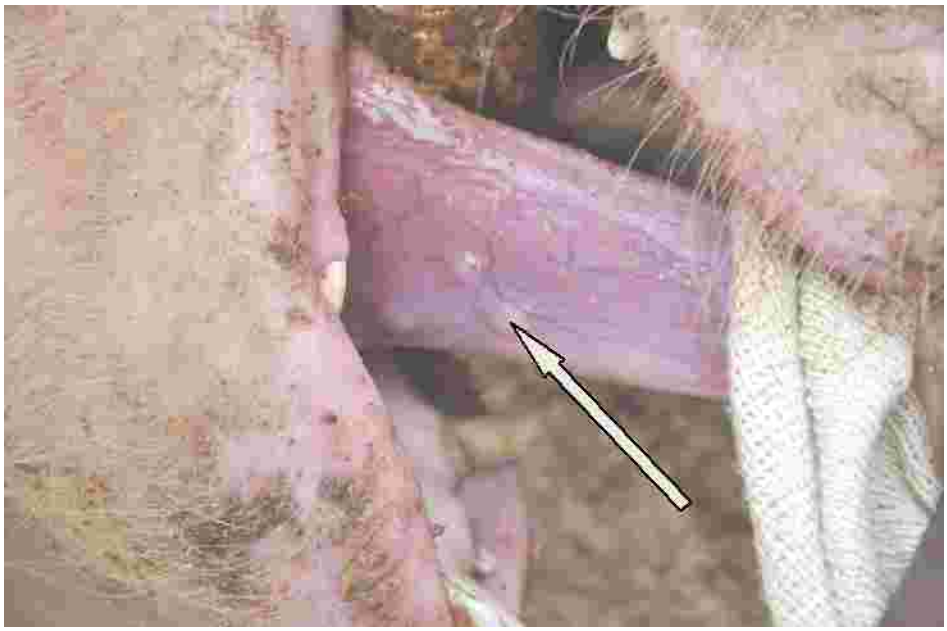

Figure 3.3: Examination of the ventral aspect of the tongue of a pig. (See arrow showing one of the three live cysts exposed).

3.5.1.3 Blood collection from pigs

To facilitate blood collection from the cranial vena cava, the pig was restrained in dorsal recumbence with the help of three people. Of the three people, one person held the hind legs, the second person held the forelegs and the third held the head at the level of the mandible. Using an 18 gauge hypodermic needle and a 20 ml syringe, the 4th person collected the blood from the cranial vena cava (Figure 3.4) and slowly transferred it into a properly labeled plain vacutainer tube.

The collected vacutainer blood tubes were kept in a cool box to allow the blood to clot.

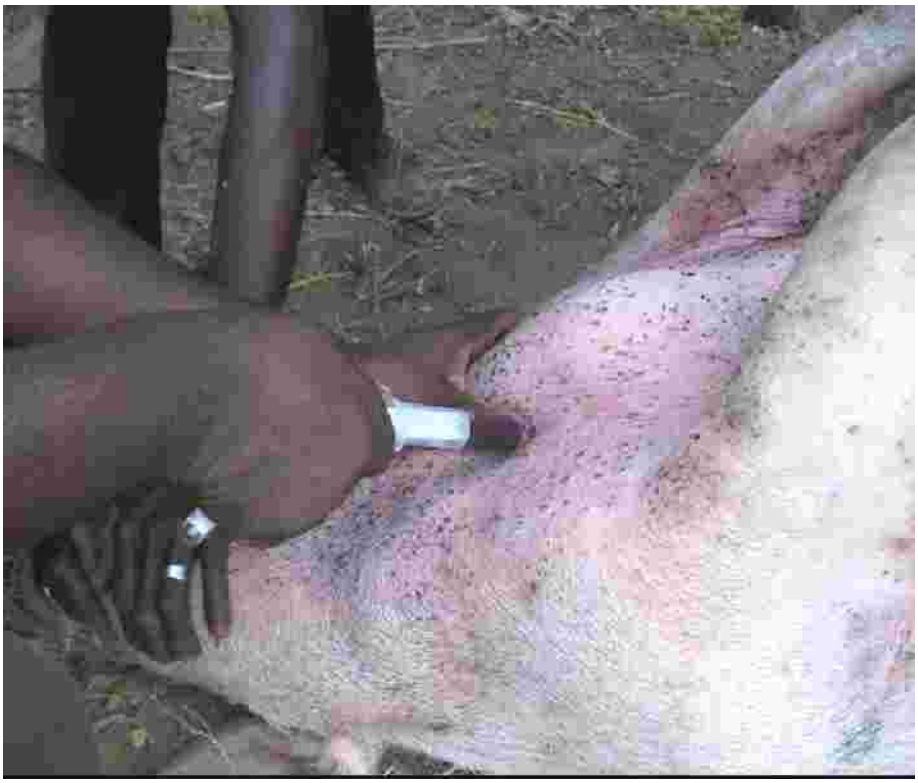

Figure 3.4: Blood collection from the cranial *vena cava* of a pig

To obtain the maximum amount of serum, the blood was allowed to stand overnight at 4°C and then centrifuged at 3000g for 15 minutes. The supernatant (serum) was aliquoted in well labeled 1.8 ml cryogenic vials and stored at -20 °C until use.

3.5.2 Humans

3.5.2.1 Stool sample collection

The participants were provided with a labeled and codified sample bottle and a black plastic bag. They were asked to at least half-fill the sample bottle with their respective stool samples and submit the sample bottles enclosed in the provided black plastic bag to the sampling center either the same day or the following morning. Submitted stool

samples were kept in a cooler box and taken to the laboratory for coproscopic examination and divided into two aliquots; one placed in 10% formalin and the other in 70% ethanol and these were stored at 4°C until use.

3.5.2.2 Blood sample collection

About five ml of blood was collected into a sterile plain blood collecting tubes by qualified local medical personnel from the cephalic and median cubital veins using 21-Gauge needles and 5 ml syringes (Figure 3.5) and allowed to clot.

Figure 3.5: Collection of blood from a participant by a health practitioner from cephalic vein.

To obtain maximum amount of serum, the blood tubes were allowed to stand at 4°C and then centrifuged at 3000 g for 15 minutes. The supernatant (serum) was aliquoted into 1.8 ml vials and transported to Lusaka where they were stored at -20°C until analysis.

3.6 Laboratory analysis of samples

3.6.1 Stool samples

3.6.1.1 McMaster method for detection and quantification of STH eggs

The collected stool samples were examined to detect and quantify STH eggs using the McMaster method (Levecke *et al.*, 2011). About two grams of each stool sample were weighed in a plastic beaker to which 58 ml of saturated sodium chloride (NaCl) solution was added and properly mixed. The mixture was passed through a sieve into another plastic beaker. The remaining solution from the stool was transferred into the first beaker or container. This was repeated three times. The remaining stool was then discarded. The solution was mixed 10 times by transferring from one beaker to the other. Immediately after mixing, a sample of the mixture was pipetted off and transferred into one chamber of the McMaster slide. The procedure was repeated to fill the other chamber. The slide was allowed to stand on the stage of the microscope for 1 minute and then the total numbers of eggs for each helminth species under both of the etched areas on the slide were counted. To get the eggs per gram (EPG) of each faecal sample for each worm species in the 2 chambers, the number of eggs counted was multiplied by 100. A sample was positive if at least one egg was detected.

