

**UNIVERSITY OF ZAMBIA**

**SCHOOL OF MEDICINE**

**DEPARTMENT OF PUBLIC HEALTH**

**THE EVALUATION OF COMMUNITY LED TOTAL SANITATION AS AN  
INTERVENTION MEASURE FOR THE CONTROL OF PORCINE  
CYSTICERCOSIS IN KATETE DISTRICT IN THE EASTERN PROVINCE OF  
ZAMBIA**

**BY**

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**A dissertation submitted to the University of Zambia in partial fulfillment of the award  
of the degree of Master of Science in Epidemiology**

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## **DECLARATION**

I **Carol Bulaya** do hereby declare that this dissertation 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.

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## **DEDICATIONS**

This dissertation is dedicated to my parents **Mr Kingsley Bulaya** (deceased) and **Ms Dorothy Kabange** who instilled in me the importance of education. I also dedicate this to my sister **Ms Jacqueline Nampungwe Bulaya** for her support and patience in my life. And finally to all my family and friends who supported and encouraged me during the course of my studies.

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## LIST OF DEFINITIONS, ABBREVIATIONS AND SYMBOLS

%	Percentage
=	Equal to
>	Greater than
≥	Greater than or equal to
<	Less than
≤	Less than or equal to
$\chi^2$	Chi-square
µg	Microgram
µl	Microlitre
+ve	Positive
Ab	Antibody
Ag	Antigen
Ag-ELISA	Antigen Enzyme-Linked Immunosorbent Assay
Ab-ELISA	Antibody Enzyme-Linked Immunosorbent Assay
EITB	Enzyme Immunoelctrotransfer Blot
ELISA	Enzyme-Linked Immunosorbent Assay
WB	Western-Blot
CLTS	Community-Led Total Sanitation
ODF	Open Defecation Free
KAP	Knowledge, Attitudes and Practices
STHs	Soil Transmitted Helminth Infections
NTDs	Neglected Tropical Diseases
HH	Household

Gp	Glycoprotein
H <sub>2</sub> O <sub>2</sub>	Hydrogen peroxide
H <sub>2</sub> SO <sub>4</sub>	Sulphuric acid
IgG	Immunoglobulin G
M	Molarity
ml	Milliliter
mm	Millimetre
MoAb	Monoclonal antibody
N	Normality
n	Sample size or number examined
NBCS	New born calf serum
NCC	Neurocysticercosis
nm	Nanometers
OD	Optical density
OPD	Orthophenylene diamine
<i>p</i>	Probability
PBS	Phosphate buffer saline
rpm	Revolutions per minute
STATA	Statistics/Data Analysis Software
T20	Tween 20
TCA	Trichloroacetic acid
TsCF	<i>Taenia solium</i> cyst fluid

## ABSTRACT

*Taenia Solium* cysticercosis is a zoonotic infection endemic in many developing countries. It occurs mainly in rural communities and is associated with poverty, poor sanitation and free-range pig management. Humans act as the definitive host (taeniasis) and pigs and humans as the intermediate hosts (cysticercosis). Infection of the central nervous system leads to neurocysticercosis which manifests as epilepsy, seizures and eye blindness and may lead to death. Studies have been conducted in Zambia showing that the disease is endemic with a high prevalence of porcine cysticercosis 19.6% (Sikasunge *et al.*, 2008) and human taeniasis 11.6% (Mwape *et al.*, 2013). However despite the evidence, very few intervention measures against the tapeworm have yet been formulated nor implemented. The proven control measures for parasitic and infectious diseases transmitted by faeces are the improvement of basic sanitation, hygiene and health education. Community Led Total Sanitation (CLTS) is an innovative community based sanitation programme being that aims at reducing open air defecation in rural communities. It is assumed that the success of CLTS will lead to control of poor sanitation related diseases including porcine cysticercosis. The objective of this study was to determine the seroprevalence of porcine cysticercosis before and after CLTS. The study also undertook to assess any difference in the risk factors and cysticercosis awareness in pig farmers before and after CLTS.

A comparative study was conducted with pre- and post-intervention assessments in the same villages to evaluate CLTS as an intervention measure for porcine cysticercosis in Katete District in the Eastern Province of Zambia. The B158/B60 Ag-ELISA was used to detect cysticerci antigens in serum from pigs. A household questionnaire was administered to respondents whose pigs were examined in order to obtain information on general characteristics, pig husbandry practices, sanitation and awareness of *T. solium* infections.

The Wald test p-values were computed in Stata v11 to assess significant differences in the variables. A total of 379 pigs were examined (104 pre-intervention and 275 post-intervention). Of the pigs examined 14 (13.5%) and 45 (16.4 %) were *T. solium* positive after Ag-ELISA examination pre and post-intervention respectively showing no significant difference (p-value=0.473). From the questionnaire a total of 153 respondents were examined (64 pre-intervention, 89 post-intervention). Sanitation as a risk factor showed that the presence of latrines pre and post-intervention were 43(67.2%) and 74(84.1%) respectively, showing an increase of 31 latrines constructed in the villages and was statistically significant (p-value of 0.027). However; the proportion of latrine usage was at 41(93.2%) at baseline and lower 62(84.9%) post-intervention and this was statistically insignificant (p-value=0.151). Other important risk factors i.e. knowledge and awareness of cysticercosis, consumption and selling of infected pork, free-range pig husbandry also showed no change after the intervention (p > 0.05).

The study revealed that CLTS as an intervention did not lead to a decrease porcine cysticercosis infections in pigs (p > 0.05). The study also revealed that some of the risk factors and awareness of *T. solium* control were not significantly improved (p > 0.05). It is recommended that besides CLTS; health education, mass drug treatment and veterinary control of pigs be incorporated, particularly to pig farmers as an essential component of prevention and control programmes.

## CHAPTER ONE: INTRODUCTION

### 1.1 Background

Neglected Tropical Diseases (NTDs) are a group of parasitic and bacterial diseases that cause illness for more than one billion people globally and kill an estimated 534,000 people worldwide every year. The infections are widely distributed in tropical and subtropical areas, with the greatest numbers occurring in sub-Saharan Africa, the Americas, China and East Asia (WHO, 2012) and affect the world's poorest people. The most common of the NTDs are soil-transmitted helminths (STHs) of which worldwide more than 1.5 billion people, or 24% of the world's population are infected. The most prevalent soil-transmitted helminths (STH) are: Ascariasis, Hookworm, and Whipworm. Others STHs of public health importance are; Guinea Worm, Lymphatic Filariasis, Onchocerciasis, Schistosomiasis, Trachoma and Cysticercosis (CDC, 2011). STH infections can contribute to anaemia, vitamin A deficiency, malnutrition and impaired growth, delayed development, and intestinal blockages (CDC, 2011).

*Taenia solium* cysticercosis and taeniasis are zoonotic infections endemic in many developing countries, with humans acting as the definitive host (taeniasis) and pigs and humans as the intermediate hosts (cysticercosis) (Flisser *et al.*, 2003). Infection of the central nervous system leads to neurocysticercosis (NCC) which causes various neurological disorders, the most common being epileptic seizures, papilledema, headache and vomiting due to increased cerebral intracranial pressure (Lobato *et al.*, 1981). These cause considerable morbidity and even mortality (Garcia *et al.*, 1993). Worldwide, people with epilepsy have poorer employment status, less education, poorer housing and greater food insecurity. There is also a greater possibility of accidents during seizures (WHO Technical Report, Series 971).

The prevalence of *T. solium* infection varies greatly according to the level of sanitation, pig husbandry practices and eating habits in a region (Murell *et al.*, 2005); most of them being rural communities where pigs are left free roaming and sanitation is low. In endemic regions, several factors such as open defecation, poor personal hygiene, free ranging pigs, and lack of safe drinking water have been found to facilitate the occurrence of cysticercosis. These factors facilitate both human and animal host access to human faecal materials, which at times may contain *T. solium* eggs (Ngowi *et al.*, 2012). Consequently, many epidemiological studies have been carried out in rural populations.

In Africa, data has shown that *T. solium* is an emerging and expanding zoonosis (Geerts *et al.*, 1995). Pig keeping is popular in many African countries with many of these kept under the free range management system which allows pigs access to human faeces. Africa is one of the regions where the full cycle of *T. solium* is occurring because of the favourable environmental conditions and poverty, which inhibit application of effective control measures for the parasite. Thus, Africa provides a suitable ecology for *T. solium* endemicity (Ngowi *et al.*, 2012).

Pig keeping and pork consumption in Zambia have increased significantly during the past two decades (Phiri *et al.*, 2003). The main reason for this is the increased deaths of cattle due to theileriosis in Eastern and Southern provinces and the recognition by farmers of a quicker and more impressive return on their investment from raising pigs. In addition, the increased demand for pork in urban areas of the country has resulted in the transportation of pigs from these rural smallholder communities to large population centres (Phiri *et al.*, 2002). Most of these smallholder pig producers are so poor that they cannot afford to confine and feed their pigs and as such the pigs are allowed to roam about (scavenge). This free-range management system exposes pigs to consume human faeces often contaminated with tapeworm eggs and hence become infected. The lack or absence of meat inspection regimes and disease control



in certain illegal livestock and livestock products markets exacerbates public health risk to *T. solium* cysticercosis in the urban areas where many infected pigs are transported and consumed by unsuspecting people. Survey records in six of the nine provinces from 2003 to 2008 based on meat inspection, tongue palpitation and serological tests give a prevalence of 7.3 - 34 % for porcine cysticercosis confirming its endemicity in Zambia.

Until the last decade, there was a general lack of data available for this disease condition in both the country and region; however research has been conducted and there now currently exists information on the occurrence, transmission dynamics, socioeconomic impact and burden of disease (Phiri *et al.*, 1998; Krecek *et al.*, 2003; Pondja *et al.*, 2007; Sikasunge *et al.*, 2007; Mwape *et al.*, 2012; 2013). These have led to awareness that the best way to control this type of faecal-oral infection is through education and sanitation programs.

The significant results have been demonstrated by Community led Total Sanitation (CLTS) in South Asia through the provision of subsidy-led toilets for individual households and emphasizing not merely behaviour change by individuals in general but of an entire community, to achieve 'open defecation-free' (ODF) villages. There is a growing recognition that this evolving approach which offers tremendous potential not only for achieving, but even for surpassing, the relevant Millennium Development Goals targets set for 2015 (Water and Sanitation Program-South Asia, Field Note, 2007). In Zambia, this approach has been implemented in pilot in Choma district, in the Southern Province in 2007 with tremendous success. It is now being rolled out in seven provinces with over 1,000 villages reporting ODF in January, 2013 (Zambia National Sanitation Programme Newsletter –No.4). It is however important to evaluate whether this type of intervention is effective in the control of porcine cysticercosis.

### **Historical Perspectives in the study of *Taenia spp***

The scientific study of the taeniid tapeworms of humans can be traced to the late 17<sup>th</sup> century. 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). According to Cox (2002), Edward Tyson was the first person 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 for the current knowledge on the biology of the taeniid tapeworms of humans. Although there were differences between the broad tapeworm and the taeniid tapeworms that were identified, the distinction between *Taenia solium* and *Taenia 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 19<sup>th</sup> century that Kuchenmeister confirmed the differences based on the morphology of the scolex (Cox, 2002).

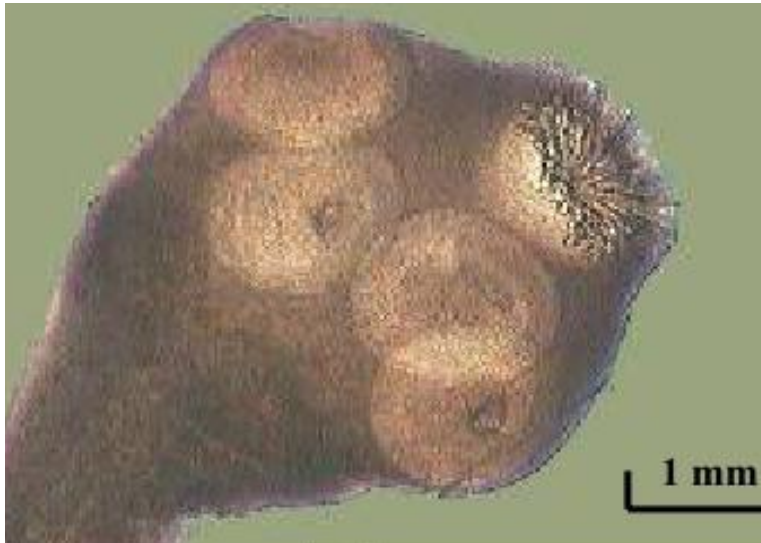
The first indication that intermediate hosts were involved in the life cycles of taeniid tapeworms emerged in 1784 from studies using the pork tapeworm. German pastor, Johann August Ephraim Goeze observed that the scolices of the tapeworm in humans resembled cysts in the muscle of pigs (Kean *et al.*, 1978). Some 70 years later, Kuchenmeister, in much criticised experiments, fed pig meat containing cysticerci of *T. solium* to criminals condemned to death and recovered adult tapeworms from the intestine at post-mortem (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).

## 1.2 *Taenia solium* Parasitology

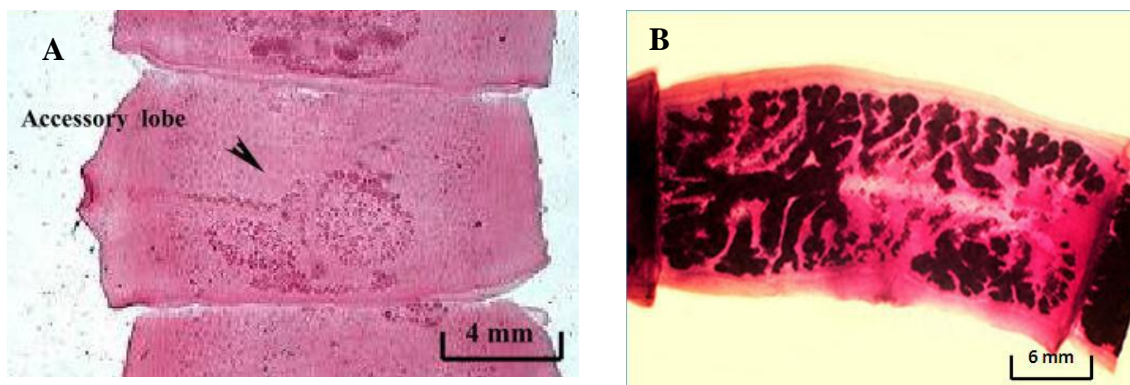
### *Taenia solium* Morphology

*Taenia solium* is a tapeworm belonging to the family *Taeniidae* of the order *Cyclophyllidae* in the *Cestoidae* class of the phylum *Platyhelminthes* (Soulsby, 1982). The adult mature worms are only found in humans in the upper small intestine and they grow to approximately 4- 8 metres. The scolex or head is globular and less than 1 mm in diameter and has 4 suckers and two crowns of horns or rosetellum numbering 26-28 (Figure 1.1). After the scolex is the neck which is long and links to a segmented body is called the strobila which is thin and resembles a ribbon. Each segment is called a proglottid, with those closest to the neck being immature followed by the mature reproductive proglottids. The gravid proglottids are approximately 1 by 1 centimetre and each proglottid is a hermaphrodite. The ovary consists of two lobes, one an accessory lobe and one a genital pore (Figure 1.2A). The gravid proglottids of *T. solium* have 12 lateral branches and no vaginal sphincter muscles (Figure 1.2B). The gravid proglottids, each containing about 40,000 eggs, do not leave spontaneously and are voided in faeces, frequently in chains. The eggs are 26-34  $\mu\text{m}$  in diameter. The larvae or cysticerci are known as *Cysticercus cellulosae* and are approximately 8-10 mm in size. The cysticercus is a vesicle that is whitish, semitransparent measuring approximately 1 cm in diameter and has a spherical scolex inside (Figure 1.3A). They are found in the muscles of the intermediate hosts and in the brain in the case of neurocysticercosis. The eggs of *T. solium* are spherical with a diameter of 30-40 $\mu\text{m}$ . They have a thick yellow-brown radiated shell (Figure 1.3B) and contain a 6 hooked embryo (oncosphere) and are infectious to humans (Garcia *et al* 2007). Cysticercosis of pigs is caused by the presence of cysticercal larvae, *Cysticercus cellulosae*