3.6.1.2 Formol-ether concentration technique for detection of *Taenia* eggs of and those of other helminths

To investigate for eggs of *Taenia* species and other helminths, the formal-ether concentration technique was used as described by Richie (1948). Two grams of stool sample was transferred into a centrifuge tube containing 8 ml of 10% formal saline

solution and properly mixed using bamboo skewers. In addition, 2 ml of ether were added to the centrifuge tube and was tightly closed with a stopper. For thorough mixing, the tube was vigorously shaken and later centrifuged at 2500 rpm for 5 minutes. The supernatant was decanted leaving the sediment in the tube. After tapping the tube, a drop of the sediment was obtained and placed on a slide. A cover slip was placed onto the drop to facilitate for microscopic examination. To enhance sensitivity, duplicate smears were made for each sample. The slides were systematically examined under a microscope using 10X objective. For more detailed examination of any unclear object seen, the 40X objective was used.

3.6.1.3 Enzyme-linked-immunosorbent assay for the detection of *Taenia* copro-antigens

A copro-antigen detection ELISA (Co-Ag ELISA) as described by Allan *et al.* (1990), and modified by Mwape *et al.* (2012), was performed on the stool samples for the diagnosis of taeniosis. Samples were prepared by mixing equal volumes of phosphate buffered saline (PBS) and faecal samples in falcon tubes. The samples were left to soak for 1 hour with intermittent shaking. The tubes were then spun at 2000g for 30 minute. For coating the plates, Nunc® Maxisorp plates were used. One hundred µl of the hyper immune rabbit anti-*Taenia* IgG polyclonal antibody diluted at a concentration of 2.5µg/ml in coating buffer (0.05M carbonate/bicarbonate buffer, pH 9.6) was added to all wells including the conjugate control (CC) except the substrate control (SC), where only coating buffer was added, and incubated on a shaker at 37 °C for 1 hour. The plates were washed once with PBS in 0.05% Tween 20 (PBS-T20) and all wells blocked by adding blocking buffer (PBS-T20+2% New Born Calf Serum) After

incubating at 37 °C for 1 hour and without washing, 100 µl of the supernatant was added to each well of the coated plates except for SC and CC where blocking buffer was added and incubated on a shaker at 37 °C for 1 hour. The plates were washed 5 times in PBS-T20 and dried by vigorously beating them on blotting paper. As a detector, 100 µl of biotinylated polyclonal (0.219mg/ml) at a concentration of 2.5 µg/ml in blocking buffer was added to all wells, except for the SC where blocking buffer was added, and incubated at 37°C on a shaker for 1 hour. The plates were washed 5 times in PBS-T20 and dried. One hundred µl of streptavidin diluted at the rate of 1 µl/10 ml blocking buffer was added to all wells except the SC where only blocking buffer was added, and incubated at 37°C on a shaker for 1 hour. The plates were washed 5 times in PBS-T20 and later dried. After that, 2 tablets of the chromogen/substrate, orthophenylenediamine (OPD) (DAKO, #S2045) were added to 12 ml of distilled water, to which 5 µl of H_2O_2 was added. One hundred µl of this solution was added to each well and incubated at room temperature in the dark without shaking for 15 minutes. To stop the reaction, 50 µl of 4N H_2SO_4 was added to each well. The plates were read using an automated spectrophotometer (LabsystemMultiskan RC) at 492 nm with a reference of 655 nm.

To determine the test results, the optical density of each stool sample was compared with the mean of a series of eight *Taenia* negative stool samples plus 3 standard deviations (cutoff).

3.6.2 Serum samples

3.6.2.1 Pre-treatment of sera for Ag-ELISA

The B158/B60 Ag-ELISA, initially developed for *T. saginata* cysticercosis (Brandt *et al.,* 1992), was performed as described by Dorny *et al. (*2000) with slight modifications. The sera were pre-treated using freshly prepared 5% trichloroacetic acid (TCA) (Sigma, Chemical Co.) w/v dissolved in distilled water. The pre-treatment was done to remove non-specific immune complexes to increase the sensitivity and specificity of the assay. A 5% TCA solution was made by dissolving 1 gram of TCA in 20 ml of distilled water. The serum samples were thus pre-treated by mixing an equal volume of serum and 5% TCA. For the negative control sera, 75 µl of serum was used while 150 µl of serum was used for the pre-treatment of positive control and the test sera. These mixtures of sera and 5% TCA solution were incubated for 20 minutes at room temperature. After incubation the mixtures were centrifuged at 12,000 rpm for 9 minutes and 150 µl of the supernatant was removed and aliquoted into microtitre tubes. The pH of the collected supernatant was raised by adding an equal volume of 75 µl sodium carbonate/bicarbonate buffer (0.610M) at pH 10.0 (neutralization buffer) to the supernatant of the negative control sera and 150 µl of neutralization buffer to the supernatant of the positive control and the test sera. One hundred µl of this mixture at final sera dilution of 1:4 was used in the Ag-ELISA protocol.

3.6.2.2 Enzyme-linked-immunosorbent assay for the detection of circulating cysticerci antigens

The human and pig serum samples were examined for the presence of cysticerci antigens using a double monoclonal antigen-based sandwich Ag-ELISA as described by

Dorny *et al.* (2000) with minor modifications. The Ag-ELISA protocol for both human and pig sera were basically the same except that human controls were used for human sera and pig controls for pig sera. This technique involves trapping the antigen (Ag) between two monoclonal antibodies (MoAb). The MoAbs were obtained from the Prince Leopold Institute of Tropical Medicine, Nationalestraat 155, and B-2000 Antwerp, Belgium. The assay involved coating 96 well polystyrene ELISA plates (Nunc ®Maxisorp). Monoclonal antibody B158C11A10 was used as 1st MoAb and was followed by biotinylated MoAb B60H8A4 as the detector antibody (2nd MoAb). The plates were coated with 100 µl of MoAb B158C11A10 diluted at 5µg/ml in carbonate buffer (0.06M, pH 9.6) (except for the two wells used as substrate control (SC) where only 100 µl of coating buffer were added), and incubated at 37°C on a shaker for 30 minutes. After coating, the plates were washed once with PBS-T20 and dried by beating the plates vigorously on blotting paper. Blocking to avoid non-specific reactive sites was done by adding 150 µl of PBS-T20/1%NBCS (Blocking buffer) and then the plates were incubated on a shaker for 15 minutes at 37°C. Thereafter, the plates were emptied and dried. Without washing the plates, 100 µl of pre-treated sera, including the weak and strong positive and negative controls, at a dilution of ¼ was added to each well (except for the SC and conjugate control (CC) wells where 100 µl of Blocking buffer was added) and incubated at 37°C on a shaker for 15 minutes. After washing the plates five times with PBS-T20 , they were dried followed by the addition of 100 µl of biotinylated MoAb B60H8A4 diluted at 1.2µg/ml in blocking buffer to each well (except for the SC wells where 100 µl of blocking buffer were added) and the plates incubated at 37°C on a shaker for 15 minutes. The plates were then washed five times

with PBS-T20 and then dried. One hundred µl of streptavidin-horseradish peroxidase (Jackson Immunoresearch Lab, Inc.) diluted at 1/10,000 in blocking buffer was added to each well (except for SC where blocking buffer was added) to act as conjugate after which the plates were incubated on a shaker at 37°C for 15 minutes. Then the plates were washed five times with PBS-T20 and then dried. After that, two tablets of the chromogen/substrate, orthophenylenediamine (OPD) (DAKO, #S2045) were added to 12 ml of distilled water, to which 5 µl of H_2O_2 was added. To each of the wells was then added 100 µl of this solution and incubated at room temperature in the dark without shaking for 15 minutes. To stop the reaction, 50 µl of 4N H_2SO_4 was added to each well. The plates were read using an automated spectrophotometer (LabsystemMultiskan RC) at 492 nm with a reference of 655 nm.

The optical density of each serum sample was compared with the mean of 8 reference negative sera samples at a probability level of $p = 0.001$ to determine the results in the test (Dorny *et al.*, 2004).

3.6.2.3 Interpretation of the results

All positive and serum samples were done in duplicate. The two wells containing the same sample were checked that they gave roughly the same optical density. The average optical density was calculated for every sample. The cut off was calculated based on the optical densities of the negative samples using a variation of the student T-test (Sokal and Rohlf, 1981). The cut off that was determined was used to calculate a ratio (Ratio = average optical densities/cut off). When the ratio was greater than one, the sample was considered positive with 99.9% certainty.

3.7 Statistical analysis

All the collected data were entered into a Microsoft Excel sheet and later exported to SPSS Version 16.0 for analysis. Cross tabulations were done in SPSS, while 95% Confidence Intervals (CI) for prevalence rates were computed using the McCallum Layton online calculator (http://www.mccallum-layton.co.uk). Fisher's exact test or Yates corrected $?^2$ were used to assess for association between human cysticercosis, taeniosis, STHs and porcine cysticercosis and the factors (sex, age and breed) at a 95% confidence level.

CHAPTER FOUR

4.0 RESULTS

4.1 Prevalence of porcine cysticercosis

4.1.1 Overall prevalence

A total of 275 pigs were tongue examined and blood sampled from 11 villages and 139 households. Of the 275 pigs examined, 225 were from three months to 12 months old whereas 50 were older than 12 months. Of the 275 pigs sampled, 94 were females while 181 were males. Of the sampled pigs, 6 (2.2%) were positive on tongue examination and 32 (11.6%) were positive on Ag-ELISA. Simuyandi village had comparatively the highest prevalence of porcine cysticercosis both by tongue examination and Ag-ELISA (Table 4.1). There were no significant differences ($?^2$ = 9.64, p = 0.233) in the prevalence of porcine cysticercosis by tongue examination among the villages. However, significant differences ($?^2$ = 36.08, $p < 0.05$) in the prevalence of porcine cysticercosis by Ag-ELISA among the villages sampled were observed.

Table 4.1: Prevalence of porcine cysticercosis in Monze district by village on tongue examination and Ag-ELISA (n = 275).

Village	N	Prevalence n (%) (tongue examination)	95% CI	Prevalence n(%) (Ag-ELISA)	95% CI
Lumamba	29	0(0.0)	0	2(6.9)	0 – 16.1
Kabimba	22	0(0.0)	0	0(0.0)	0
Mpokota	39	0(0.0)	0	1(2.6)	0 - 7.6
Chaamwe	11	0(0.0)	0	2(18.2)	0 - 41
Mainga	49	1(2.0)	0 – 5.9	2(4.1)	0 – 10.2
Sikabenga	27	2(5.4)	0 – 12.7	10(27.0)	12 – 41.3
Mayoba	6	0(0.0)	0	0(0.0)	0
Hamunyanga	11	0(0.0)	0	1(9.1)	0 – 26.1
Chilala	25	0(0.0)	0	3(12.0)	0 – 24.7
Halwindi	20	0(0.0)	0	0(0.0)	0
Simuyandi	26	3(11.5)	0 – 23.8	11(42.3)	23.3 – 61.3
Total	275	6(2.2)	0.47 – 3.9	32(11.6)	7.8 – 15.4

N = number of individuals sampled per village
n = number of positive individuals
CI = Confidence interval

The prevalence of porcine cysticercosis by Ag-ELISA in Simuyandi village was significantly different from Halwindi, Mainga, Mayoba, Mpokota, Kabimba and Lumamba but not significantly different from Chilala, Hamunyanga, Sikabenga and Chaamwe. On the other hand, Sikabenga village was significantly different from Kabimba, Mpokota, Mainga, Mayoba and Halwindi but not significantly different from Lumamba, Chaamwe, Hamunyanga and Chilala.

4.1.2 Porcine cysticercosis prevalence by age on Ag-ELISA

Of the pigs aged between three to 12 months, 26 (11.6%), were positive for cysticercosis while the prevalence in older pigs was 12.0% (6/50). There was no significant difference ($?^2 = 0.01$, $p = 1.000$) in the prevalence of porcine cysticercosis by "3 = 12" and ">12" months old age categories.

4.1.3 Porcine cysticercosis prevalence by sex on Ag-ELISA

The prevalence of porcine cysticercosis by Ag-ELISA in females was 10.6% (10/94) while the prevalence in males was 12.2% (22/181). Although in comparative terms there were more male pigs diagnosed positive than females on both tests (Figure 4.1), there was no statistical difference on tongue examination ($?^2$ = 0.14, p < 0.843) and on Ag-ELISA ($?^2$ = 0.002, p < 1.000).

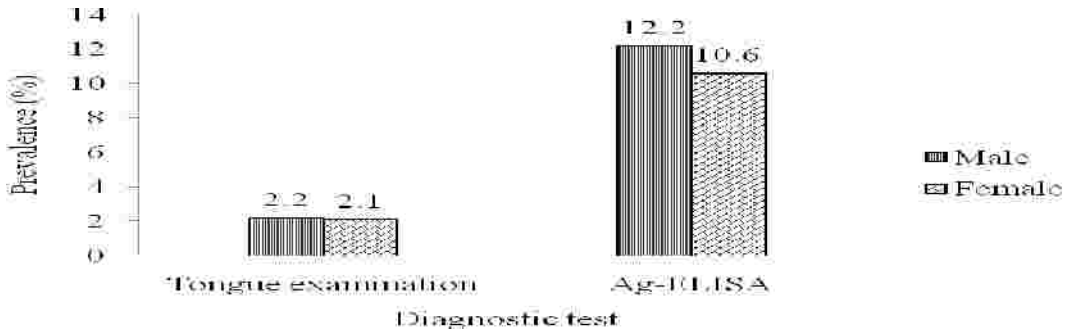

Figure 4.1: Prevalence of porcine cysticercosis in Monze district by sex on tongue examination and Ag-ELISA (n = 275).

4.2 Prevalence of *T. solium* infections in humans

Of the total 163 humans sampled, 59 were males while 104 were females. The age range of the participants was from six to 80 years old.

4.2.1 Prevalence of human cysticercosis

4.2.1.1 Prevalence of cysticercosis in humans by village

The overall prevalence of cysticercosis among the 163 humans sampled from the five villages was 14.7%. There was no significant difference ($?^2$ = 4.84, p = 0.290) in the prevalence among the villages on Ag-ELISA (Table: 4.2).

Table 4.2: Sero-prevalence of cysticercosis in humans in Monze district by village on Ag-ELISA (n = 163)

Village	N	Prevalence n (%)	95% CI
Lumamba	35	5(14.3)	2.7 – 25.9
Kabimba	18	0(0)	0
Mpokota	53	11(20.8)	9.9 – 31.7
Chaamwe	24	3(12.5)	-0.7 – 25.7
Mainga	33	5(15.2)	3 – 27.7
Total	163	24(14.7)	9.7 – 21.1

N = number of individuals sampled per village
n = number of positive individuals
CI = Confidence interval

4.2.1.2 Prevalence of human cysticercosis by sex

Overall, the observed prevalence was marginally lower (9/59, 14.4%) in males than in females (15/104, 15.3%). There was, however, no statistically significant difference ($?^2$ = 0.02, p = 1.000) in the prevalence of cysticercosis in humans on Ag-ELISA by sex.