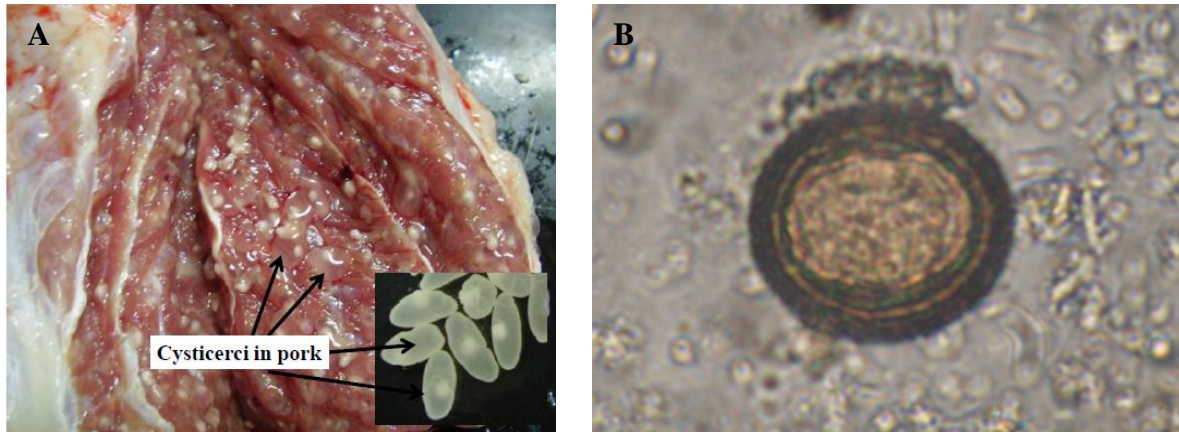
the metacestode of *T. solium* of man (Schantz *et al.*, 1992). According to Gracey and Collins (1992), the *Cysticercus cellulosae* in pig muscles measures between 0.2cm when young and 2cm at full growth. Each cysticercus has the appearance of a white vesicle with the lateral invaginated scolex appearing as a white spot. The scolex, similar to the adult tapeworm, possesses four suckers and double crown of 26-28 hooks (Gracey and Collins, 1992).



**Figure 1.1:** The armed scolex of *T. solium* with four suckers without staining at 40X magnification (Source: [www.stanford.edu](http://www.stanford.edu))



**Figure 1.2.:** The three lobed ovary (A) and 7-20 Uterine branches (B) of *T. solium*. (Source; [www.dpd.cdc.gov](http://www.dpd.cdc.gov) and [www.stanford.edu](http://www.stanford.edu); 2013)



**Figure 1.3.:** *T. solium* cysticerci (*Cysticercus cellulosae*) in muscle tissue (A) with a transparent membrane containing an invaginated scolex (Source; Mwape K.E., 2009) and The rounded or sub-spherical taeniid egg (B) with a thick radially striated embryophore,(Source; Mwape K.E., 2009)

### **Life Cycle of *Taenia solium***

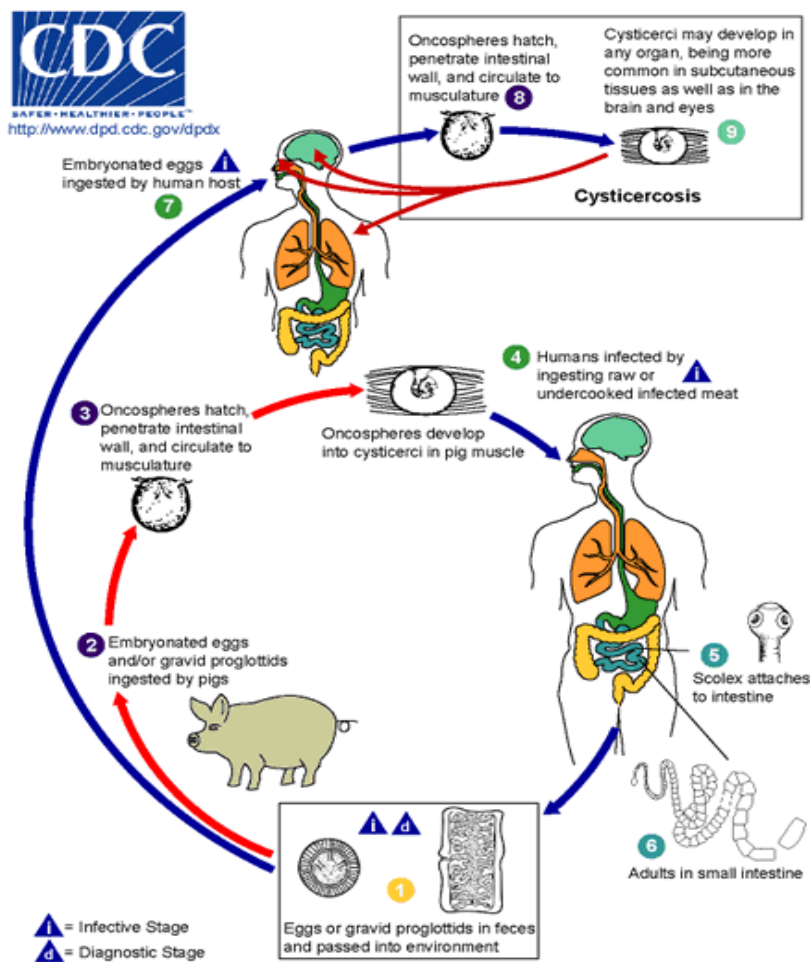
The life cycle of *T. solium* (Figure 1.4) includes pigs as the natural intermediate hosts, harbouring the larval vesicles or cysticerci (cysticercosis), and humans as the definitive host, harbouring the intestinal adult form or tapeworm (taeniasis). Humans can also serve as the intermediate host and develop the cysticercosis by accidental ingestion of *Taenia* eggs (Garcia *et al.*, 1998). The gravid segments of *T. solium* usually leave the host with the stools; often several attached proglottids may leave at the same time (Peters and Pasvol, 2002). The gravid proglottids, which are shed with faeces, contain approximately 50,000 eggs each.

When a pig ingests human faeces containing eggs and proglottids, eggs called the embryophore are released in the intestine, where bile and enzymes trigger the disaggregation of the embryophoric blocks and digest the oncospherical membrane. The oncospheres (hexacanth embryos) hatch, are activated by the digestive enzymes, cross the intestinal wall

and are then transported through the blood or lymphatic's to lodge in muscles, eyes and central nervous system, where they transform into cysticerci, which are the larval stage or metacestode. The size of *C. cellulosae* varies with its stage of development; by 20 days the cyst is the size of a pinhead, by 60 days the size of a pea with the head visible, while by 110 days all cysts are approximately of the same size and the scolex is developed and invaginated (Gracey and Collins, 1992). The cysts remain viable for at least one year within the pig, when they are usually slaughtered. In older pigs, the inflammatory reaction surrounding the cysticerci becomes evident with time.

When a person eats raw or undercooked pork meat that contains cysticerci, the scolex evaginates, attaches to the intestinal wall and over 3 to 4 months develops into a new adult tapeworm measuring 8-10 meters. Intestinal taeniasis is generally asymptomatic and so is swine cysticercosis, which seemingly does not cause neurological problems to pigs. *T. solium* only causes disease to humans that harbour the larval stage, which occurs when humans ingest *T. solium* eggs (Gemmell *et al.*, 1983; Aluja *et al.*, 1998; Flisser, 1988; 1994; 1998). When accidentally ingested by man; the eggs of *T. solium* develop into metacestodes in muscles, subcutaneous tissues or central nervous system (CNS) leading to cysticercosis. Human cysticercosis may occur if eggs are conveyed to the mouth by unclean fingers after defaecation or other oral contamination on swallowing of *T. solium* egg contaminated soil, water or vegetation are the most likely routes of human infection (Schantz *et al.*, 1992). Flisser (1994) reported that retrograde movement of intestinal contents may cause autoinfection, as the oncospheres are released from the eggs by successive exposure to the acid stomach and alkaline intestinal juices. After infection with eggs, the highest number of cysts establishes in the subcutaneous tissue, then the brain, but cysts may be found in muscle, particularly of the thigh or calf, or in the heart, liver, lungs and eye (Gracey and Collins,

1992). Brain localisation of cysticerci causes neurocysticercosis (NCC) which is a major cause of neurological disease in humans. Between 5% and 40% of adult carriers of *T. solium* develop cysticercosis (Carpio *et al.*, 2002). Soulsby (1982) stated that the cysticerci are infective after about nine to ten weeks and that though the longevity of cysticerci is not known, however, the young age at which pigs are slaughtered means that the majority of cysts in pork would be viable.



**Figure 1.4:** Life Cycle of *Taenia Solium* includes pigs as the natural intermediate hosts, harbouring the larval vesicles or cysticerci (cysticercosis), and humans as the definitive host, harbouring the intestinal adult form or tapeworm (taeniasis). (Source; [www.cdc.gov/parasites/cysticercosis](http://www.cdc.gov/parasites/cysticercosis))

## **Porcine cysticercosis**

In geographical areas or locations that have indiscriminate or inadequate disposal of human faeces, pigs ingest stools containing *T. solium* eggs. Localization of cysticercii in pigs is mostly in the muscle and subcutaneous fat which post-mortem show as “measly pork”. For pigs with heavy infections it shows prominently in the brain (Cysticercosis Working Group in Peru 2002, Unpublished Data). Although some pigs have massive infections, porcine cysticercosis is rarely associated with symptoms of any kind. Most pigs are killed before the age of 9 months, which is too short a time for cysts to reach a degenerative stage that is associated with symptoms in human beings (Garcia *et al.*, 2003).

Physical inactivation of cysticerci can be achieved by irradiation and cooking. A minimum of 60°C is required for inactivation. Freezing at temperature of -10°C will also inactivate cysticerci. Cysticerci can also survive upto 30 days in the carcass of pigs at 4°C and their eggs can persist in the environment for months (Gamble *et al.*, 1997).

## **Impact on pig production**

Porcine cysticercosis is an economically important parasitic disease because it affects a large number of pigs, making their meat unfit for human consumption and thereby incurring sizable economic losses. The presence of cysts in the live animal or in the meat greatly reduces its market value causing economic losses to the producer (Widdowson *et al.*, 1999). According to legislation in many African countries meat of infected pig should be destroyed, but due to lack of well-organised meat inspection and very common illegal slaughtering, almost all infected carcasses are marketed and/or consumed (Zoli *et al.*, 2003). Usually in Africa, a pig carcass with cysticercosis is sold at a reduced price thereby causing a loss to either the farmer or the intermediary agent (Phiri *et al.*, 2003; Zoli *et al.*, 2003; Ngowi *et al.*,



2012).

In 2002, ten countries in West and Central Africa based on a conservative economic estimate, estimated the annual losses due to slaughter of unfit pigs at Euro 25 million (Zoli *et al.*, 2003). Studies have been conducted in Cameroon and South Africa to determine the economic burden of porcine cysticercosis (Carabin *et al.*, 2006; Praet *et al.*, 2009) and they show that infected pigs have been found to contribute 4.7-26.9% of the overall costs of the infections, resulting in total annual losses of €10 million and US\$18.6 to US\$34.2 million respectively. These cost estimates in the pig industry have been entirely based on the prevalence of porcine cysticercosis and the economic loss of the pig's value due to the infection. Another factor that needs to be taken into consideration is the loss due to slaughter or undervaluing due to presence of disease (Zoli *et al.*, 2003). These estimates suggest that inactivity bears a considerable monetary cost to *T. solium* endemic regions.

Studies have established that cysticercosis has a possible impact on the endocrine and hormonal levels of pigs by significantly reducing the serum level of testosterone while increasing significantly the level of follicle stimulating hormones in males (Cárdenas *et al.*, 2012) whilst in females it reduces the level of progesterone (Escobedo *et al.*, 2010 and Escobedo *et al.*, 2011). These endocrinological changes are thought to exert significant inhibitory action in the reproduction function of the infected individuals' effect is more intense in male than female hosts. This effect can reduce the productive and economic impact of small holder pig farmers who are already at a disadvantage in resource-poor, endemic areas (Ngowi *et al.*, 2011).

### **Impact on public health**

The most serious form of human cysticercosis is NCC a leading cause of late onset epilepsy

and ocular cysticercosis which causes eye blindness (Flisser *et al.*, 2003; Engels *et al.*, 2003). Studies conducted by Zoli *et al.* (2003) noted that the importance of cysticercosis on human health is rather difficult to estimate because of the highly variable clinical picture of the disease. It ranges from asymptomatic to severe headache, epilepsy and even death. Furthermore, the cost of several visits to the physician, the costs for serology and/or CT-scan, transport and drugs have to be taken into account. Zoli *et al.*, (2003) further stated that although in many African countries, patients are not hospitalised during the treatment of NCC, losses due to the disease are thought to be quite insidiously significant.

Preux *et al.* (2000) stated that the social stigma of epilepsy must also be taken into account and that most communities cast out epileptic patients, because epilepsy is considered a contagious and/ or a shameful disease. In these communities, epileptics are often isolated to prevent the spread of the ailment. According to surveys done by Preux *et al.*, (2000) in West Cameroon only 27% of epileptics get married and 39% fail to enter into any professional activity.

Cysticercosis has enormous socio-economic relevance which includes the cost of medical treatment, loss in man hours and direct losses due to condemnation of infected carcasses. In Mexico, 10-12% of neurological admissions are thought to be attributable to NCC infections (Flisser, 1988). A minimum estimate of the cost of admission to hospital and wage loss for NCC in the United States (a non-endemic country) was US\$8.8 million annually whereas treatment costs in Mexico were estimated at US\$89 million while Brazil was US\$85 million (Roberts *et al.*, 1994).

### **1.3 Epidemiology of *Taenia solium***

The World Health Organization (WHO) estimates that approximately 50 million people

worldwide have cysticercosis infection, although estimates are probably low since many infections are subclinical and there are relatively few population based data on prevalence (Ndimubanzi *et al.*, 2010). Cysticercosis is endemic in many regions of Central and South America, Sub-Saharan Africa, India, and Asia. The prevalence of cysticercosis varies within these countries is often higher in rural or peri-urban areas where pigs are raised, insufficient health infrastructure exist, poor health education, sanitation and especially inadequate disposal of human excrement sanitary conditions are suboptimal (Budke *et al.*, 2009). In some communities the rate of epilepsy approaches 3% and 25 to 40% of these cases have evidence of cysticercosis (Medina *et al.*, 2005; Mwape *et al.*, 2013). However due to international migration some infections are detected in developed economies and furthermore, carriers of the intestinal *T. solium* have also been found even in Muslim countries that, in principle, do not eat pork (Flisser, 2011).

In Africa, few countries in the West and Central regions have studied in detail *T. solium* cysticercosis in both pigs and humans during the past decade, particularly in Cameroon, Zambia and Burkina Faso (Zoli *et al.*, 2003; Sikasunge *et al.*, 2008; Carabin *et al.*, 2009; Assana *et al.*, 2010). The most recent important foci of porcine and human cysticercosis have been identified in the Democratic Republic of Congo (Praet *et al.*, 2010; Kanobana *et al.*, 2011), Burkina Faso (Ganaba *et al.*, 2011; Carabin *et al.*, 2009) and Senegal (Secka *et al.*, 2011). In Eastern and Southern African countries, *T. solium* has been reported as a serious public health and agricultural problem (Mafojane *et al.*, 2003; Phiri *et al.*, 2003; Carabin *et al.*, 2006; Krecek *et al.*, 2008). The epidemiological data on cysticercosis clearly indicates that, with the exception of Muslim countries in North Africa, *T. solium* cysticercosis is endemic in the all regions of Africa.