4.2.1.3 Prevalence of human cysticercosis by age

The prevalence of cysticercosis in humans was proportionally highest in children younger than 12 years old followed by humans from 12 years to 50 years old. The lowest prevalence was among those older than 50 years (Figure 4.2). There was no significant difference ($?^2$ = 0.12, p = 0.941) in the prevalence of cysticercosis in humans according to age.

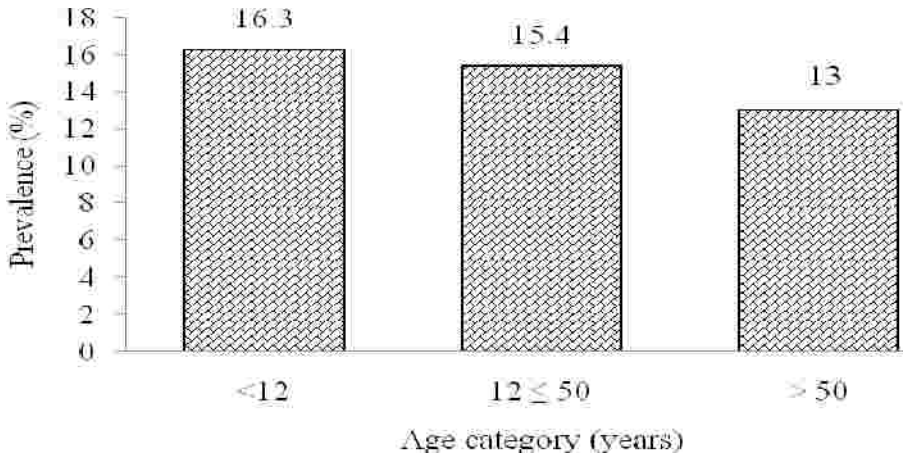

Figure 4.2: Prevalence of cysticercosis in humans in Monze district by age on Ag-ELISA (n = 163).

4.2.2 Prevalence of taeniosis

4.2.2.1 Prevalence of taeniosis by village

A total of 133 samples were examined using formol-ether concentration method (Ritchie, 1948). Only one sample (0.8%) revealed *Taenia* species eggs (Figure 4.3).

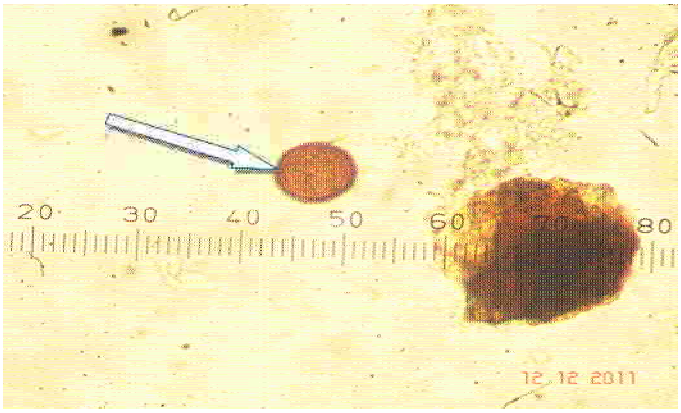

Figure 4.3: An egg of *Taenia* sp. as observed under a microscope (x 10) by formol-ether concentration method.

A total of 131 humans were examined for taeniosis by copro-antigen ELISA (Co-Ag-ELISA). Of these, 9.9% (13/131) were positive. There were no significant differences ($?^2$ = 5.06, p = 0.247) in the prevalence of taeniosis by Co-Ag-ELISA among the five villages sampled. Each village had at least one positive individual (Table 4.3).

Table 4.3: Prevalence of taeniosis in Monze district by village by Co-Ag-ELISA (n = 131)

Village	N	Prevalence n (%)	95% CI
Lumamba	27	6(22.2)	6.52 – 37.88
Kabimba	12	1(8.3)	0 – 23.91
Mpokota	46	3(6.5)	0 – 13.62
Chaamwe	24	1(4.2)	0 – 12.23
Mainga	22	2(9.1)	0 – 12.12
Total	131	13(9.9)	4.79 – 15.01

N = number of individuals sampled per village
n = number of positive individuals
CI = Confidence interval

4.2.2.3 Prevalence of taeniosis by sex on Co-Ag-ELISA

From the total 131 humans sampled, comprising 84 females and 47 males, it was observed that a relatively higher proportion of males (12.8%) were positive for taeniosis on Co-Ag-ELISA than in females (8.3%). However, there was no significant difference ($?^2$ = 0.66, p = 0.544) in the prevalence of taeniosis by Co-Ag-ELISA according to sex.

4.2.2.4 Prevalence of taeniosis by age on Co-Ag-ELISA

The prevalence of taeniosis according to age category was as presented in Figure 4.4. There were no significant differences ($?^2$ = 2.79, p = 0.253) in the prevalence of taeniosis by Co-Ag-ELISA among the "<12", "12=50" and ">50" years-old age categories.

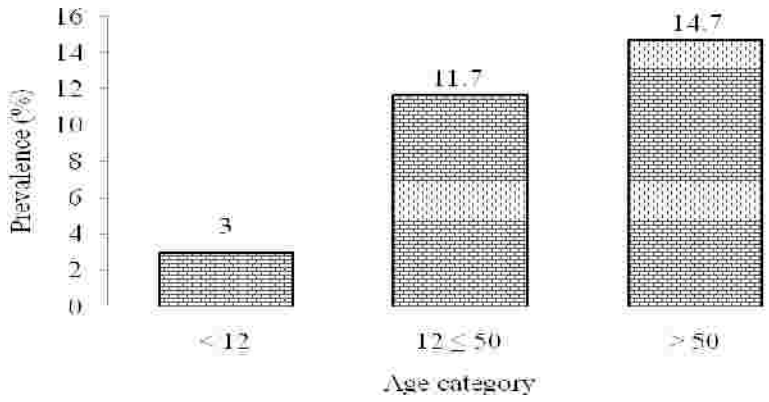

Figure 4.4: Prevalence of taeniosis by age after Co-Ag-ELISA (n = 131).

4.3 Prevalence of soil transmitted helminths

4.3.1 Overall prevalence

Of the total 133 individuals examined from the five villages, 84 were females while 49 were males. Of these, 30 were less than 12 years old, 71 were between 12 and 50 years old and 32 were more than 50 years old. The overall prevalence of STHs among the 133 (There was not enough stool sample from two individuals to aliquot for Co-Ag-ELISA, hence the 131 samples above) individuals examined from the five villages using McMaster method was 16.5% (22/133). These comprised two nematode species, namely, *Ancylostoma* spp. (Figure 4.5 A) (15.04%) and *Trichuris* spp. (Figure 4.5 B) (1.5%).

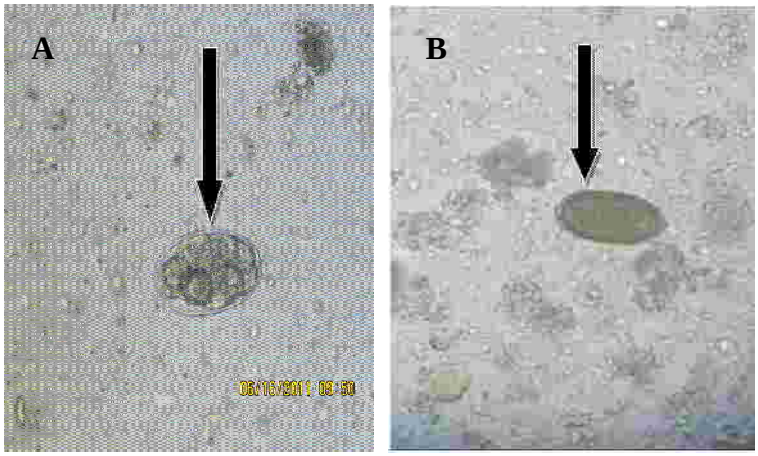

Figure 4.5: Egg of *Ancylostoma* spp. (A) and egg of *Trichuris trichiura* (B) by McMaster method.

4.3.2 Prevalence by village

Soil transmitted helminths were detected from all the five villages sampled with varying prevalence rates as shown in table 4.4. Kabimba village had the highest prevalence of STHs while Chaamwe village had the lowest. The two cases of *Trichuris trichiura* that were detected came from the same village (Mpokota).

Table 4.4: Prevalence of STHs in Monze District according to village (n = 133) by McMaster method

Village	N	Prevalence n (%)	95% CI
Lumamba	27	7(25.9)	9.38-42.42
Kabimba	12	4(33.3)	6.63-59.97
Mpokota	47	6(12.8)	3.25-22.35
Chaamwe	24	3(12.5)	0-25.73
Mainga	23	2(8.7)	0-20.22
Total	133	22(16.5)	10.19-22.81

N = number of individuals sampled per village
n = number of positive individuals
CI = Confidence interval

There was no significant difference ($?^2$ = 5.60, p = 0.214) in the prevalence of STHs among the villages sampled.

4.3.3 Prevalence by sex

The prevalence of STHs in males (18.4%) was comparatively higher than in the females (15.5%). However, there was no significant difference ($?^2$ = 0.190, p = 0.809) in the prevalence of STHs according to sex.

4.3.4 Prevalence by age

The prevalence of STHs according to age category was as presented in Figure 4.6**.** The highest prevalence (11.4%) was detected in children below 12 years old. There were no significant differences ($?^2$ = 2.79, p = 0.253) in the prevalence of STHs among the "<12", "12=50" and ">50" years-old age categories.

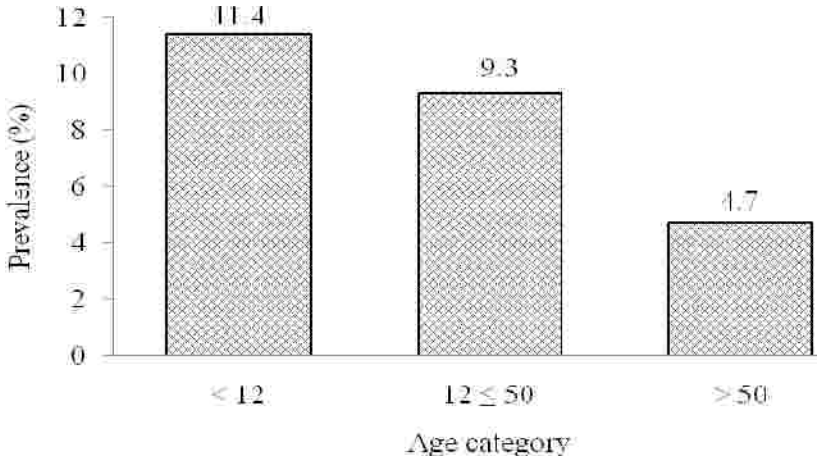

Figure 4.6: Prevalence of Soil Transmitted Helminths by age (n = 133) (McMaster method).

CHAPTER FIVE

5.0 DISCUSSION

To the best of my knowledge, this is the first study in Zambia to simultaneously look at two sanitation- related neglected diseases of which one is a zoonosis. So far, the studies on *T. solium* infections in Zambia have either investigated the problem in humans or pigs only and not both. To the best of my knowledge, it is also the first time *T. solium* infections have been studied together with STHs in humans in Zambia.

5.1 Prevalence of porcine cysticercosis

This study revealed a 2.2% and 11.6% prevalence of porcine cysticercosis on tongue examination and serum Ag-ELISA respectively in a total of 275 pigs examined from 11 villages in the study area. Sero-positive pigs were detected in 72.7% of the sampled villages. The results confirmed that porcine cysticercosis continues to be endemic in the study area as was reported by Phiri *et al.* (2002) and Sikasunge *et al.* (2008a). The prevalence of porcine cysticercosis in this study, however, was lower than the 5.2% and 11.1% reported by Phiri *et al.* (2002) and Sikasunge *et al.* (2008a), respectively on tongue examination. The 11.6% prevalence on Ag-ELISA found in this study is also lower than the 20.8% and 23.3% reported by Phiri *et al.* (2002) and Sikasunge *et al.* (2008a), respectively. The higher prevalence in earlier studies could probably arise from the wider survey coverage and the larger sample sizes than in the current study. Another reason for the lower prevalence in the current study could be due to the spillover effects of the Community-led Total Sanitation (CLTS) which were being undertaken by the Monze District Council and the United Nations Children's Fund (UNICEF) in the

nearby villages and many other places in the province (Monze District Council Secretary, Pers. Comm., 2011). Community-Led Total Sanitation (CLTS) is a new approach to sanitation promotion that encourages community self-analysis of existing defecation patterns, their health risks and promotes local solutions to reduce and ultimately eliminate the practice of open The Ministry of Local Government and Housing in Zambia, in conjunction with UNICEF piloted CLTS in Choma District in Southern Province in 2007 where the initial sanitation coverage was only 27%. After three months, the coverage increased to 88% and most of the villages were verified open defecation free (ODF) (Zulu *et al.*, 2010). Drawing lessons from Choma, the CLTS approach has now been implemented in many districts in Zambia and has been adopted as one of the national strategies for rural sanitation promotion (Zulu *et al.*, 2010). The CLTS has thus resulted in an increase in levels of hygiene including increased construction and use of pit latrines (Zulu *et al.*, 2010), thereby reducing the pigs' access to human stool that could be contaminated with eggs of *T. solium*.

The prevalence of porcine cysticercosis detected by Ag-ELISA in this study was higher than that detected by tongue examination. Porcine cysticercosis on tongue examination was detected from only 3 (27.3%) of the 11 villages sampled while on Ag-ELISA cysticercosis was detected from 8 (72.7%). There has been varying information regarding the sensitivity of lingual examination in detecting porcine cysticercosis. The study in Nigeria found that lingual examination could detect 14.9% of truly infected pigs (Onah and Chiejina, 1995) while in Peru, Gonzalez and others found that 70% of the infected pigs were detected by lingual examination (Gonzalez *et al.*, 1990). A study in Zambia estimated a sensitivity of about 21% of lingual examination as compared to

meat inspection (Dorny *et al.*, 2004). The finding in this study are in agreement with Sarti-G *et al.* (1992) and Phiri *et al.* (2006b) who reported that tongue examination only offers an estimate of infection levels, primarily only heavy infections, as lightly infected pigs tend not to have cysts on the tongue. Sarti-G *et al.* (1992) further reported that infection intensity could be the most important factor determining whether cysts were discernible on tongue examination or not. Prevalence obtained by tongue examination should thus be regarded as an underestimate of the true prevalence. Nevertheless, lingual examination is a relatively quick and inexpensive way of doing a rapid assessment of the presence and level of infection of porcine cysticercosis (Gonzalez *et al.*, 1990).