The important risk factors for porcine cysticercosis have been identified as free range pig

husbandry, allowing pigs access to human faeces, home slaughtering of pigs without meat inspection, selling and consumption of infected pork, absence of latrines (Njila *et al.*, 2003; Boa *et al.*, 2006; Sikasunge *et al.*, 2006; Secka *et al.*, 2010) and presence of tapeworm carrier in a household (Eshitera *et al.*, 2012)..

These studies have shown that the maintenance of the taeniasis/cysticercosis complex is key in perpetuating the life cycle of the parasite and hence the disease. In Mexico, Garcia-Garcia *et al.* (1999) demonstrated that the presence of tapeworm carriers in households is the main risk factor attributed to human cysticercosis. Persons infected with *T. solium* tapeworms intermittently shed proglottids and/or substantial numbers of infective eggs in their faeces thereby exposing the majority of the victims to cysticercosis by the faecal-oral route. They further observed that migration of tapeworm carriers from rural areas to the cities predisposes a higher transmission risk of cysticercosis when it involves the presence of poor environmental and social conditions.

A study conducted by Eastern Cape of South Africa (Krecek *et al.*, 2012) established that an overall prevalence of porcine infection at 57- 41% by B158/B60 Ag-ELISA and HP10 Ag-ELISA respectively. It also showed a significant association between infection and the lack of a pit latrine contributing to an 89% increase in the likelihood of porcine cysticercosis infection due to the easy access to human faeces for the pigs in these areas.

The knowledge and awareness of the cysticercosis in an endemic area also contributes to levels of infection. The practice of free range farming or semi-indoor system of pig rearing and lack of pit latrines allows pigs to have free access to human faeces. Additionally; the practice of backyard slaughter of pigs with no meat inspection and local consumption of the contaminated meat contributes to the maintenance of the life cycle (Maridadi *et al.*, 2011).

## **Prevalence of porcine cysticercosis in some selected Latin American, Asian and African**

### **Countries**

Extensive epidemiological and clinical studies demonstrating the endemicity of *T. solium* have been carried out in many Latin American countries and a few in Asia. These studies involved sampling in both humans and pigs. In Africa, baseline data on the prevalence of *T. solium* has been collected in pigs and humans. Table 1.1 shows prevalence of porcine cysticercosis in some Latin American, Asian and African countries.

**Table 1.1** Pig population estimates of selected countries in 2012 and the current status of porcine cysticercosis (PCC) caused by *Taenia solium*.

Country	Pig Population (2010)	Porcine cysticercosis prevalence (%)	Diagnostic Method	Reference
Burkina Faso	1,920,200	0 - 39.6	Ag-ELISA	Zoli <i>et al.</i> ,(2003)
South Africa	1,594,490	54.8 - 57	Ag-ELISA	Krecek <i>et al.</i> , (2012)
Gambia	28,500	4.8 (n=371)	Ag-ELISA	Secka <i>et al.</i> ,(2010)
Senegal	346,681	6.4 - 13.2(n=1334)	Ag-ELISA	Secka <i>et al.</i> ,(2010)
Angola	791,000	0 - 6.8	Meat inspection	Zoli <i>et al.</i> ,(2003)
Cameron	1,680,000	11 - 39.8	Ag-ELISA	Assana <i>et al.</i> ,(2010)
DR Congo	967,000	41.2 (n=153)	Ag-ELISA	Praet <i>et al.</i> ,(2010)
Tanzania	495,000	30.7 - 32(n=600)	Ag-ELISA	Komba,(2008)
Kenya	347,400	4.0	Ag-ELISA	Kagira <i>et al.</i> ,(2010)
Zambia	500,000	8.2 - 23.3(n=98-1691)	Ag-ELISA	Phiri <i>et al.</i> ,(2002) Sikasunge <i>et al.</i> ,(2008)
Cambodia	ND*	7.4 - 15.7(n=432)	Meat inspection	Sovyra,(2005)
Vietnam		0.9 (n=891)	Meat Inspection	Huan,(1998)
China	ND	5.4 (0.8-40)	ND	Rajshekhhar <i>et al.</i> (2003)
Mexico	ND	4	ND	Sarti <i>et al.</i> , (1992b)
Peru	ND	61	ND	Garcia <i>et al.</i> , (1999)
	ND	43	ND	Diaz <i>et al.</i> , (1992)

\* ND indicates no data

### Prevalence of porcine cysticercosis in Zambia

Extensive studies have been carried out in pigs in six districts of three provinces of the country namely, Eastern, Southern and Western provinces. These studies have indicated very high prevalence's of porcine cysticercosis (Table 1.2) (Phiri *et al.*, 2002; Sikasunge *et al.*, 2008b) and have highlighted the importance of free-range pigs and absence of latrines in maintaining the infection in pigs (Sikasunge *et al.*, 2007). Using the Bayesian approach, the porcine cysticercosis prevalence has been estimated as very high with 64.2% (Dorny *et al.*,

2004b).

**Table 1.2** Prevalence of porcine cysticercosis after tongue examination and Ag - ELISA by district in Eastern, Southern and Western provinces of Zambia

Province	District	N	Tongue examination +ve (%)	Ag-ELISA +ve (%)
Southern	Gwembe	385	83 (21.6)	131 (34)
	Kalomo	98	8 (10.3)	20 (20.8)
	Monze	387	34 (8.8)	88 (22.7)
	Sub-total	870	125 (14.4)	239 (27.5)
Eastern	Petauke	384	25 (6.5)	56 (14.6)
	Katete	385	29 (7.5)	74 (19.2)
	Sub-total	769	54 (7.0)	130 (16.9)
Western	Mongu	150	11 (7.3)	45 (30.0)
	Sub-total	150	11 (7.3)	45 (30.0)
Total		1789	190 (10.7)	414 (23.3)

Sources; Phiri *et al.* (2002) and Sikasunge *et al.* (2008). Ag-ELISA = Enzyme-linked immunosorbent assay for the detection of circulating antigens of the metacestode of *T. solium*. +ve = positive test

The high porcine cysticercosis prevalence's recorded in pigs are an indication of the presence of adult tapeworm carriers within the communities. The prevalence in humans has now been documented to range from 5.8 - 12.4% for cysticercosis and 6.3 - 11.9% for taeniosis (Mwape *et al.*, 2012; 2013). These studies highlight the endemicity of *T. solium* infections in Zambia and the urgent need for control.

#### 1.4 Diagnosis of porcine cysticercosis

The diagnosis of cysticercosis in pigs is based on parasitological, meat inspection and immunodiagnosis methods. The parasitological method involves tongue palpitation which allows for the detections of cysts usually in the ventral side of the tongue and ante-mortem examination of the meat. The immunodiagnostic methods involve the detection of detection of specific cysticercosis antibodies or antigens circulating in blood sera.

### **a) Parasitological Methods**

The most common method of diagnosing porcine cysticercosis *in vivo* at the village level is tongue examination. However; tongue examination requires technical skills and has low sensitivity (Sciutto *et al.*, 1998a). The vesicular metacestodes can be palpated and easily seen. However, fibrous or calcified larvae (cysts) are more difficult to detect, as they tend to be quite small (Sciutto *et al.*, 1998b).

Onyango-Abuje *et al.* (1996) and Sciutto *et al.* (1998a) further estimated that more than 50% of pigs that harbour metacestodes are seen in the tongue. Sciutto *et al.* (1998a) also reported that the maximal sensitivity obtained by the tongue examination were 71% in experimentally infected pigs. Gonzalez *et al.* (1990) also noted that the *in vivo* examination of the tongue and post-mortem anatomopathological examination are common procedures used in the diagnosis of porcine cysticercosis. However, he reported that tongue examination shows high specificity and low sensitivity or about 70% of the anatomopathological examination, which has a disadvantage of requiring cuts in the meat. Gonzalez *et al.* (1990) further noted that although tongue examination is one of the methods used to detect cysticercosis infection in pigs, which is cheap and easy to perform, it can easily miss mild infections.

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 (Walther and Koske, 1980; Gonzalez *et al.* 1990). Meat inspection is done at ante-mortem examination of the carcass on several predetermined sites such as the heart, diaphragm, masseter muscles, tongue, neck, shoulder and intercostal and abdominal muscles (Soulsby, 1982). However; the efficacy of meat inspection is highly dependent in the thoroughness of the meat inspection and the degree of infection of the animal. A lightly



infected carcass will most likely not show any viable cysts to the naked eye and meat inspection in these areas will seriously underestimate the real prevalence of porcine cysticercosis (Murrell *et al.*, 2005). Another disadvantage of the current meat inspection procedures is that infection is detected after the death of an animal, which is too late to make any decisions over treatment (Onyango-Abuje *et al.*, 1996).

#### **b) Immunodiagnostic Techniques**

Immunodiagnosics are often necessary to identify *T. solium* cysticercosis. Serological tests have recently been developed for the detection of specific cysticercosis antibodies or antigens (Geerts *et al.*, 1981; Harrison *et al.*, 1989; Dorny *et al.*, 2003). The tests are important tools for epidemiological studies since they can be applied to living animals on a large scale (Dorny *et al.*, 2003). 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 both human and pig cysticercosis (Diaz *et al.* 1992).

Recent advancements in immunodiagnosics have led to the development of serological tests for the detection of specific cysticercosis antibodies or antigens of *T. solium* in pig serum. The serological tests available for *T. solium* taeniasis and cysticercosis are; Enzyme-linked-Immuno-electro Transfer Blots (EITB) or Western Blot Assay (WB), Antibody Enzyme-linked-Immuno-sorbent Assay (Ab-ELISA) and Antigen Enzyme-linked-Immuno-sorbent Assay (Ag-ELISA). These tests are more sensitive than tongue examination however; the sensitivity and specificity may be affected by some factors such as low cyst burden; transient antibodies and cross reaction of maternal antibodies with young pigs (Sciutto *et al.*, 1998). Although (EITB) requires whole cysticerci as their source of antigen; comparison studies

have shown that ELISA is less sensitive and specific than EITB assay for epidemiological studies (Schant *et al.*, 1994). They are also expensive and inaccessible for most poor countries.

### **Antibody Enzyme-Linked Immunosorbent Assay (Ab-ELISA)**

Serodiagnosis of cysticercosis through detection of anti-parasite antibody has been widely evaluated using several target antigens, ranging from total *T. solium* extracts (Flisser *et al.*, 1994) of the metacestodes to selected preparations, such as cyst fluid, scolex or extracts of external membranes (Larralde *et al.*, 1986). Pinto *et al.* (2000) conducted a study to evaluate antigens of *T. solium* and *T. crassiceps* cysticerci using Enzyme-Linked Immunosorbent Assay (ELISA) test for the diagnosis of porcine cysticercosis. Four antigens; (i) vesicular fluid and (ii) crude *T. crassiceps* antigens and, (iii) scolex and (iv) crude *T. solium* antigen preparations were assayed. Pinto *et al.* (2000) found that though all the antigens showed good performance, the vesicular fluid of *T. crassiceps* was the best followed by crude *T. crassiceps*. A separate study conducted by Nunes *et al.* (2000) also found similar results as those obtained by Pinto *et al.* (2000). According to the study by Nunes *et al.* (2000), 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. Indirect ELISAs based on the detection of the host's (porcine) anti-cysticercal antibodies have not been very reliable in individual animals due to many cross-reactions observed (Geerts *et al.* 1981; Harrison *et al.*, 1989). Garcia *et al.* (2001) noted that antibody detection has an important drawback, in that, it may indicate exposure to infection and not necessarily the presence of an established, viable infection, resulting in transient antibodies; and also that antibody may persist long after the parasite has been eliminated by immune mechanisms and/or drug therapy (Harrison *et al.*, 1989; Garcia *et al.*, 1997). Harrison *et al.*, (1989) stated that, the indirect ELISAs do not

differentiate between recent infections with live metacestodes and older infections with degenerated metacestodes, which are no longer infective. Pinto *et al.* (2000) reported that when using *T. solium* antigen, the occurrence of cross-reactions with other diseases such as hydatidosis and ascariidosis have been observed. Immunodiagnosis based on serum antibody detection is an efficient marker of contact with the parasite, permitting the identification of endemic areas in which control and preventive measures must be intensified (Fleury *et al.*, 2013). However, this technique is expensive and not readily available even in urban areas.

### **Antigen Enzyme-Linked Immunosorbent Assay (Ag-ELISA)**

Several researchers including (De Jonge *et al.*, 1987; Harrison *et al.*, 1989; Brandt *et al.*, 1992); Draelants *et al.*, 1995; Onyango-Abuje *et al.*, 1996 ;Van Kerckhoven *et al.*, 1998 and Dorny *et al.*,2000) have contributed to the development of antigen detecting ELISAs. Harrison *et al.* (1989) developed an antigen detecting ELISA (HP10 antigen ELISA) based 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 recognised 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. Similar Ag-ELISA (B160/B60 antigen ELISA) has been used in sero-epidemiological studies for *T. saginata* and *T. solium* cysticercosis in Zambia (Dorny *et al.*, 2002; Phiri *et al.*, 2002; Sikasunge *et al.*, 2008). Harrison *et al.* (1989) reported that 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. It is also reported to give fewer cross-reactions with other helminth infections (Dorny *et al.*, 2000). There are

unfortunately, also cross reactions with antigens from *T.hydatigena* metacestodes (Dorny *et al.*, 2004) and *T.s.asiatica* metacestodes (Fall *et al.*, 1996; Geerts *et al.*, 1992) in pigs. Therefore in regions where these parasites are endemic, the detection of *T.solium* cysticercosis is restricted (Dorny *et al.*,2001) and accurate data on the prevalence are not easily available or of questionable reliability (Rajshekhar *et al.*,2003).

Harrison *et al.* (1989) showed that when the drug praziquantel killed the cysticerci, the Ag-ELISA assay became negative, presumably because parasite products were no longer produced by the dead cysticerci. Similar findings were observed by Aluja *et al.* (1999) that Western blot gives positive results as long as the metacestodes are in the vesicular stage, but when they become caseous the result tends to be negative and that results using the ELISA show the same tendency. A small trial conducted by Rodriquez *et al.* (1989) in pigs naturally infected with *T. solium* indicated that the Ag-ELISA also had potential in the diagnosis of viable infection in pigs. Sciutto *et al.* (1998b) used both antigen and antibody ELISA to analyse pig sera, in experimentally infected pigs all of which were infected on necropsy examination, and showed that 83.7% and 86.0% pigs were positive to Ag-ELISA and Ab-ELISA respectively. However, Sciutto *et al.* (1998b) found that neither Ag-ELISA, Ab-ELISA nor Enzyme-Linked Immunotransfer blot (EITB) are adequate for the diagnosis of porcine cysticercosis in lightly infected village pigs (pigs with low cyst burdens) and that such pigs may escape detection by meat inspection thereby maintaining parasite transmission by allowing lightly infected carcasses to remain in the food chain.

### **Enzyme-Linked Immunotransfer Blot (EITB) or Western Blot**

Currently immunoblot using a *T. solium* glycoprotein antigen extract (LL-Gp) consisting of seven major glycoproteins, which are species-specific, has been successfully used for

antibody detection of *T. solium* cysticercosis in humans and pigs (Tsang *et al.*, 1989; 1991). The LL-Gp 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). It has a specificity of 100%, and sensitivity for 98% for patients with multiple cerebral lesions, and a sensitivity of 60-85% for patients with single cystic lesions. The assay is also highly reproducible and simple to perform and the reagents are very stable (Tsang *et al.*, 1989; Diaz *et al.*, 1992). Other researchers have been able to produce similar results in children at a sensitivity and specificity of 72% and 96% respectively. The sensitivity was higher 100% in cases with multiple cysts and multiple sites (Aguiler-Rebolledo *et al.*, 2002).

The major disadvantages of the test however, are the complicated nature of antigen preparation and 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). 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. According to Wilkins *et al.* (1999), 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. And additionally it is an antibody test that may only detect exposure and not necessarily active infection (Dorny *et al.*, 2003).

Two Western Blots, Eluate-WB and Vesicular Fluid Western Blot (VF-WB) using purified parasite glycoprotein antigens are also used and give similar results (Tsang *et al.*, 1989). Their sensitivity as compared to that of the EITB assay is 98% and 100% respectfully. These

therefore can reliably be used for diagnosis in developing countries where access to the EITB assay is difficult (Guido *et al.*, 2003).

### **1.5 Prevention and Control Strategies of *T.solium* infections**

The growing interest of the research and public health sectors in control of taeniosis/cysticercosis was increased in 1993 by an opinion of the International Task Force for Disease Eradication, that *T. solium* infection was potentially eradicable, even though greater experience in evaluating the efficacy and feasibility of control measures was required (CDC 1993, Recommendations of the International Task Force for Disease Eradication). This statement was based on the following reasons; tapeworm infection in humans is the only source of infection for man and pigs, the reservoir of infection in pigs is restricted by the life span of these animals (rarely exceeding one year), there is no significant reservoir of infection in wildlife, effective, inexpensive, easily deployed drugs are available for the treatment of human infections and finally *T. solium* infections already have disappeared from most of Europe and the feasibility of significant reduction of *T. solium* transmission by population-oriented chemotherapy has already been demonstrated.

The statement of the International Task Force for Disease Eradication was a turning point in designing control measures against *T. solium* infections. For more than a century, the preventive and control measures were the responsibility and domain of veterinary services, but now the main responsibility for eradication of *T. solium* infections had shifted to medical services, which should be involved in the treatment of human taeniosis because it is the only source of cysticercosis in humans and in pigs. It has to be emphasised that medical control strategies should be focus oriented not just on the general population. It should be focused on the locality of the porcine cysts, taeniasis and epilepsy. Control measures that can be used are

as follows:

1. Pig prophylaxis with a veterinary benzimidazole oxfendazole at 30mg/kg resolves the presence of cysts and is an inexpensive, cost-effective way of controlling porcine cysticercosis in endemic low-income areas (Sikasunge *et al.*, 2008, Pondja *et al.*, 2012), however control measures aimed at pigs can, however, only prevent new *T. solium* taeniasis infections in humans leaving the reservoir in humans unaffected (Pawlowski, 2008).
2. Immunity to the oncosphere stage plays a central part in the regulation of transmission of taeniid cestodes and oncosphere-based vaccines have proved partially protective to *T. Solium* infections in healthy pigs (Verastegui, 2002, Lightowlers, 2003) Vaccination with TSOL18 an oncosphere antigen vaccine has proven to induce complete protection against *T. solium* infection in pigs in Cameroon (Assana *et al.*, 2010).
3. Accurate laboratory diagnosis and screening processes which comes in two types: (i) Low sensitivity, low cost - lingual and microscopic examination - (Morgan and Hawkins, 1949; WHO 1983). (ii) High cost, High sensitivity (ELISA/EITB/WB) - this however is highly effective in detecting asymptomatic cases in endemic areas. (Shiguekawa *et al.*, 2000; Tsang *et al.*, 1989)
4. Human prophylaxis in asymptomatic cases is important. Chemotherapy with taeniocides such as Niclosamide and Praziquantel (10mg/kg) directly destroys the source of infection by eliminating tapeworm carriers and therefore prevents the spread of cysticercosis, both in humans and pigs (Allan *et al.*, 2002; 2007; Pawlowski, 2006; Pawlowski *et al.*, 2005; Sarti *et al.*, 2000). Mass therapy of humans has, however, not been shown to be consistently effective (Pawlowski *et al.*, 1991; Flisser, 1994; Cruz *et al.*, 1989; Diaz *et al.*, 1991;1989; Sarti, 1997; Sarti *et al.*, 2000) .This has been difficult

to achieve in the context of persistence of free-roaming pig production and lack of health education (Sarti et al., 2000). Additionally, taeniocides can actually cause inflammation and calcification of cysts lodged in the central nervous system, leading to an early onset of epilepsy (Ndimubanzi *et al.*, 2010).

However, most of the above mentioned control strategies are expensive for resource-poor communities this has led to the search for alternative solutions into the prevention of infections in both humans and pigs. The most workable control measure is the improvement of basic sanitation, hygiene and health education which has proved to be an efficient strategy for many parasitic and infectious disease transmitted by faeces (Fleury *et al.*, 2013).

To meet the complexities and possible migration of *T.solium*, multidisciplinary regional networks of specialists which act as interlocutors of the local government and international organizations have been formed. They actively participate in applicable preventive measures and most adequate measures for their respective countries. They are found in Africa, Asia, Latin America and Europe.

In Africa, The Cysticercosis Working Group Eastern and Southern Africa (CWGESA) was established in 2002 to promote communication, collaboration and coordination of integrated research and control activities to combat cysticercosis. The 7<sup>th</sup> CWGESA General Assembly provided an opportunity to review the *Regional Action Plan for Combating Cysticercosis in Eastern and Southern Africa* formulated during the International Action Planning Workshop on *Taenia solium* cysticercosis/taeniosis (TSCT) held in Arusha, Tanzania, in August 2002. Participants of the 6<sup>th</sup> General Assembly held in Nairobi, Kenya in October 2009 were able to review and to adopt the document outlining the Roadmap for Elimination of TSCT in Eastern and Southern Africa. This was jointly presented by the CWGESA Chairperson, Prof. S.



Mukaratirwa, and Prof. A.L. Willingham at the Interagency Meeting on Planning Neglected Zoonotic Diseases Prevention and Control held in Geneva from 5 – 6 July 2011, organised by the World Health Organization (WHO/NTD), UNICEF/UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR), the Food and Agriculture Organization of the United Nations (FAO) and the World Animal Health Organization (OIE).(CWGESA Press Release., 2011).

The following strategies describe how the provision of health education sanitation and legislative enforcement can be used to control *T.solium* infections in endemic populations.

**a) Health Education Programs:**

In Mexico, a comprehensive study was undertaken in a rural community to evaluate the effect of health education, in both the short and the long term (6 and 42 months), as an intervention measure against *T. solium* (Sarti, 1997). It was a successful intervention strategy because almost 4 years after the health education was implemented, no infected pigs could be identified in these communities (swine cysticercosis rate before intervention was 2.6%). As a step towards eliminating the disease, farmers who raise pig in resource-poor areas need to be convinced that better-quality pork products can fetch higher prices, so an incentive to raise pigs hygienically can be created. A study conducted in Mbulu District of Northern Tanzania (Ngowi *et al.*, 2007) showed that basic financial analysis of a health and pig management education intervention indicated a significant financial benefit of Net Present Value US \$3507.00 and internal rate of return of 370% as compared to those who had no intervention with Net Present Value US \$1815.00 and internal rate of return of 200%. This also showed that health education interventions have a significant financial benefit and can be used as an incentive for smallholder pig farmers.

A comparative study conducted in the Iringa and Chunga Districts of Tanzania (Ngowi *et al.*, 2011) with pre-and post intervention assessments showed that health education intervention significantly improved the knowledge and attitude variables i.e. nature of pig cysts, nature of porcine cysticercosis transmission and the actual impact of the parasite to humans. It recommended that health education be integrated as an essential component of prevention and control programmes for *T. solium* infections. Further recommendations from this study were that technological tools such as local radio programmes and mobile phones be used to deliver information to the local populace.

#### **b) Sanitation Programs:**

The provision of basic sanitary facilities is an important step in the prevention of a number of faecal transmitted diseases like cysticercosis. Water and sanitation programs have therefore attracted donor focus, and a number of affordable models of latrines have been developed. Such projects have had a varying degree of success, with a change in focus from being basically donor-driven to the more sustainable community participation.

However, in poor communities latrines have not always been high on people's priorities, and some projects have been abandoned. This is due to the fact that these projects did not address the needs of the people prior to construction of latrines. The campaigns should commence with health education and the solving of some of the more immediate priorities within a community (White, 1997). An integrated approach to water and sanitation is also needed, one that provides for latrines and improved water supply and health education.

Worldwide, most sanitary intervention programs have been carried out in Latin America and Asia. A project specifically targeting cysticercosis was undertaken in the community of Chalcatzingo, Morelos state, Mexico involving 2000 inhabitants to study the local knowledge

of both infections (taeniosis and cysticercosis) and both parasites (the tapeworm and the cysticercus). This intervention attempted to bring about change in knowledge, attitudes and practices of the community by health education with community participation; ultimately to stop new infections in people and swine. This was initiated by in-depth questionnaires developed by anthropologists. One of the main indicators of success of intervention in this zoonosis is environmental contamination as measured by swine cysticercosis (Gonzalez *et al.*, 1994). By this criterion intervention through education was extremely successful since no pigs with cysticercosis were identified in the whole community later on (Sarti *et al.*, 1997). Other studies have been done in Peru (Garcia *et al.*, 2003; Gonzalez *et al.*, 1990; Diaz *et al.*, 1992) with similar results.

In Bangladesh since 2007, the UK Government has been supporting the SHEWA-B (Sanitation, Hygiene Education and Water Supply in Bangladesh) programme to improve the access to safe water, sanitation and hygiene of approximately 30 million people in 19 districts across Bangladesh (SHEWA-B Annual Review, 2012). However the major indicators for evaluation which have been used are the drop in diarrhoeal disease and infant/child mortality rates (WaterAid, Global Synthesis Report, 2009).

### **c) Intervention Measures of International and National Health Authorities:**

Despite the fact that several international initiatives have been concerned with the problem of cysticercosis for many years; with strategies for the prevention and control of taeniasis and cysticercosis being analyzed and recommendations made, the control of cysticercosis in developing countries has not been achieved (Flisser *et al.*, 2006). According to a World Health Assembly Report (2003), economic losses in the veterinary field also urged national

authorities to establish monitoring systems and notification and to take action towards prevention and control of cysticercosis and taeniasis through research and interventions in the field. However despite these recommendations; at national health level in most developing countries, not much has been done.

In only very few countries have specific norms have been recommended. In Latin America only Mexico has an official policy for the vigilance, the prevention and the control of taeniasis and cysticercosis in the first level of attention which was published in 1994 and modified in 2004 (NOM, 2004). This policy is mandatory throughout the country for all health staff providing care in public, social and private institutions as well as for professional and technical agricultural personnel, animal husbandry veterinarians engaged in private practice in pig farms, producers, pig owners and anyone involved in the transport and commercialization of this species (Flisser, 2011). Unfortunately, despite the substantial improvement in the social, economic and health conditions of the population; 15 years after its promulgation, cases of swine and human cysticercosis are still being diagnosed and not notified in Mexico. Thus indicates that cysticercosis in Mexico has been controlled but not eradicated. This phenomenon can be explained to a number of factors; inaccessibility in rural areas, lack of health facilities in rural areas, lack of qualified staff to monitor legislature and lack of monetary compensation to farmers for any pigs confiscated (Flisser,2011 and Fleury *et al*,2013).

It is clear that programs such as these require an established structure supported by a recognised local authority. To address these needs the Mexican government since 2009, an extensive pilot control program is under way, based on health and sanitary education and associated with vaccination of pigs. Local authorities are part of the efforts, helping with the identification of the endemic areas, by furnishing sera collected from the pigs, and by funding

the program. The results so far are encouraging .It has become clear is that programs must be of long duration, at least 5 years. It is of little use to visit communities, give talks, and vaccinate pigs and leave. People in far-flung rural areas need long-lasting help, advice and supervision. Therefore, without the active participation of the governments, failure of any control program is predictable because scientists cannot apply it at large enough scales and sufficient time (Fleury *et al.*, 2013).

## **1.6 Community Led Total Sanitation (CLTS)**

The Community-Led Total Sanitation (CLTS) approach was first pioneered in 1999 by Kmal Kar (a development consultant from India) in a small community in Bangladesh. Since then CLTS has rapidly spread to over 20 countries in Asia, Africa, Latin America and the Middle East, and has been championed by various international non-governmental organisations (NGOs), namely the Water and Sanitation Program (WSP), Plan International, Wateraid and the United Nations International Children’s Emergency Fund (UNICEF).

The intermediate goal of the CLTS is to modify the normative environment in which open defecation occurs thereby altering community norms, beliefs and attitudes. CLTS involves facilitating a process to inspire and empower rural communities to stop open defecation and to build and use latrines, without offering external hardware subsidies. Communities are encouraged to appraise and analyse their own sanitation profile, including the extent of open defecation and the spread of faecal-oral contamination. This approach ignites a sense of disgust and share among the community. The community then collectively realises the impact of its unsanitary practices and this realisation mobilises and initiates collective action to improve the existing sanitation profile. ([www.communityledtotalsanitation.org](http://www.communityledtotalsanitation.org))

Until recently, there had been little success in increasing access to sanitation in Zambia both

with and without subsidy. Therefore, in 2007, UNICEF in conjunction with the Government of Zambia decided to pilot the CLTS approach in Choma district in Zambia's Southern province, where the coverage was 40%, in order to see whether CLTS could offer an effective strategy for rural sanitation implementation in the country. Twelve communities were triggered by trained CLTS facilitators. Within the period of two months, sanitation coverage increased from 23% to 88% within a population of 4,536 and 75% of the villages were verified as open defecation free (ODF), surpassing the MDG target for sanitation in the pilot area in just two months. The role of traditional leaders was crucial in ensuring sustained action from communities and the chiefs involved are keen to scale up the approach to all the communities in their respective chiefdoms (Zulu *et al.*, 2010).

Following the success of the pilot project, "The 3 Million People Sanitation Program" was launched in April 2012 by the Minister of Local Government and Housing in Zambia. After the launch, 12 districts including Katete in Eastern Province of Zambia were selected for the pilot that took place between April and June 2012. A review meeting was held to review implementation and based on the impressive results from the pilot; another 20 districts were identified for the roll-out. To date, 32 districts have been trained and triggered and the team is preparing to scale up from April 2013 to cover a greater part of the country. As of December 2012, over 1,000 villages have reported to have achieved open defecation free (ODF) status and are awaiting verification and certification (Zambia National Sanitation Programme Newsletter - No.4). However, the program processes that include inputs, process outputs as well as impact have not been evaluated to inform the intervention. This is seen as a critical gap raising questions on how informed the CLTS intervention is. It therefore seems logical to propose that CLTS needs to be evaluated as a control tool for pork tapeworm infections in Zambia urgently.



## CHAPTER TWO: RESEARCH FOCUS

### 2.1 The Problem Statement

The high porcine cysticercosis (19.6%) in Katete district (Sikasunge *et al.*, 2008) and that of human taeniosis (11.8%) (Mwape *et al.*, 2013) is an indication of a high level of infection in both humans and pigs. These studies show that the disease is endemic and despite the evidence; very few intervention measures targeting *T.solium* infections have been formulated or implemented in Zambia. From the standpoint of disease transmission to humans and maintenance of the parasite's life cycle, the adult tapeworm is of primary importance. The prevention of infection of humans and subsequent environmental contamination with *Taeniid* eggs is of paramount importance in both prevention and control strategies. The development of improved sanitation and hygiene practices have had a major impact on the occurrence of cysticercosis in developed countries and also among urban dwellers in developing countries, because of their effect on the transmission of taeniid eggs (Gilman *et al.*, 1999). Community Led Total Sanitation (CLTS) currently being implemented in the district as a means of improving sanitation and subsequently may interrupt the transmission cycle of *T.solium* which is highly prevalent. It is with this fact that CLTS needs to be evaluated as an intervention measure using the output impact of porcine cysticercosis which is prevalent in Katete District of Eastern Province.