The sero-prevalence of porcine cysticercosis found in this study is similar to the 11.0% obtained by Pouedet *et al.* (2002) in a large scale survey in West-Cameroon. Cameroon is one of the countries where the taeniosis-cysticercosis complex has been examined more in detail both in pigs and humans (Zoli, *et al.*, 2003). The porcine cysticercosis sero-prevalence in the current study is also within the village specific range of 3.2-46.7% reported by Ngowi *et al.* (2004) in Mbulu District in Tanzania. In Democratic Republic of Congo (DRC), a country that lies north of Zambia and with which Zambia shares the largest border, a porcine cysticercosis sero-prevalence range of 10-30% was reported (Chartier *et al.*, 1990). In Mozambique, abattoir records indicated that porcine cycticercosis was present in all provinces of the country (Afonso *et al.*, 2001). A cross-sectional study carried out in Angónia District, Tete Province in North-western Mozambique, using Ag-ELISA reported a sero-prevalence of 34.9% (Pondja *et al.*, 2010). Earlier, a sero-prevalence study using antibody-detecting ELISA results in 11

districts of rural Tete Province showed that 15% (n = 387) were positive (Afonso *et al.*, 2001). The diagnostic method (Ab-ELISA) used in this study, however, has some weaknesses. It measures exposure of the host to antigen rather than the presence of the actual infection and the occurrence of transient antibody response to *T. solium* infection (Gonzalez *et al.*, 1999) and thus tends to exaggerate the prevalence. The sero-prevalence, based on antigen detection, observed in the current study is far below the 64.6% and 64.2% (both estimated by the Bayesian approach) reported by Krecek *et al.* (2008) and by Dorny *et al.* (2004) in South Africa and Zambia respectively. In Zimbabwe, a retrospective study in official abattoirs around the country from 1994 to 2001 reported a mean prevalence of 0.43% based on meat inspection. This figure, however, is probably biased because it included official records from commercial pigs. In contrast, a postmortem survey in 1999, showed that the prevalence of porcine cysticercosis in rural west Zimbabwe where small holder pig keeping is popular was 28.6% (Matenga *et al.*, 2002).

The present study observed a very marginally lower prevalence (11.6%) of porcine cysticercosis in young pigs (= 12 months old) than that in older pigs (> 12 months old) but the difference ($?^2$ = 0.01, p = 1.000) was not statistically significant. Similarly, Sikasunge *et al.* (2008a) did not find a significant difference in the prevalence of porcine cysticercosis by age. Other studies have reported differences in prevalence of porcine cysticercosis by age. In Peru (Garcia *et al.*, 2003), Mexico (Sarti *et al.*, 1992), Cameroon (Pouedet *et al.*, 2002) and in Mozambique (Pondja *et al.*, 2010) the authors elucidated that porcine cysticercosis prevalence increased with the age of pigs. This may indicate either that older animals might have had longer exposure than the younger

ones or that younger pigs are protected through the initial exposure period, perhaps via maternal transfer of antibodies, but become susceptible later. Maternal antibodies are protective for larval cestode infections but slowly decrease in piglets born to cysticercosis infected sows (Sikasunge *et al.*, 2007; Gonzalez *et al.*, 1999). However, other studies have not reported positive association (Sakai *et al.*, 2008; Ngowi *et al.*, 2004). These observations underline important regional differences in the risk factors for the transmission of porcine cysticercosis and that one should be careful in generalizing these factors from one culture to another.

The comparatively higher porcine cysticercosis prevalence of 22 out of 181 (12.2%) in males than the 10 out of 94 (10.6%) obtained on Ag-ELISA in females could be due to the larger number of male pigs that were examined in this study. This finding agrees with that of Sarti-G *et al.* (1992) who found that the prevalence of cysticercosis was slightly higher in male than in female pigs. This finding also agrees with the results obtained by Sarti *et al.* (1992) who reported that there was no significant difference between male (4.2%) and female (3.9%) pigs after tongue examining 571 pigs. Rodriquez-Canul *et al.* (1998) also found that there was no significant difference in prevalence by sex (n = 75) whether estimated by tongue palpation ($?^2$ = 0.07; p = 0.789), or by immunoblot ($?^2$ = 1.42; p = 0.233). Another possible explanation for our findings is that male pigs tend to move about more than the females within the community in search of partners to mate with (pigs on heat). It could also mean that male pigs are genetically more active than female pigs thereby increasing their chances of coming across *T. solium*-egg-contaminated human faeces. Another likely explanation

for our findings could be that male pigs tend to bully the female counterparts as they scavenge for food.

The current study found a higher prevalence of cysticercosis in humans, who are 'accidental hosts' that get infected by contaminated food, water and hands than in the naturally coprophagic pigs. This, rather paradoxical result, could be due the higher number of older pigs that were sampled in the 6 villages where humans were not sampled than in the 5 villages where both humans and pigs were sampled and comprised mainly young (< 12 months old) pigs. Older pigs tend to be more prone to cysticercosis infection due to longer exposure (Pouedet *et al.*, 2002) than young pigs.

5.2 Prevalence of *T. solium* infections (cysticercosis/taeniosis) in humans

The sero-Ag-ELISA detected an overall prevalence of 14.7% of cysticercosis among the 163 individuals sampled from the 5 villages with no significant difference ($?^2$ = 4.84, p = 0.290) among the villages. This indicates the presence of viable cysts in these sero-positive individuals. There were sero-positive individuals in 4 of the 5 villages where humans were sampled but none in one village (Kabimba). The sero-prevalence of human cysticercosis in this study is within the range for the sub-region of 5.8% to 20.5% on Ag-ELISA (Sacks and Berkowitz, 1990; Vilhena *et al.*, 1999; Afonso *et al.*, 2011; Mwape *et al.*, 2012; Mwape *et al.*, 2013). The human cysticercosis sero-prevalence in the current study is much higher than the 0.7% - 2.4% range in the West Province of Cameroon (Zoli *et al.*, 1987; Nguekam *et al.*, 2003) and the 1.3% of the general population in Benin (Houinato *et al.*, 1998). The human 14.7% sero-prevalence observed in this study is, however, lower than the antigen detecting sero-prevalence of

21.6% in the Democratic Republic of Congo (Kanobana *et al.*, 2011) and the antibody detecting sero-prevalence of 33.5-38.5% reported by Mwape *et al.* (2013) in Katete district of Zambia. It is also lower than 31.5% and 16.3%, also based on antibody ELISA, reported in the Burundi and Mbulu district of Tanzania respectively (Nsengiyunvia *et al.*, 2003; Mwan'gombe *et al.*, 2012). However, antibody detection indicates exposure to the parasite and not necessarily established infection and hence is likely to detect more positives than the antigen detecting assay used in the current study (Garcia *et al.*, 2001; Dorny *et al.*, 2003).

In this study, a marginally lower prevalence of cysticercosis (9/59, 14.4%) in males than in females (15/104, 15.3%) was observed although this difference was not statistically significant ($?^2$ = 0.02, p = 1.000). Other studies have also found a statistically insignificant difference between sero-prevalence in males and females (Allan *et al.*, 1990; Sanchez *et al.*, 1998; Mwape *et al.*, 2012). The findings of this study agree with those of Mwape *et al.* (2006) who found a lower cysticercosis prevalence in males (7.7%) than in females (9.7%), although this difference was not statistically significant. Garcia *et al.* (1998), however, demonstrated a strong relationship between being female and EITB-based seropositivity. The reason given for the increased risk in females recorded by Garcia *et al.* (1998) was that more females handled raw, infected pork more frequently than did males. Garcia *et al.* (1998) further explained that the increased pork contact might predict increased consumption of undercooked pork by the females and subsequent establishment of the intestinal infection with the adult tapeworm. As a result, this would increase the faecal-oral exposure to *T. solium* eggs for the individuals (external autoinfection) or their close contacts.

The marginally higher prevalence in females could be due to the proportionally larger number of females (104) that were sampled than the males (59). In contrast, this result does not agree with the report in Benin in a nation-wide survey which revealed a significantly higher prevalence of cysticercosis in men (1.9%) than in women (0.8%) (Houinato *et al.*, 1998).