### 2.2 Justification of Study

Concurrent with the reported increase in smallholder pig keeping and pork consumption in the past two decades, have been increasing reports of porcine cysticercosis (Phiri *et al.*, 2003 and Sikasunge *et al.*, 2008).The increase in porcine cysticercosis indicates a strong presence of *T. solium* in humans with studies reporting high pig and human infection prevalence's



(Sikasunge *et al.*, 2008 and Mwape *et al.*, 2013). However; more research is required in Zambia to assess the extent of the impact of *T. solium* infection in order to determine whether and what prevention and control methods are needed. The analysis of the porcine cysticercosis infections before and after the CLTS program will help with the following; 1) knowledge on the performance of the program in terms of the burden of pig infections of *T. solium*; 2) assessment of the determinants will provide intervention-targeted evaluation of the program ; 3) Policy - advise government on further control measures needed to lessen the burden of the disease ; 4) Research-Analysis of CLTS will give data on its impact on pig and human health and pave way for further research on the best way to eliminate the disease and 5) meeting the MDG Goal 7 of ensuring environmental sustainability with the specific target of halving by 2015, the proportion of the population without sustainable access to safe drinking water and basic sanitation .

### **2.3 Research Question**

What is the impact of CLTS as an intervention using the seroprevalence of *Taenia solium* porcine cysticercosis as an output in Katete District?

### **2.4 Hypotheses**

**Null Hypothesis (H<sub>0</sub>)** : The success of CLTS will not lead to the control of poor sanitation related diseases which includes pork tapeworm infections

**Alternative Hypothesis (H<sub>1</sub>)**: The success of CLTS will lead to the control of poor sanitation related diseases which includes pork tapeworm infections

### **2.5 Study Objectives**

**General Objective**

To evaluate the impact of CLTS intervention using the seroprevalence of *T. solium* porcine cysticercosis as an output in Katete District of the Eastern Province of Zambia.

### **Specific Objectives**

The specific objectives included the following:

- (a) To determine the prevalence of porcine cysticercosis before and after CLTS.
- (b) To assess awareness of porcine cysticercosis among the study population before and after CLTS.
- (c) To determine if there is a difference in risk factors associated with prevalence of porcine cysticercosis before and after CLTS.

### **2.6 Theoretical Framework**

To help answer these questions, a theoretical framework was proposed. Theoretically, due to the biology and transmission of *T. Solium* eradication is possible. The main hindrances hinge on lack of sanitation, health promotion and public health advice. The accurate diagnosis and treatment of adult stage tapeworm is important in breaking the parasites life cycle. The lack of access to proper sanitation is linked to a variety of diseases including diarrhoea, soil transmitted helminths (STHs) infections, taeniasis /cysticercosis especially that more than one million deaths per year can be attributed to poor water, sanitation and hygiene conditions. To reduce this number, one of the United Nations Millennium Development Goals (MDGs) is to halve, by 2015, the proportion of the population without sustainable access to safe drinking water and basic sanitation. Despite the enormous potential, surprisingly few studies have tried to quantify the associated health effects (Caincross *et al.*, 2010). The few observational studies of adequate quality available are focussing consistently either on diarrhoea or STHs.

A contaminated domestic environment, which is exacerbated by open defecation, affects both animal and human at the same time.

Community Led Total Sanitation (CLTS) schemes have already been implemented in certain regions of Zambia, namely Choma, but data quantifying the impact of such schemes on measurable variables such as disease prevalence are lacking. It is hoped that CLTS will eradicate the practice of open defecation and ultimately encourage rural communities to achieve an open defecation free (ODF) status. This will reduce the access of human excrement to pigs and thereby reduce the rate of porcine cysticercosis infections. It should be noted that the effect on human health varies and is hard to measure. It can vary from asymptomatic to death at various stages; the most definitive diagnosis being neurocysticercosis-NCC-which manifests as Epilepsy, Spinal Cysticercosis or Paraplegia (Parmar *et al.*, 2001). It is however hoped that this program will in turn (with a predicted prolonged lag time) impact on prevalence of human cysticercosis and human taeniasis.

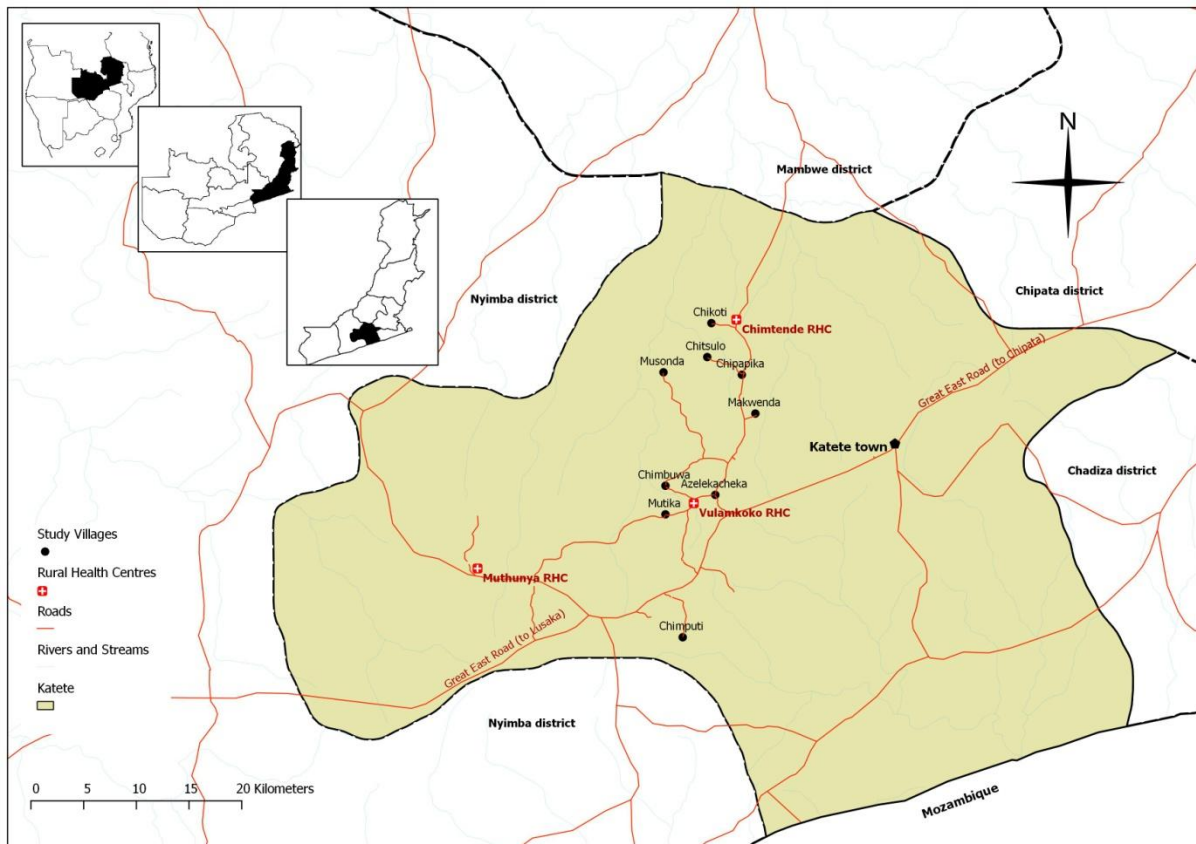
## CHAPTER THREE: MATERIALS AND METHODS

### 3.1 Study area

The Eastern province of Zambia occupies a triangular wedge between the Zambezi Valley to the south and the Luangwa Valley to the north. It lies between latitudes 10°S and 15°S and between longitudes 30°E and 33°40'E. The study was conducted in Katete District (Figure 3.1) situated between 31°47' and 31°55' E and between 31°55' and 14°12'S. The rainy season in this province starts in November and ends in April. Annual rainfall from 2005 to 2012 in Katete ranged from 593.9mm to 1104.4mm (mean 908.7mm). Mean annual maximum temperatures in the same period ranged from 29.1°C to 30.2°C (mean 29.4°C) in the district, while the mean annual minimum temperatures ranged from 16.9°C to 18.4°C (mean 17.5°C). The vegetation in Eastern province includes “Miombo” woodland dominated by *Brachystegia* and *Julbernardia* species, the “Munga” woodland, where the principal tree species are *Acacia*, *Combretum* and *Terminalia* and the “Mopane” woodland with *Colophospermum mopane* being the dominant tree species.

Katete District is one of the districts in the initial UNICEF/Ministry of Local Government Project-Community Led Total Sanitation (CLTS) Programme. For this study two catchment areas were chosen namely Vulamkoko and Chimtende from which nine villages namely; Musonda, Chimputi, Mutika, Chimbuwa, Chitsulo, Chikoti, Azelekacheka, Makwenda and Chipapika were chosen for this cross sectional survey based on their pig population, predominance of free range pig husbandry, observation of cysts in pigs slaughtered in backyards and reported high prevalence of porcine cysticercosis 19.6% (Sikasunge *et al.*, 2008) and human taeniasis 11.6% (Mwape *et al.*, 2013) in the district. The pigs are left free roaming in search of feed during the daytime, confined at night and usually kept for local

consumption.



**Figure 3.1.:** Map of Katete District showing study area (Source: Dr Chitwambi Makungu., 2014)

### 3.2 Study Population

According to the 2010 Central Statistics Report, the Eastern province has a total population of 1,592,661 with 87.4% (1,391,986) in rural areas, 12.6% (200,675) in urban areas and a population density is 30.9 people/ km<sup>2</sup>. Katete District has a total population of 234,585 with 91.31 % (214,218) in rural areas and 20,367 (8.7%) in urban areas. The Vulamkoko and Chimtende Rural Health Centres (RHC) provide health care in these communities with a total catchment population of 25,553 and 22,846 (clinic headcount records) respectively. Agriculture employs 90.1% of the economically active population with an increase in pig rearing from 63,627 in 2003 to 89,467 in 2010. (PHS 2001/2 Small and Medium Scale Index Table, Zambia 2010 Census of Population and Housing Descriptive Tables). According to personal communication with the Katete Department of Veterinary Services; the 2013 pig census in the area estimates the current pig population at 65,865. Approximately 98% of all pigs are reared in small-scale household settings. In Eastern province, pigs are normally kept in small shelters or Kraals called *makola* (*kola* = singular) during the rainy season and are left free to scavenge during the dry season.

### 3.3 Study Design

The study was a comparative cross-sectional study nested in an Integrated Control of Neglected Zoonoses (ICONZ) project which is evaluating the CLTS Program in Katete District using *Taenia solium* in human and pig populations as output indicators.

- a) ICONZ project: Cluster Randomised Trial design in which the unit of randomization was the village and the unit of analysis will be the individual person or pig. Villages were matched into pairs based on STH prevalence. The villages within each pair were randomized as an intervention or control using a randomising table. There are 11

villages for the intervention and 11 villages for the control. The baseline survey was conducted in April of 2012.

Villages were included in the study on their willingness to collaborate, accessibility by road all year round even during the wet season, no current promotion of water, sanitation or hygiene programs, a rural setting, a minimum of 10 pig-keeping households (HHs) and a minimum of 25 HHs.

- b) Cross-sectional study: This study was conducted in December, 2013 in only nine of the intervention villages. The study was conducted by comparing variables of interest from the same villages before and after CLTS as an intervention had been implemented. This study was implemented at the 8 month post- intervention stage and was part of the evaluation and monitoring of the ICONZ project. Pig serum was sampled to the seroprevalence of porcine cysticercosis and a household questionnaire was administered to household heads or a valid proxy (household member aged above 18 years) to obtain information on general household characteristics, pig management and sanitation practices. The following eligibility criteria was used:

### **3.3.1 Inclusion Criteria**

Households that gave written consent to participate in the study, a respondent must have been resident in the household and aged between 18 – 80 years of age. All pigs had to be older than three months in selected household. A village must have been CLTS triggered by at least 8 months. It also had to have a champion (i.e. trained CLTS local leader to monitor the building of toilets) available.

### 3.3.2 Exclusion Criteria

Households that did not to give consent to the study, households that did not have an adult aged 18 years or above available, a respondent who did not live in the household, piglets younger than three months (to avoid false positives due to passive immunity acquired from the mothers) and pregnant sows (to avoid stress to the sows which could lead to abortion).

### 3.4 Pig Cross - sectional Survey

#### 3.4.1 Pig selection and sample size calculation

Qualified veterinary personnel from the School of Veterinary Medicine of the University of Zambia were recruited for the pig blood collection. Additional veterinary assistance was sought from the District Veterinary Office. A modified simple proportion formula developed by Cochran in 1963 was used to determine the difference in the prevalence of porcine cysticercosis in the intervention villages after 8 months of follow up with a power of 80% and a confidence level of 95%.

The standard formula required for a total of **n** is as follows:

$$n = Fp(1-p) / d^2$$

Where:

- n**        Approximate random sample (size) to be followed
- p**        Proportion in the absence of the intervention
- d**        allowable error
- F**        F value

Given that:  $p = 0.155$  (2013 ICONZ baseline prevalence data prevalence = 15.5%); F-value at



80% power and 0.05 level of significance = 7.85 and d = maximum tolerable error for the prevalence estimate = 0.05. Thus approximately 411 pig samples were required for post intervention sampling.

**Table 3.1.:** F-values at different power and levels of significance to detect a difference between groups of a specified sample size in a corresponding population

Significance level	Power			
	80%	90%	95%	99%
0.1	6.19	8.56	10.82	15.77
0.05	7.85	10.51	12.99	18.37
0.025	9.51	12.41	15.10	20.86
0.01	11.68	14.88	17.81	24.03

### 3.4.2 Pig Blood sample collection and storage

The blood samples were obtained from the cranial vena cava into plain blood collecting tubes and allowed to clot in a cool box. On submission the clotting blood samples were preserved on ice. The clotted blood was then separated by centrifugation at 3000 rpm for 15 min to obtain sera. The sera was apportioned into 2 ml aliquots and stored in labelled vials. The aliquots were then stored at -20°C for laboratory testing. Cysticerci antigens were tested for in sera by a monoclonal antibody-based sandwich Enzyme-linked immunosorbent (Ag-ELISA) as described by Brandt *et al.*, (1992) and Dorny *et al.* (2004). The serum was pre-treated before analysis for presence of cysticerci antigens. A pig was categorized as positive for cysticercosis if they tested positive for Ag-ELISA.

### 3.4.3 Pre-treatment of pig sera for Ag-ELISA

The B158/B60 Ag-ELISA for the detection of cysticerci antigens in serum was applied as a cysticercosis diagnostic test during this survey. It was 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. Two hundred µl of this mixture at final sera dilution of 1:4 was used in the Ag-ELISA protocol.

#### **3.4.4 Enzyme-linked-immunosorbent assay for the detection of circulating *T. solium* cysticerci antigens (Ag-ELISA) in serum of pigs**

The Ag-ELISA 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 ELISA plates (Nunc<sup>®</sup> Maxisorp). Monoclonal antibody B158C11A10 was used as 1<sup>st</sup> MoAb and was followed by a biotinylated MoAb B60H8A4 as the detector antibody (2<sup>nd</sup> 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 Phosphate buffer saline Tween 20 (PBS - T20) and dried by beating the plates vigorously on blotting paper. Blocking to avoid non-specific reactive sites was done by adding 150  $\mu$ l of PBS - T20/1% New Born Calf Serum (NBCS) -a 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  $\mu$ l of pre-treated sera, including the weak and strong positive and negative controls, at a dilution of  $\frac{1}{4}$  was added to each well (except for the SC and conjugate control (CC) wells where 100  $\mu$ l 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  $\mu$ l of Biotinylated MoAb B60H8A4 diluted at 1.2 $\mu$ g/ml in blocking buffer to each well (except for the SC wells where 100  $\mu$ l 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  $\mu$ l 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  $\mu$ l of H<sub>2</sub>O<sub>2</sub> was added. To each of the wells was then added 100  $\mu$ l of this solution and incubated at room temperature in the dark without shaking for 15 minutes. To stop the reaction, 50  $\mu$ l of 4N H<sub>2</sub>SO<sub>4</sub> was added to each well. The plates were read using an automated spectrophotometer (Tecan Sunrise) 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). 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). A serum sample was considered as positive when the ratio (optical density of test sample/optical density cut-off) was  $\geq 1.0$  (Dorny *et al.*, 2004). In addition to the above a questionnaire was also used in order to determine the general household characteristics, pig management and sanitation practices of the farmers.

### **3.5 Human Survey-Household Questionnaire for KAP**

In addition to the above a household questionnaire was administered in order to determine the general household characteristics, pig management and sanitation practices of the farmers. The households who own pigs were identified using the snowballing technique (Sikasunge *et al.*, 2006; Pondja *et al.*, 2010). Snowballing is a technique for developing a research sample where existing study subjects recruit future subjects from among their acquaintances (Salganik and Heckathorn, 2004). Trained personnel were used to administer the questionnaires in the local language *Chichewa*. Upon obtaining informed written consent; the household questionnaire was administered to the household head (HH) or, if not available, any member above 18 years addressing the collection of data on variables of interest on the knowledge, attitudes and sanitation practices (KAP) as well as pig rearing and husbandry habits in study communities (see Table 3.1) . All the questionnaires were coded and kept anonymous to protect participants' details; they were also locked in a safe and secure place. Analysis was conducted by comparing the current results with the ICONZ baseline survey already conducted in April and May of 2012. This study involved the use of secondary data from the baseline ICONZ data already collected and comparing it with the primary 8 months post-intervention data collected in this study. The questionnaire used for primary data collection was a subset of the one used for the secondary data. This is because the latter was

also looking at economic factors which were not needed for this study.

### **3.6 Ethical Considerations**

The study sought ethical approval from Excellence in Research Ethics and Science Converge Institutional Review Board (Ref No.:2013-June-010). Further approval was sought from The Ministry of Agriculture and Livestock, Department of Veterinary Services (Ref.: DVS/53/2/10), local district veterinary authorities and local community leaders. Meetings were held with the community leaders to explain the purpose of the study and request their permission to conduct the study in their area. Finally written consent was sought from the individual participants in the households to take part in the questionnaire study; stating the nature and purpose of the study.

### **3.7 Data Analysis**

The data collected in the household survey was entered into EpiData v3.1 and exported to Stata for Windows version 11.0 (Stata Corp; College Station, TX, USA, 2009) for statistical analysis. The data collected for the pig samples was analyzed using the Student T-test in Microsoft Excel 2007 and exported to Stata for Windows version 11.0 (Stata Corp; College Station, TX, USA, 2009) for further statistical analysis. Univariate analysis was conducted to obtain the frequencies for cysticercosis seroprevalence, household and pig management characteristics. In the case of the secondary baseline data it was performed in only nine of the intervention villages. Then pig and household survey data from both arms of the study were then combined and matched to only the nine intervention villages sampled for further analysis.

The Wald Test statistical test was used as a pair-wise comparison of the variables of interest in both arms of the study. This was applied to the dependant variable - proportions of the

positive cysticercosis and independent variables-transmission risk factors i.e. pig management, pig keeping practices, home slaughter, sanitation practices and cysticercosis awareness in pigs .The Chi-Square test was used to assess the association between porcine *T. solium* infection and the sex of the pig and stepwise regression analysis to adjust for confounding factors. The levels of significance were set at  $p \leq 0.05$  for all statistical analyses and confidence intervals were set at 95% level.

**Table 3.2.:** Variables of study

VARIABLE	INDICATOR	TYPE	MEASURE OF INTEREST
<b>Pig Data</b>			
<b>Dependent Variable</b>			
Positive porcine cysticercosis	Positive (1= Positive, 0= Negative)	Proportions/Percentages	Categorical
<b>Independent Variables</b>			
Sex	(1= male, 0= female)	Proportions/Percentages	Categorical
<b>Household Survey Data (Questionnaire)</b>			
Household Head Sex	(1= male, 0= female)	Proportions/Percentages	Categorical
Household Head Age	1=20-29 2=30-39 3=40-49 4=50-59 5=>60	Proportions/Percentages	Categorical
Level of education	0= None, 1 = Primary School 2 =Middle School ,3 = High School" 4 =Tertiary education	Proportion/Percentages	Categorical
Occupation	1=Crop farming, 2=Livestock 3= Paid employment 4= Other Business	Proportions/ Percentages	Categorical
Pig Herd Size	1= 0-3 2= 4-6 3= 7-10 4 = >11	Proportions/Percentages	Categorical
Pig Husbandry System	1= indoor ,2= semi-intensive 3= Free-range,4= tethering	Proportions/Percentages	Categorical
Pig Feeding	1=Crop Residues, 2=Maize Bran 3=Sunflower Cakes 4= Seasonal Fruits	Proportions/Percentages	Categorical
Consume Pork	(1= Yes, 0= No)	Proportions/Percentages	Categorical
Home Slaughter of Pigs	(1= Yes, 0= No)	Proportions/Percentages	Categorical
Knowledge of cysticercosis	(1= Yes, 0= No)	Proportions/Percentages	Categorical
Observed cysticercosis in pork?	(1= Yes, 0= No)	Proportions/Percentages	Categorical
Sell meat with cysticercosis	(1= Yes, 0= No)	Proportions/Percentages	Categorical
Observed epilepsy in communities	(1= Yes, 0= No)	Proportions/Percentages	Categorical

Presence and usage of latrines (assessed by direct observation)	1=Present and used ,2=present but NOT used ,3=absent	Proportions/Percentages	Categorical
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## CHAPTER FOUR: RESULTS

### 4.1 Baseline Results

#### 4.1.1 Prevalence of porcine cysticercosis at baseline:

A census of the villages gave a total of 344 pig-owning households of which a total of 64 were sampled. Of the **overall (n=104)** pigs examined 14 (13.5) % were found positive using the Ag-ELISA, see Table 4.1

**Table 4.1: Prevalence of porcine cysticercosis on Ag-ELISA at baseline in Katete**

<b>District</b>				
<b>Village</b>	<b>Pig owning HH</b>	<b>(n)pigs</b>	<b>Ag-ELISA +ve (%)</b>	<b>95% C.I</b>
Chipapika	9	19	2 (10.5)	10.7-25.8
Chikoti	3	7	1 (14.3)	1.8-11.6
Chitsulo	4	12	0 (0.00)	-
Makwenda	11	16	2 (12.5)	8.3-22.4
Musonda	11	15	2 (13.3)	7.6-21.3
Chimbuwa	7	7	4 (57.1)	1.8-11.6
Chimputi	8	14	2 (14.3)	6.8-20.1
Mutika	5	8	1 (12.5)	2.5-12.9
Azele kacheka	6	6	0 (0.0)	-
<b>TOTAL</b>	<b>64</b>	<b>104</b>	<b>14 (13.5)</b>	<b>6.8-20.1</b>

#### 4.1.2 Socio-demographic Characteristics of Households:

A total of 64 households in the intervention villages were visited with 16 (25%) male and 48 (75%) female respondents. On average each household had between 4 to 6 members with a mean number of individuals in each household was determined as 6. The highest number of

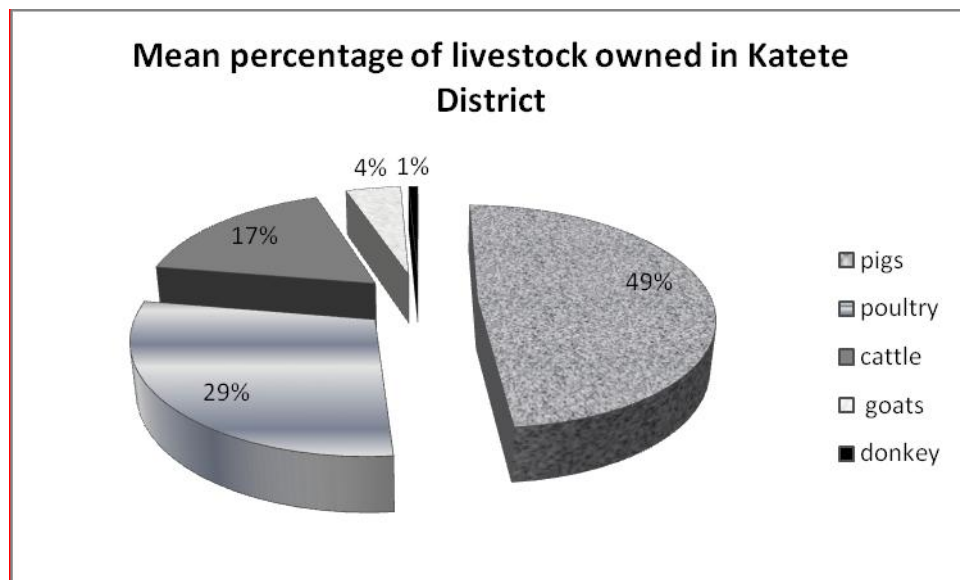
people in a household recorded was 10 and the lowest 1. The majority of the people in these households had only attained primary school education (62.5%), with none reaching tertiary school education. Most of the households interviewed depend on crop farming (79.7%) as their main source of income and within that population (20.3%) supplemented their income with livestock farming, formal employment and some form of business (Table 4.2). The mean household assets show that pigs (Figure 4.1) were the most common livestock owned with (33.9%) of the households in Katete District owning a pig herd size of 3 pigs per household.

**Table 4.2: Baseline Household Characteristics in Katete District from 64 household heads**

<b>Characteristic</b>	<b>Study population n (%)</b>
<b>Sex</b>	
Female	48 (75)
Male	16 (25)
<b>Distribution by Age Group</b>	
20-29	8 (12.5)
30-39	23 (35.9)
40-49	15 (23.4)
50-59	9 (14.1)
>60	9 (14.1)
<b>Mean Household Size</b>	
1-3	8 (12.5)
4-6	30 (46.9)
7-9	24 (37.5)
≥10	2 (3.1)
<b>Level of Education</b>	
None	13 (20.3)
Primary	40 (62.5)
Middle	9 (14.1)
High	2 (3.1)
Tertiary	0 (0.0)
<b>Main Income</b>	
Crop farming	51 (79.7)
Livestock	6 (9.4)
Other business	7 (10.9)

### 4.1.3 Pig Management System:

The percentage of livestock owned per household indicate that pigs (Figure 4.1) are the most common (49%); with (33.9%) of the households in Katete District owning a pig herd size of 1-3 pigs per household. From the total of 64 respondents 40 (67.8 %) kept pigs on free-range, 18 (30.5 %) were kept in pens during the crop planting season; whilst 43 (72.9 %) kept pigs in free-range, 6 (10.2 %) were kept in pens in the crop growing season respectively. In the crop harvest and dry seasons, the majority of the pigs are kept free-range 53 (89.8 %) and only 6 (10.2 %) were kept in pens (Table 4.3).



**Figure 4.1:** The percentage of livestock owned per household in Katete District (note pigs are the most common)

**Table 4.3: Baseline Characteristics of Pig Management in Katete District from 59****household heads**

<b>Characteristic</b>	<b>Number of respondents (%)</b>
<b>Pig Herd Size</b>	
0-3	20 (33.9)
4-6	15 (25.4)
7-10	13 (22.0)
>11	11 (18.6)
<b>Pig Husbandry System</b>	
<b>Planting Season</b>	
In Pens	18 (30.5)
Free range	40 (67.8)
Tethered	1 (1.7)
<b>Crop Growing Season</b>	
In Pens	16 (27.1)
Free range	43 (72.9)
Tethered	0 (0.0)
<b>Crop Harvest Season</b>	
In Pens	6 (10.2)
Free range	53 (89.8)
Tethered	0 (0.0)
<b>Pig Feed</b>	
Crop Residues	39 (66.1)
Maize Bran	27 (45.8)
Kitchen leftovers	21 (35.6)
Commercial feed	22 (37.3)
Sunflower cake	6 (10.2)
Seasonal fruits	7 (11.9)

## 4.2 Eight Months Post-Intervention Results

### 4.2.1 Prevalence of Porcine Cysticercosis

In the post-intervention survey a total of 275 pigs were examined in 9 of the intervention villages. Of the total pigs examined 45 (16.4 %) were found to be positive on Ag-ELISA. The data is presented below (Table 4.4).

**Table 4.4: Prevalence of porcine cysticercosis on Ag-ELISA eight months post-intervention in Katete District**

Village	Pig owning HH	pigs (n)	Ag-ELISA +ve (%)	95% C.I
Chipapika	11	38	10 (13.8)	9.7-17.9
Chikoti	09	41	8 (14.9)	10.7-19.1
Chitsulo	14	42	5 (15.3)	11.0-19.6
Makwenda	10	32	6 (11.6)	7.8-15.5
Musonda	10	31	2 (11.3)	7.5-15.0
Chimbuwa	10	23	3 (8.4)	5.1-11.7
Chimputi	05	16	5 (5.8)	3.0-8.6
Mutika	10	20	1 (7.3)	4.2-10.4
Azelekacheka	10	32	5 (11.6)	7.8-15.6
<b>TOTAL</b>	<b>89</b>	<b>275</b>	<b>45 (16.4)</b>	<b>12.0-20.8</b>

### 4.2.2 Socio-demographics Characteristics of Households

A total of 89 households in the intervention villages were visited with a total of 47 (52.8%) male and 42 (47.2%) female respondents. On average each household had between 4 to 6 members with a mean number of individuals in each household was determined as 7. The highest number of people in a household recorded was 13 and the lowest 1. The majority of the people in these households had only attained primary school education (57.3 %); with none reaching tertiary school education and about (98.9 %) of the households interviewed

depend on crop farming.

**Table 4.5: Post-Intervention Household Characteristics in Katete District from 89 respondents**

<b>Characteristic</b>	<b>Study population n (%)</b>
<b>Sex</b>	
Female	42 (47.2)
Male	47 (52.8)
<b>Distribution by Age Group</b>	
20-29	27 (30.3)
30-39	27 (30.3)
40-49	15 (16.9)
50-59	10 (11.2)
>60	10 (11.2)
<b>Mean Household Size</b>	
1-3	4 (4.6)
4-6	41 (46.6)
7-9	33 (37.5)
≥10	10 (11.4)
<b>Level of Education</b>	
None	16 (18.0)
Primary	51 (57.3)
Middle	15 (16.9)
High	7 (7.9)
Tertiary	0 (0.0)
<b>Main Income</b>	
Crop farming	88 (98.9)
Livestock	1 (1.1)
Other business	0 (0.0)

### 4.2.3 Pig Management System

Our results indicate that 33 (37.0 %) of the households interviewed had a pig herd size of 7-10 pigs. From the total of 89 respondents 27 (30.3 %) kept pigs free-range, 23 (25.8 %) in pens and 39 (43.8 %) were kept in a semi-intensive system of free range during the day and in pens at night. It must be noted that this was during the crop planting season. Most of the farmers practiced semi-intensive management system by enclosing pigs at night and leaving them to scavenge during the day (Figure 4.1). The pigs when enclosed were generally kept in small shelters called *kola* (multiple = *makola*) in *Ichichewa* (Figure 4.2). The pigs were given supplementary feeding of agricultural by-products such as maize bran locally known as *gaga* and sunflower cake (Figure 4.3 and Table 4.6).