The current study revealed similar sero-prevalence of human cysticercosis in all age categories by Ag-ELISA, although marginal differences were observed in age categories (<12 years old (16.3%), 12=50 years old (15.4%) and >50 years old (13.5%)). Praet *et al.* (2011), however, found that most of the cysticercosis infected individuals belonged to the age group above 60 years and attributed this to longer exposure period and higher susceptibility with age. In a change point analysis of the association of antigen seropositivity and age, Mwape *et al.* (2012) reported that the numbers of individuals in which circulating antigens were detected was significantly higher in people older than 30 years indicating that viable cysts were more frequent in people older than 30 years.

Taenia solium taeniosis tends to have low prevalence, typically less than 1%, even in endemic communities (Allan *et al.*, 1996); a higher prevalence is considered hyper endemic (Cruz *et al.*, 1999). The current study revealed a taeniosis prevalence of 9.9% by copro-antigen ELISA (Co-Ag-ELISA) from the total of 131 humans examined with no statistical difference ($?^2 = 5.06$, $p = 0.247$) among the 5 villages sampled with each village having at least one positive individual. This is in agreement with the study by Mwape (2006) (based on microscopy) who reported that taeniosis was hyper endemic in the area. In the Eastern province of Zambia, Mwape *et al.* (2012) and Mwape *et al.*

(2013) reported prevalence of 6.3% and 11.9% respectively (both based on copro-Ag ELISA), showing that taeniosis is also hyper endemic in that part of Zambia. On the other hand, the taeniosis prevalence (0.008%) by microscopic examination (formol-ether sedimentation method) in the current study, was lower than the 2.1% reported by Mwape (2006) in Monze district of Zambia, the 0.3% reported by Mwape *et al.* (2012) in Eastern Zambia, the 4-10% by Wolgenut *et al.* (2010) in Kenya, the 0.33% by Praet *et al.* (2010) in Democratic Republic of Congo, the 0.11% by Vondou *et al.* (2002) in Cameroon and the 11.5% by Dada *et al.* (1993) in Nigeria, all based on coproscopic examination. Comparing the taeniosis prevalence by the two methods used in this study (microscopy and Co-Ag-ELISA), the taeniosis prevalence (0.008%) by formol-ether sedimentation method (microscopy) was much lower than the 9.9% by Co-Ag-ELISA. Garcia *et al.* (2003b) reported that Co-Ag detection consistently detected 2 times more tapeworm carriers than microscopy alone. Allan *et al.* (2006) also reported that copro antigen tests have reliably detected more tapeworm carriers than microscopy. Thus microscopy underestimates real prevalence of taeniosis and would be a poor monitoring tool for control purposes. Allan *et al.* (2006) further outlined other characteristics of Co-Ag-ELISA which include high sensitivity (96 to 98%) and specificity (98 to 100%), parasite antigens can be detected in faeces weeks prior to patency, levels of antigens are independent of egg output, coproantigens are stable for days at a wide range of temperatures (-80°C to 35°C) for several months in formalin-fixed samples and that coproantigen levels drop rapidly (1 to 5 days) following successful treatment.

However, Co-Ag-ELISA is genus-specific (Allan *et al.*, 2006) and thus the possibility of some positive cases being those of *T. saginata* instead of *T. solium* cannot be

completely ruled out. In Zambia, bovine cysticercosis has been reported in Central, Southern provinces (Dorny *et al.*, 2002), Western province (Phiri *et al.*, 2006a; I.K. Phiri, Pers. Comm., 2012) and more recently in Eastern province (Praet *et al.*, 2013).

Like in many other studies, no significant association was determined in the current study between gender and taeniosis positivity (Rodriquez-Canul *et al.*, 1999; Sanchez *et al.*, 1999; Garcia *et al.*, 2003). Comparatively, more males (12.8%) than females (8.3%) were positive for taeniosis. This could be due to more males consuming undercooked *T. solium* infected pork than females. A possible explanation could be the habit of eating undercooked pork. Boa *et al.* (2006) reported that the habit of eating barbecued pork at local brew shops, a habit that is also observed in Zambia, practiced mainly by men during drinking of local brew is a risk factor to taeniosis. This method of preparing pork was believed not to kill the cysticerci (Dada *et al.*, 1993). In Tanzania, Ngowi *et al.*, (2004) observed that some of the detected infected pigs were sold at lower price (discounted by 60%) to butchers who normally slaughter the pigs at home and sell the meat at local brew shops.

In this study, no significant difference in the prevalence of taeniosis by age on Co-Ag-ELISA was found although, in comparative terms, the prevalence increased with age (3% in < 12, 11.7% in 12 = 50 and 14.7% in >50 years old). This is in agreement with many other studies that have not found significant association between age and taeniosis positivity (Rodriquez-canul *et al.*, 1999; Sanchez *et al.*, 1999; Garcia *et al.*, 2003; Mwape *et al.*, 2012). However, the Co-Ag testing for *T. solium*, mainly undertaken in Latin America, has indicated that human taeniosis has highest prevalence rates in individuals between their mid-teens and early 40s (Allan *et al.*, 1996).

5.3 The implications of the co-endemicity of *Taenia solium* infections in pigs and in humans in Monze district

This study partly aimed at determining the prevalence of *T. solium* infections both in pigs and humans in the study area in order to better understand the epidemiology of this parasite. The results in both the intermediate host (pig/human) and the final host (human) imply that the factors that maintain the lifecycle of the *T. solium* parasite are present in this study area. These results are also in conformity with the risk factors that were reported by Sikasunge *et al.* (2007) in the study area. Among the reported risk factor were the free range pig keeping, backyard (clandestine) slaughtering of pigs without meat inspection, openly defecating in nearby bush with very limited use of latrines, insufficient knowledge about the tapeworm, poverty and poor sanitation standards and eating of insufficiently cooked, especially lightly cysticercosis infected pigs. These should be some of the factors contributing to the maintenance of the *T. solium* lifecycle in this study area. Although CLTS programs are taking place, which could explain the lower prevalence in the current study area than in Sikasunge *et al.* (2008a), change in human behaviour and attitudes is usually gradual. Since humans are the only definitive hosts for the *T. solium* cestode, it implies that the tapeworm carriers that are defecating openly in the nearby bush are providing pigs' easy access to the *T. solium*-egg-contaminated faeces resulting in porcine cysticercosis. The humans that consume the insufficiently cooked infected pork end up with the taeniosis. The poor sanitation and hygiene are the factors contributing to human cysticercosis which could most likely lead to high prevalence of NCC in the study area. Ndimubanzi, *et al.* (2010) reported that NCC accounts for about 30% of epilepsy cases. The current study results

are in agreement with the reports by Diaz *et al.*, (1992) and Sciutto *et al.*, (2000) that in regions where cysticercosis is common in pigs, human cysticercosis is also high.