**Table 4.6: Post-Intervention Characteristics of Pig Management in Katete District from 89 household heads**

<b>Characteristic</b>	<b>Number of respondents' n (%)</b>
<b>Pig Herd Size</b>	
0-3	11 (12.4)
4-6	26 (29.2)
7-10	33 (37.1)
>11	19 (21.4)
<b>Pig Husbandry System</b>	
<b>Planting Season</b>	
In Pens	23 (25.8)
Free range	27 (30.3)
Semi-intensive	39 (43.8)
<b>Pig Feed</b>	
Crop Residues	0 (0.0)
Maize Bran	88 (98.9)
Kitchen leftovers	0 (0.0)
Commercial feed	0 (0.0)
Sunflower cake	11 (12.4)
Seasonal fruits	5 (5.6)





**Figure 4.2:** Scavenging pigs on free-range as seen in Chipapika village in Katete District  
(Note: background bushes which could easily serve as toilets for the villagers).



**Figure 4.3:** Pig enclosed in a *kola* in Chimbuwa Village, Katete District



**Figure 4.4:** Makwenda Village-feeding time with maize bran locally known as *gaga* (Note: pigs, dogs, and sheep).

### **4.3 Comparison of Results before and after CLTS**

#### **4.3.1 Porcine Cysticercosis Prevalence**

Overall, there was an insignificant difference in the sero-prevalence of porcine cysticercosis in pigs before (13.4%) and after (16.4%) CLTS using Ag-ELISA. The Wald test (to test the true value of the parameter based on the sample estimate = prevalence) was applied to the positive samples from both intervention periods yielded a p-value of 0.473, showing that the result was not significant. The Ag-ELISA results at baseline show evidence that male pigs had a higher prevalence of 6 (15.4%) compared to female pigs 7(11.5%) ;( $\chi^2 = 26.34$ ;  $p = 0.00$ ), however evidence from the post-intervention indicated no significant difference in male pigs 14 (21.5%) in comparison to female pigs 31 (14.8%) ;( $\chi^2 = 1.67$ ;  $p = 0.197$ ). (Table 4.7)

**Table 4.7: Comparison of prevalence of porcine cysticercosis on Ag-ELISA and by sex pre and post CLTS Intervention**

<b>Variable</b>	<b>Baseline (%)</b>	<b>Post-intervention (%)</b>	<b>p-value</b>
Overall Prevalence (n <sub>0</sub> =104, n <sub>1</sub> =275 ) <sup>a</sup>			
Positive	13.5	16.4	0.47*
<b>Sex</b>			<b>p-value</b>
Male (n <sub>0</sub> = 36, n <sub>1</sub> = 65 ) <sup>a</sup>			
Positive	15.4	21.5	0.00**
Female(n <sub>0</sub> = 61, n <sub>1</sub> = 210) <sup>a</sup>			
Positive	11.5	14.8	0.19**

<sup>a</sup> n<sub>0</sub> means the number of pigs before CLTS

n<sub>1</sub> is the number of pigs after CLTS in 9 of the intervention villages

\*Wald Test

\*\*Chi-Square Test

#### **4.3.2 Investigation of Awareness and Risk factors of Cysticercosis:**

The assessment of the response rates from the human survey pre and post-intervention were 19% and 26% respectively. (Appendix 3) .The individual village response rates are also indicated and they show marked increase due to the higher number of households sampled. Comparison of awareness and risk factors were assessed using the Wald Test on the same type of questions from both arms of the study (Table 4.8).

With regards to whether the respondents had ever heard of porcine cysticercosis or seen porcine cysticercosis infected pork, a majority of the respondents (over 80%, p-value=0.441) in either arm of the study stated they had. Furthermore a substantial proportion of respondents at baseline (70.9%) were more aware of cysticercosis as a pig disease as compared to those post-intervention (43.2%) with a p-value=0.001, whereas 56.8% had completely no idea of what cysts seen in pork were .Our study also revealed significant

difference with home slaughter of pigs (p-value=0.0001) and pork consumption (p-value=0.003). The results were also insignificant (p-value=0.679) to residents who sell pig meat with cysts. A significant number of respondents in both arms of the study think that African Swine Fever is the most important pig disease that they encounter in comparison with cysticercosis and other pig diseases. It was also noted that post-intervention (96.4%) of the respondents had observed someone in their community with epilepsy. In addition, all of the respondents (100 %) depended on borehole water as their water source.

**Table 4.8:** Comparison of proportions of risk factors and awareness variables before and after CLTS intervention in Katete District

Variables	Baseline (%)	Post-intervention (%)	P-value
Observed cysticercosis in pig meat (n <sub>0</sub> = 64, n <sub>1</sub> = 89 ) <sup>a</sup>			
No	14.1	11.2	
Yes	84.4	88.8	0.441*
Missing	1.5	-	
Knowledge of what pig cyst is (n <sub>0</sub> =55, n <sub>1</sub> = 81 ) <sup>a</sup>			
Don't know/Other	29.1	56.8	
Pig Disease	70.9	43.2	0.001*
Practise home slaughter of pigs(n <sub>0</sub> =59, n <sub>1</sub> = 89 ) <sup>a</sup>			
No	50.9	19.1	
Yes	49.2	80.9	0.0001*
Do family members consumed pig meat (n <sub>0</sub> =29, n <sub>1</sub> = 88 ) <sup>a</sup>			
No	34.1	12.8	
Yes	65.5	87.2	0.003*
Sells pig meat with cysts (n <sub>0</sub> = 61, n <sub>1</sub> = 89) <sup>a</sup>			
No	85.3	87.6	
Yes	14.8	12.4	0.679*
The disease problem considered (n <sub>0</sub> = 59, n <sub>1</sub> = 89) <sup>a</sup>			
African Swine Fever	52.5	87.6	0.000*
Cysticercosis	23.9	0.00	0.003*
Other pig diseases	23.6	12.4	
Observed Epilepsy within the community(n <sub>0</sub> = 64, n <sub>1</sub> = 89) <sup>a</sup>			
No	84.4	3.4	
Yes	14.1	96.6	0.00*
Missing	1.5	-	

<sup>a</sup> n<sub>0</sub> means the number of respondents before CLTS while n<sub>1</sub> is the number of respondents after CLTS \*Wald Test

### **4.3.3 Presence and Utilization of Latrines:**

The presence of latrines was assessed from direct observation when administering the questionnaire and is based on individuals who participated in the survey. The pre and post-intervention were 43 (67.2%) and 74 (83.1%) respectively, showing an increase of 31 latrines constructed in the intervention villages. This was statistically significant with the Wald test giving a p-value of 0.027. The proportion of latrine use was at 41 (93.2%) at baseline and 62 (84.9%) post-intervention giving a net increase of only 21 latrine. This means that there had only been a 33.9% increase in toilet usage and this was statistically insignificant (p-value = 0.151). This fact was further confirmed when administering the questionnaire by direct inspection of the latrines by the research assistants. Figures 4.5 (A and B) point out that the intervention is still on-going with the construction of latrines.



**A**



**B**

**Figure 4.5:** Covered pit latrine hole and bricks ready for construction in Mutika Village (A) and a burnt brick and log latrine (B) under construction in Chimputi Village (champion inspects the structure)

## CHAPTER FIVE: DISCUSSION

The present study has confirmed that the seroprevalence of porcine cysticercosis before (13.5%) and after (16.4%) CLTS was statistically insignificant ( $p$ -value=0.473) in the study area. The difference in prevalence rates obtained in this study could arise from the wider household survey coverage at post-intervention (89) compared to pre-intervention (64) ;a larger pig sample size in the post-intervention study (275) compared to baseline survey (104) in the intervention villages and the inclusion of pigs above the age of one in the post intervention. The inclusion of older pigs which may have had repeated exposure to infection may have also led to a higher prevalence.

After comparing prevalence by gender, there was evidence to suggest that a higher prevalence at post intervention in male pigs 6(15.4%) compared to female pigs 7(11.5%) with Ag-ELISA ( $\chi^2 = 26.34$ ,  $p = 0.00$ ). Sikasunge *et al.*, 2007 recorded similar findings, which is in agreement with the present findings. This finding also agrees with that of Sarti *et al.* (1992) who found that the prevalence of cysticercosis was slightly higher in male than in female pigs and that it increased with age. However, evidence from the post-intervention indicated no significant difference in males 14(21.5%) in comparison to females 31(14.8%); ( $\chi^2 = 1.67$ ;  $p = 0.197$ ). The difference in prevalence rates by gender obtained in this study could arise from the smaller number of male pigs (39:65) sampled compared to the female pigs (61: 210) in both arms of the study in the intervention villages only. Our post-intervention results agree with the results obtained by Bokarti *et al.*, (2012) who reported no significant difference between male (9.2%) and female (10.4%) pigs after meat inspection of 978 pig carcasses. Such a result is possible because the number of pigs examined was large compared to our study ( $n = 379$ ) .The higher prevalence in females in this study might be due to the natural



feeding behaviours of females with their litters who loiter in and around human habitats in search of garbage and generally avoid conflict with their aggressive male counterparts. Similar observations were also made by Aluja *et al.*, (1998). Another possibility could be that male pigs tend to move about a lot within the community in an attempt to find partners to mate (pigs on heat). It could also mean that male pigs are genetically more active than female pigs thereby increasing the chances of coming across human faeces contaminated with *T. solium* eggs. This study, therefore confirms that there is a high prevalence of porcine cysticercosis in the surveyed villages despite the on-going intervention.

Our study revealed that the presence of latrines in the study villages had increased from 43 to 74, indicating an increase in latrine construction. This was statistically significant at  $p=0.0185$ , this indicates that the intervention is showing an effect. However, the number of latrines which were present and being used was lower post-intervention ( $p = 0.151$ ) because most latrines are under construction and not being used. This was confirmed when inspecting latrine use, at post intervention there were 62 compared with that at pre intervention 41 giving net increase of only 21 latrines. This means that there has only been a 33.9% increase in toilet usage. This represents negative increase in use of latrines post-intervention. And since almost all toilets were being used (93.2%) the extra increase can be assumed to the new toilets. The low usage of latrines may explain the insignificant difference in prevalence of porcine cysticercosis post-intervention in Katete District. Our study agrees with those carried out by Kagira *et al.*, (2010), where lack of latrines was the only significant risk factor associated with prevalence of porcine cysticercosis, and this is similar to that reported by Mutua *et al.*, (2007). The preliminary unpublished data of the ICONZ project which is evaluating CLTS program in Katete District has revealed that the villagers lacked or had poor building materials for the construction of the latrines. This led to either no construction of

latrines or the poor construction of latrines which subsequently collapsed in the rainy season (ICONZ 2014, Unpublished Data).

The practice of open defaecation can also be attributed to the cultural practices and traditional belief system of the *Chewa* people which influence latrine usage in the Katete District. The most common belief is open use of a latrine is an act that must not be observed by other members of the community. Also not everybody in a household can or will use the same latrine i.e. adults with children or a married couple with in-laws. Therefore going into nearby bush is considered as appropriate behaviour. This was confirmed in anthropological study of the African indigenous knowledge systems (AIKS) of the *Chewa* people in Katete District, whom we quote “...to attend to a call of nature when in my community the only time you just disappear without telling anybody is when you want to answer to the call of nature...” (Banda, 2008). Our study also agrees with those done by Ngowi *et al.*, (2004) and Papafilippou *et al.*, (2010) which state that the introduction of latrines does not guarantee that they will be used with reasons given as foul odors, flies, cockroaches, flooding and prevailing social practices - most notable is the use of pigs as 'sanitary policemen' in free-range systems, taboo's on the use of latrines in communities and that leaching pit latrines were a risk for contaminating a nearby water source.

The conventional approaches on the control of cysticercosis have attempted the issue of poor sanitation by improving coverage and access with support for constructing toilets. It has been shown however; that construction of individual toilets alone does not translate to their usage (Aluja *et al.*, 1982; Ngowi *et al.*, 2004). Behavioural change must occur for any sanitation program to be successful. Sanitation programs have for some time now, incorporated the need to raise awareness and emphasize the benefits of toilet usage. Community Led Total Sanitation (CLTS) as a sanitation program is however targeted at individual demand and does

not necessarily result in significant progress in securing the desired outcome. The outcome in this case is to stop the spread of disease and infection through the bacteriological contamination of water sources and the transmission of pathogens through the faecal-oral route (Planning Commission of India, Mid-Term Appraisal of the Tenth Plan, 2005).

Additionally, results of the pig husbandry system indicated that most the pigs in this area are kept free-range which means they scavenge in the surrounding areas of the villages for food. This means that in an area where taeniasis is endemic, with the practice of open- defecation still rampant; humans may still be shedding tapeworm infected faeces and pigs still have access to infected faeces. This agrees with studies by Sarti *et al.*, (1997) and Rodriquez-Canul *et al.* (1999), which showed that extensively raised pigs (free-range type of husbandry system) had a higher seroprevalence of cysticercosis than intensively raised pigs. It has been observed that defecation in areas where pigs can access the infected human faeces can lead to increased transmission of *T. solium* (Ngowi *et al.*, 2004; Sikasunge *et al.*, 2007; Pondja *et al.*, 2010).

Our study revealed that most pigs within the district are kept free-range across all crop seasons. This may be due to lack of resources such as feed and also the labour involved in confining the pigs in houses. This is in agreement with the results of Eshitera *et al.*,(2012) who found a high prevalence of cysticercosis which was likely related to the popularity of free-range pig keeping, as the vast majority (98%) of pigs where kept under such a system for all or part of the year despite its illegal status. And since not every household has a latrine, the pigs may also serve a secondary role as sanitary-police to maintain villages free from garbage, human and animal faeces. Gilman *et al.*,(1999) reported that a pig can be fed at a little cost by allowing it to roam free in villages or on free farm land and in this way obtain a variety of food to supplement its diet. Krecek *et al.*, (2012) also reported that lack of sanitary

facilities makes for an ideal environment for the transmission of porcine cysticercosis. Studies conducted in Mexico and Cameroon indicate that pig husbandry practices were the main risk factors associated with porcine cysticercosis as free-ranging pigs have a much higher access to human faeces in communities with few or no latrines (Sarti *et al.*, 1997; Assana *et al.*, 2010). This is however in contrast with a study done by Pondja *et al.*, 2010 where despite 94.8% of the household having latrines; the prevalence of porcine cysticercosis did not differ in households without a latrine when compared to those with a latrine. The lack of association between latrines and porcine cysticercosis might have been due to the fact that pigs were allowed to roam freely in both households with and without latrines.

From observation and the baseline secondary data it was noted that there are no pig abattoirs, and there is little inspection of porcine carcasses by the veterinary or medical personnel in the study area. Many pigs were slaughtered for consumption or home income (65.5%) or sold to customers on demand without any carcass inspection. Carcass inspection was conducted by traders (69.0%) from nearby towns who buy the pig at an under-valued price if cysts are present. Under this kind of situation where the evidence of porcine cysticercosis has been demonstrated, humans are at risk of getting taeniasis even in areas where pigs are not reared.

Our study indicated that the mean number of years residents in the study villages have kept pigs is 21; this means that pig-rearing is common within the area. The fact that most pigs are kept free-range; the area lacks latrines and has no pork inspection leads to frequent infection of the pigs and subsequent infection of humans. Older pigs acquire more frequent infection through consumption of infected faeces and this leads to immunity development. It must be noted that the indigenous pigs known as the black dwarf or *Nsenga* which are predominant in Eastern province are more resistant to *T. solium* infection than the commercial breeds found in pig farms in urban areas. Their immunity to cysticercosis is evident by the calcification of

their cysts when dissected (Phiri *et al.*, 2002; Sikasunge *et al.*, 2006).