5.4 Prevalence of soil-transmitted helminth infections in Monze district

Like *T. solium* infections, the present study revealed that STH infections are endemic in the study area. The overall prevalence of STHs, comprising *Ancylostoma* spp. and *Trichuris* spp., among the 133 individuals examined from the 5 villages using McMaster method was 16.5% with every sampled village having positive cases. The co-endemicity of these infections in the same study area is a normal finding since both infections are perpetuated by open defecation, poverty, inadequate water supplies and poor sanitation. The 16.5% prevalence of STH infection found in this study is slightly lower than the recent prevalence study report (17.9%) by Siwila *et al.* (2010) but comparatively similar to the 16.4% reported by Halwindi *et al.* (2011) both by Kato-Katz method. A few prevalence studies in Zambia have reported higher prevalence rates of STHs. For example, Modjarrad *et al.* (2005) and Wenlock *et al.* (1997) reported prevalence rates of 24.9% and 48.6% respectively. On the other hand, Simoonga (2006) reported lower prevalence rates of hookworms of 11% and 4.3% in Kafue and Luangwa district respectively. The observed prevalence of STHs in this study is likely to be an underestimation of the true prevalence because only a single sample per individual and a single diagnostic method (McMaster method) was used for the detection of eggs. Use of serial faecal samples and multi-diagnostic techniques has been reported to increase detection rates especially with regard to hookworms and light helminthic infections (Glinz *et al.*, 2010; Knopp *et al.*, 2008). As evidenced from the results, the dominant

STHs detected were hookworms. This is in agreement with Brooker and Michael, (2000) who reported that hookworms are the most widespread species of STHs in sub-Saharan Africa. In Tanzania, Mazigo *et al.* (2010) reported STH prevalence of 38%, purely of hookworms. This is of concern because the hookworms are a threat to maternal and child health particularly in the light of the moderate and severe anaemia they contribute to in pregnant women, school-aged children and pre-school children (Crompton and Stephenson, 1990; Stoltzfuz *et al.*, 1997; Brooker *et al.*, 1999). Other effects such as growth stunting, diminished physical fitness including impaired memory and cognitive functions (Crompton *et al.*, 2002) that they contribute to are worrisome. Furthermore, although the 16.5% prevalence of STHs falls below the WHO recommended 20% intervention threshold for introduction of annual mass deworming (Pullan *et al.*, 2011), if the worm carriers continue defecating openly and sanitation standards continue being poor, the current prevalence has the potential to remain high. Anderson and May, (1985) stated that STH infections are often referred to as being "overdispersed" in endemic communities, such that most worms are harboured by a few individuals in an endemic area.

Of the total 133 willing participants in the study who actually presented the stool samples for examination for STHs by McMaster method, 84 were females while 49 were males. In spite of the lower number of males sampled, the prevalence of STHs was comparatively higher in males than in females although there was no significant difference ($?^2 = 0.190$, $p = 0.809$) by gender. Several previous studies have reported higher prevalence levels among males than females (Bradley *et al.*, 1992; Glickman *et al.*, 1999; Widjana and Sutisna, 2000; Keiser *et al.*, 2002; Egwunyenga and Ataikiru,

2005). Some of the reasons given for the higher prevalence in males than in females have hinged on differences in gender roles. For example, Widjana and Sutisna (2000) reported that the higher prevalence in males was that more males who normally did not use slippers worked on farms thereby rendering themselves vulnerable to hookworm larval penetration. Other studies have shown no association between gender and prevalence of STHs (Annan, *et al.,* 1986; Traub *et al.,* 2004). The higher prevalence of STHs in males than in females in the present study cannot be explained by the difference in gender roles especially concerning agricultural activities because both males and females in the study area are equally involved and do not protect their feet from hookworm larval penetration by wearing shoes or gumboots as they carry out their farming activities. A possible explanation of the lower prevalence of STHs in males than in females is that women get dewormers from clinics during pregnancy while their male counterparts hardly get any dewormers.

In this current study, the prevalence of STHs by McMaster method according to age revealed a proportionally highest prevalence in children < 12 years old, although there was no significant difference ($?^2 = 2.79$, $p = 0.253$) in the prevalence of STHs among the < 12, 12 = 50 and > 50 years old age categories. This result is a possible finding because children can be infected with hookworms as young as 6 months (Brooker *et al.,* 1999). Another possible explanation is the higher levels of exposure of the children below the age of 12 as they play in the soil, usually with bare feet in such rural areas, and become prone to hookworm larval penetration. These results may also indicate that, the school-based deworming programmes the children are subjected to may not be so effective probably because not all the children are treated. However, the results of the

current study did not conform to the report by Brooker *et al.*, (1999) that infection prevalence rises monotonically with increasing age to plateau in adulthood. The results of the current study did not also agree with evidence from studies of populations in China and South-east Asia that suggest that peak prevalence is observed among the middle aged or even individuals over the age of 60 years old (Lili *et al.*, 2000; Bentony *et al.*, 2002).

Results of this study did not find a significant difference between the prevalence of STHs in the young and the old people. While the school-based mass deworming programmes are well intended and cost effective, the treated children are prone to re-infection from environments contaminated by the worm-carrying untreated adults who openly defecate. Another drawback is that in rural areas like the current study area, quite a number of children of school-going age don't go to school and are thus not treated during the school-based deworming.

5.5 Study limitations

This study was initially targeted at collecting baseline data for a bigger study which aimed at investigating the impact of CLTS on the prevalence of *T. solium* and STHs in the study area. The design of the CLTS study was such that some of the randomly selected villages were chosen as intervention areas (where CLTS programmes would be implemented) while other villages were chosen as control villages (where CLTS programmes would not be implemented).

However, during the sampling, it was found out that the local council was carrying out CLTS programs in some of the selected study communities. This was against the

selection criteria as outlined above and would thus have compromised the impact of the CLTS study. At this point, a decision was made to change the study area from Monze district to Katete district in the Eastern province where such sanitation or hygiene programs had not yet occurred. The direct consequence of this change was that resources that were intended for the CLTS study in Monze district were shifted to Katete district. This change of the study area and the consequent shift of resources impacted negatively on the number of villages to be sampled and the sample sizes of both humans and pigs. If a similar study is to be undertaken in future, there should be better collaboration between local district council and the researchers.

Some of the submitted stool samples were insufficient to carter for all the tests that were to be done. More emphasis should be placed on the need for willing participants in the study to submit enough stool sample (at least half-filling the provided sample bottles).

5.6 Conclusions and recommendations

In conclusion, the results of the current study show that both *T. solium* and STHs are endemic in the study area. Since both infections are associated with poor sanitation, it is logical and economical to consider controlling them together. As the tapeworm carriers and the infected pigs are important in terms of transmission, practical and cost effective interventions in combating this *T. solium* cysticercosis/taeniosis complex lies in synergized control strategies among medical health workers, veterinarians, community workers, policy makers and indeed the community itself. Treatment of pigs with oxfendazole has proved efficacious against porcine cysticercosis and leaves pork with a very clean appearance, although this process takes 2 to 3 months (Gonzalez *et al.*,

1997). On the other hand, treatment of tapeworm carriers with niclosamide may be effective in preventing transmission to pigs and among humans but the tapeworm carrier may not know his status and its implications. However, treating pigs with niclosamide does not clear the cysts in the pig brain, but since the pig brains are rarely eaten, and when the head is eaten, it is thoroughly boiled, this may not be a worrisome factor. On the other hand, while it is vital to continue periodically treating school age children and pregnant mothers with safe and effective antihelmintics, for the control programmes to succeed, they should involve the education of the whole community about the importance of good hygiene and sanitation in the reduction of infections with STHs. Programmes that are community centered and driven like CLTS should be adopted and scaled up to combat not only *T. solium* infections both in humans and pigs but STH infections as well. The ongoing sanitation programmes like CLTS should incorporate veterinarians in the community education. Education of villagers at schools, village meetings and on individual basis about the parasite life cycle and connection between infected pigs and themselves and others getting cysticercosis, should be done with coordinated efforts between medical personnel and veterinarians. The members of the community should also be educated on the life cycles of STHs for them to learn how to prevent infections instead of concentrating solely on mass deworming. Further monitoring, research that uses more sensitive diagnostic methods like PCR and surveillance in both humans and pigs are recommended.

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