Results from our study show that over 50% of the respondents in both arms of the study had seen cysticercosis, however only (29.6 %, *p-value* <0.01) were aware of what it was and this is only in the post-intervention arm. Also many of the respondents had knowledge that cysticercosis is a pig disease (*p* = 0.001) but do not know that public health implications of the disease. This is because most of the respondents thought African Swine Fever (ASF) is the most common pig disease. This can be attributed to the high mortality rate of ASF especially in the hot / dry season when the disease is most common in sub - Saharan Africa (Penrith and Vosloo, 2009). The study also revealed that other risk factors of cysticercosis like home slaughter, consumption and selling of “measly” pork are still common practice. The locals called cysts in pork as *mushokwe*, *mase*, *masese* which means literally beer dregs. This is due to the fact that cysts look like the dregs of the locally brewed beer which the pigs are sometimes fed on. Additionally, the majority of the respondents had little or no formal education, aged between 20 – 36 years and farming was the main source of income. Though the male to female ratio of respondents was 47/42 and 16/48. These socio-economic factors of the respondents may be linked to the low level of knowledge of cysticercosis by the respondents.

Our findings agree with conducted by Maridadi *et al.*, (2011) who had 75% of their respondents having heard of porcine cysticercosis and seen porcine cysticercosis infected pork. The study also revealed that the knowledge on life cycle of *T. solium* was significantly influenced by sex, age, education level and tribe/ethnicity of the farmer. Male respondents (farmers) were more likely to have proper knowledge on life cycle of *T. solium* than females, similarly, for more educated farmers compared to less educated one, and for older farmers compared to younger farmers This study agrees with others that reported that the proper

knowledge of the *T. solium* cysticercosis- taeniasis complex by the community is a key factor for its eradication through the development of proper eradication strategies (Sarti and Rajshekhar, 2003; Ngowi *et al.*, 2008). Though home slaughter and pig consumption were statistically significant; the difference can be attributed to higher number of respondents in the post-intervention survey. The fact that no health education was conducted in the area means that most risk factors except for latrine presence would show insignificant differences.

The difference in awareness of epilepsy was statistically significant ( $p\text{-value} < 0.01$ ). This difference in awareness could be attributed to people in the community being actually aware that the following signs i.e. loss of consciousness, foaming of the mouth, tongue biting and convulsions can be attributed to epilepsy. The fact that most of the respondents had heard of someone suffering from epilepsy in their communities is suggestive that there is widespread human cysticercosis and/or neurocysticercosis in these surveyed villages as evidenced by studies done by Mwape *et al.*, (2013). This allows for speculations that epileptics in these communities could be stigmatised. Birbeck (2000), in her studies on prevalence of epilepsy and febrile seizures in a rural Mission Hospital in Chikankata in Southern province of Zambia, found that epilepsy and febrile seizures were responsible for a significant burden of disease in this part of Zambia. According to surveys of Preux *et al.* (2000) in West Cameroon only 27% of epileptics get married and 39% fail to enter into any professional activity. Birbeck, (2000) further reported that serious medical complications often result from seizures, especially if untreated for greater than 2 years. According to Birbeck (2000), patients with epilepsy had significantly less education than their sex-matched siblings and that there was some evidence that epilepsy is underreported, under-recognized, and undertreated in that population.

The transmission dynamics of taeniasis and cysticercosis are poorly understood by most

communities at risk especially with regard to the relationship of human and porcine life cycle under field conditions (Garcia *et al.*, 1998). Although the pig is the essential intermediate host for *T. solium*, little attention has been paid to eradicate the disease despite documented risk factors. *T. solium* is progressively being recognized as an important parasite at the global level, to date scientists in several developing countries endemic for the parasite are struggling to bring the disease to the national attention. Failure of endemic countries to recognize the importance of the parasite is attributed partly to lack of knowledge by stakeholders on the presence, magnitude and impacts of the parasite. And because *T. solium* cysticercosis is an endemic rather than epidemic disease it is difficult to appreciate its impact, especially to non-professional policy makers. Political will is important for countries to allocate necessary resources to solve community related problems such as cysticercosis and promote intersectoral collaboration towards its control (Zinsstag *et al.*, 2005).

It should also be noted that the intervention still ongoing in Katete District and this can lead to confounding. The triggering of CLTS only commenced in August of 2012 and this means that latrines are still being constructed and not every household has access to a latrine. The absolute numbers indicate more latrines present but they do not necessarily translate into them being actually used. This means that open-defecation is still being practised and transmission has not been cut.

## Study Limitations

1. This study nested in an Integrated Control of Neglected Zoonoses (ICONZ) project which is evaluating the CLTS Program currently ongoing in Katete District. This evaluation is being conducted via Cluster Randomized Control Trail in which 11 villages were controls and 11 villages were interventions. The quality and quantity of the baseline data in comparison with the post – intervention data may have been affected by this fact.
2. Nine villages were chosen for this study based on the ones that had undergone CLTS successful triggering at eight months and had some form of follow-up. This limited the survey sampling area.
3. Sample size calculations indicated a need for 411 pigs to investigate a change in seroprevalence, however only 275 pigs were sampled as these were the only ones that could be sampled in the described inclusion criteria. Additionally, analysis of the pig baseline data from only nine of the intervention villages yielded only 105 pigs. The number of positive pigs was thus reduced. This greatly restricted the number and may have led to bias in the analysis.
4. Response Rate differences pre and post intervention evaluation was different- in both overall numbers 19% to 26% and by sex (male / female) 47/89 to 16/64 respectively, this could have lead to response bias with regards to awareness, knowledge and transmission risk factors.
5. The CLTS program in Katete was not implemented as a health education program. It looks like the implementers assumed that since it had worked in Choma a rural area of Zambia it could also work in Katete District .Studies conducted in southern Africa have shown that the success of a program like this is influenced by sex, age, education level and tribe/ethnicity of the farmer. Since these factors were not taken into account, it could have led to the no change observed the prevalence, toilet usage and other associated risk factors for cysticercosis
6. Preliminary unpublished data analysis of the ICONZ evaluation of the CLTS program post 12 months of the intervention in Katete District (courtesy of Dr Chitwambi Makungu, Applied Epidemiology PhD candidate, Samora Machel School of Veterinary Medicine) have shown that CLTS in Katete has not been successful in Katete District due to the following reasons:



- a) An insufficient number of champions were trained for the monitoring of the construction of latrines .They also had to cover 10 villages with an average radius of 50 km, which with no means of transportation made their work difficult.
- b) The lack of transportations for the champions meant that follow-up of CLTS in the villages was erratic. There was also lack of accountability and monitoring from the local government authorities who were in charge of the program.
- c) The villagers lacked or had poor building materials for the construction of the latrines. This led to either the poor construction of latrines which collapsed in the rainy season or no construction of the latrines.

## CHAPTER SIX: CONCLUSION AND RECOMMENDATIONS

### 6.1 Conclusion

The study revealed that CLTS as an intervention did not lead to a reduction in *Taenia solium* infections in pigs. The high prevalence *T. solium* infection in pigs found in this study strongly suggests the presence of many human carriers of the pig tapeworm and consequent direct contamination of their environment with eggs containing oncospheres leads to infection to both humans and pigs.

The results of our study also suggests that pigs in these surveyed villages have increased exposure to *T. solium* eggs owing to a greater prevalence of human taeniasis, free-range systems of keeping pigs and lack of and usage latrines. The study also revealed that the risk factors of selling of infected pork, consumption of infected pork and backyard slaughter of pigs and awareness of *T. Solium* in the communities were not significantly improved due to the fact that the CLTS program does not incorporate health education.

### 6.2 Recommendations

The study recommends that CLTS would need a longer adoption period to achieve significant results, hence the need for a longer term evaluation inconjunction with other prevention and control initiatives. Technical support is necessary for the CLTS programme if improved sanitation is to be achieved and long-term sustainability is to be ensured. Monitoring and surveillance of both human and pig health must be undertaken when the villages have been declared ODF in order to assess the reduction in prevalence of *T.solium* cysticercosis, other sanitation related diseases and their associated risk factors. Additionally the study recommends that besides CLTS; health education, veterinary control of pigs, mass treatment of tapeworm carriers and community (civic) education be incorporated, particularly to pig

farmers as an essential component of prevention and control programmes for *T. solium* infections. The migratory factors that support transmission in non-endemic areas need to be curtailed and resource poor communities need to be educated on the harmful effects raising pigs free range especially without proper sanitation and prophylaxis. Food hygiene and safety must be a priority through the provision of abattoirs' or legal slaughter houses. Without an integrated approach the risk factors will keep on being practised making the program futile. It is important to ensure people in far-flung rural areas are given long-lasting help, advice and supervision and this entails the involvement of a strong primary and environmental health care network that would be involved in maintaining hygienic and sanitary conditions in the communities. The Government of Zambia through its veterinary and public health services must revise its pork inspection regulations to include practical ways of controlling porcine cysticercosis. In order for efforts to be effective and sustainable, policy makers at local and national levels need to play an active role as stakeholders ensuring that needed legislation concerning surveillance, prevalence and control of *T. solium* is enacted as well as implemented. Therefore, without the active participation of the governments, failure of any control program is predictable because scientists cannot apply it at large enough scales, sufficient time and resources.

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## APPENDICES

### Appendix 1: Household Questionnaire

Household Code:

#### Introduction to Study

1. Title of Study: Evaluation of Community Led Total Sanitation as an Intervention Measure for the control of Porcine Cysticercosis in Katete District in the Eastern Province of Zambia.

Principal Investigator: Carol Bulaya

Written Consent from community members who agree to participate in this research through individual interviews.

2. The aim of this questionnaire is to gain knowledge on the burden of porcine cysticercosis in your community and your knowledge, attitudes and practices to sanitation-related issues. The study is being conducted by The University of Zambia, School of Medicine, Department of Public Health and School of Veterinary Medicine, Department of Clinical Studies.

3. The study has been approved by the Excellence in Research Ethics and Science Converge of Lusaka, Zambia

Date .....		Persons interviewing:.....
A1. District: .....		.....
A2. Village: .....		.....
<b>B. HOUSEHOLD DESCRIPTION</b>		
B1. Household identification number		
B2. Name of household head: .....		
B3. Ethnic background: .....		
B4. Main income: .....		

B5. Number of inhabitants: .....

**C RESPONDENT**

C1. Name:.....

C2. Age: .....

C3. Sex: .....

C4. Position in the household: .....

C5. Highest education level in household (1)None..... (2)Primary School.....  
(3) Middle School..... (4)High School.....

(5)Higher Education. Please Specify e.g. college, university, technical/vocational):  
.....

C6. Occupation: .....

C7. Experience on pig management (years): .....

**D. PIG MANAGEMENT**

D1. Number of pigs: .... (1) Breeders: ..... (2) Piglets:..... (3) Fattener:.....

D2 Number of females that have farrowed .....

D3. Breed(s): .....

D4. Husbandry system: (1) indoor (2) semi-intensive (3) Free-range (4) tethering

D5. What do you feed the pigs: (1) pasture..... (2)Kitchen leftovers.....  
(3)Commercial feed..... (4) other feeds: .....

D6. What is the aim of keeping pigs: (1) sale (2) home consumption  
(3) Both 1 and2

D7. What is the mean slaughter age of your pigs: .....

D8. What are the main problems encountered with pig rearing (management ):  
1. .... 2. .... 3.....

D9. What are the main problems encountered with pig rearing (disease):  
1. .... 2. .... 3.....

**E. POSSIBLE TRANSMISSION FACTORS**

E1. Do you or any member in the family consume pork? (Y/N)

E2. Have you slaughtered (a) pigs at home? (Y/N)

E3. If "Yes", was the meat inspected by a meat inspector? (Y/N)

E4. Presence and usage of latrine to be assessed by direct observation

(1) Present and used (2) present but NOT used (3) absent

E5. Is your drinking from (1) river (2) bore-hole (3) well

#### F. AWARENESS OF TAENIASIS AND/OR CYSTICERCOSIS IN MAN

F1. Have you ever heard of tapeworm infection in humans? (If "no", go to f6)

F2. Have you heard or met anyone complaining of having tapeworm infection in the village?

F3. How does one know that he/she has a tapeworm infection? .....

F4. How can people acquire tapeworm infection? .....

F5. What should people do who have tapeworm infection?

(1) Go to hospital (2) use traditional medicine. (3) Do nothing

F6. Have you heard of anyone saying or complaining in the village of following diseases:

(1) Skin nodules? (Y/N) (2) Chronic headache (Y/N)

(3) Epilepsy (Y/N) (4) Madness (Y/N)

#### G. AWARENESS OF CYSTICERCOSIS IN PIGS

G1. Have you observed "measles" (*Cysticercus cellulosae*) in pig meat: (Y/N)

G2. If "yes", do you know what these measles are? .....

G3. If "Yes", do you know how a pig acquires this infection? .....

G4. When you see measles in the meat: (1) Do you eat the meat? (Y/N)

(2) Do you sell the meat? (Y/N)



**Appendix 2: Comparison of baseline and post-intervention prevalence of porcine cysticercosis by Ag-ELISA**

Village	Pig owning HH		No Pigs sampled		Prevalence			
					Baseline		Post intervention	
	baseline	post inter	baseline	post inter	+ve (%)	95% C.I	+ve (%)	95% C.I
Chipapika	9	11	19	38	2 (10.5)	10.7-25.8	10 (13.8)	9.7-17.9
Chikoti	3	9	7	41	1 (14.3)	1.8-11.6	8 (14.9)	10.7-19.1
Chitsulo	4	14	12	42	0 (0.0)	-	5 (15.3)	11.0-19.6
Makwenda	11	10	16	32	2 (12.5)	8.3-22.4	6 (11.6)	7.8-15.5
Musonda	11	10	15	31	2 (13.3)	7.6-21.3	2 (11.3)	7.5-15.0
Chimbuwa	7	10	7	23	4 (57.1)	1.8-11.6	3 (8.4)	5.1-11.7
Chimputi	8	5	14	16	2 (14.3)	6.8-20.1	5 (5.8)	3.0-8.6
Mutika	5	10	8	20	1 (12.5)	2.5-12.9	1 (7.3)	4.2-10.4
Azele kachekeka	6	10	6	32	0 (0.00)	-	5 (11.6)	7.8-15.5
<b>TOTAL</b>	<b>64</b>	<b>89</b>	<b>104</b>	<b>275</b>	<b>14 (13.5)</b>	<b>6.8-20.1</b>	<b>45 (16.4)</b>	<b>12.0-20.8</b>

**Appendix 3: Comparison of baseline and post-intervention sampling and response rates of pig owning households**

Village	Human HH <sup>a</sup>	Human HH <sup>a</sup>	Pig Owing HH <sup>a</sup>	Pig Owing HH <sup>a</sup>	Response Rate (%)	Pig Owing HH <sup>a</sup>	Response rate (%)
	Census	Sampled (Baseline)	Census	Sampled	(%)	Sampled	(%)
				Baseline			
Chimputi	112	30	15	8	53	5	33
Mutika	26	26	15	5	33	10	67
Chimbuwa	60	31	29	7	24	10	34
Chitsulo	92	22	51	4	8	14	27
Chikoti	68	14	47	3	6	9	19
Musonda	35	31	22	11	50	10	45
Azelekachekeka	86	32	30	6	20	10	33
Makwenda	176	43	109	11	10	10	9
Chipapika	64	32	26	9	35	11	42
<b>TOTAL</b>	<b>719</b>	<b>261</b>	<b>344</b>	<b>64</b>	<b>19<sup>b</sup></b>	<b>89</b>	<b>26<sup>c</sup></b>

HH<sup>a</sup> = household

19<sup>b</sup>=Overall baseline response rate

26<sup>c</sup> = Overall post – intervention response rate