THE PREVALENCE AND TRANSMISSION RISK FACTORS OF PORCINE CYSTICERCOSIS IN EASTERN AND SOUTHERN PROVINCES 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 Veterinary Parasitology.

THE UNIVERSITY OF ZAMBIA

SCHOOL OF VETERINARY MEDICINE

DEPARTMENT OF PARACLINICAL STUDIES

LUSAKA

DECLARATION

I **Chummy Sikalizyo Sikasunge** 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 indeed any other university.

Signature: ----------

Date: 18/04/05

DEDICATIONS

What we learn we learn from others; this dissertation is dedicated to my parents, Mr. and Mrs. Bottomley Sikasunge Sikalizyo who struggled to give me the opportunity to be what I am and for nurturing the concept that learning is a life long process. I also dedicate it to my wife Mainza, my three children, Violla, Munji and Chinene.

APPROVAL

This dissertation of Chummy Sikalizyo Sikasunge is approved as fulfilling the requirements for the award of the Degree of Master of Science in Veterinary Parasitology of the University of Zambia.

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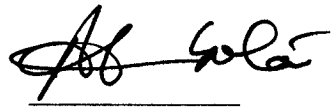
(Chairperson Board of Examiners)  Signature 18-04-05 Date

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ABBREVIATIONS AND SYMBOLS

%	Percentage
=	Equal to
>	Greater than
≥	Greater than or equal to
<	Less than
≤	Less than or equal to
χ^2	Chi-square
µg	Microgram
µl	Microlitre
+ve	Positive
Ab	Antibody
Ab-ELISA	Antibody Enzyme-Linked Immunosorbent Assay
Ag	Antigen
Ag-ELISA	Antigen Enzyme-Linked Immunosorbent Assay
EITB	Enzyme Immunotransfer blot
ELISA	Enzyme-Linked Immunosorbent Assay
Gp	Glycoprotein
H_2O_2	Hydrogen peroxide
H_2SO_4	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
No.	Number
OD	Optical density
OPD	Orthophenylene diamine
p	Probability
PBS	Phosphate buffer saline
rpm	Revolutions per minute
SPSS	Statistical programme for social sciences
T20	Tween 20
TCA	Trichloroacetic acid
Ts	*Taenia solium*
TsCF	*Taenia solium* cyst fluid

ACKNOWLEDGEMENTS

I am very grateful to the Enhancement of Research Capacity, Livestock Helminth Research Project (ENRECA-LHRP) of the Danish International Development Aid (DANIDA) who fully sponsored me and financed most of this work. I am also grateful to the UNZA-VLIR IUC Veterinary Project at the School of Veterinary Medicine, University of Zambia for equipping the Parasite Immunology Laboratory I was able to use during laboratory phase of this work.

I am greatly indebted to the Samora Machel School of Veterinary Medicine of the University of Zambia for giving me the opportunity out of the many others, to conduct this research under the Staff Development Fellowship (S.D.F.) programme in the department of Paraclinical Studies.

I am extremely indebted to my supervisor and Zambian co-ordinator of the ENRECA-LHRP, Dr. Isaac K. Phiri, who has with great care and patience, guided me through the proposal formulation, the carrying out of work and during writing up of this dissertation and made numerous contributions to its improvement. I want to acknowledge him too, for the encouragement and support and for helping me recognize and pursue my true interests. It was he who sent me into doing this research, thereby planting the seed that led to this dissertation. I appreciate the helpful and constructive suggestions made by my co-supervisor Professor Seter Siziya especially on the statistical analysis of the data presented herein.

Dr. Andrew Phiri is greatly appreciated for his valuable effort and advice during my field work. We benefited much from each other during the write-up and his constructive suggestions will not be forgotten.

I especially want to recognize Professor Pierre Dorny (my supervisor's collaborating partner) at Tropical Institute in Antwerp, Belgium for the fruitful suggestions and for the provision of Monoclonal antibodies for the antigen ELISA and other reagents during the laboratory phase of the work, without him my work would have been but with difficult.

I am indebted to Dr. S. Mukaratirwa the Dean of the School of Veterinary Science, University of Zimbabwe for having invited me to attend a workshop on Advances on Diagnosis of *T. solium*/Cysticercosis and Trichinellosis held at his institution. I benefited greatly from this workshop and the fruits can be seen from this work.

I wish to acknowledge with heart-felt thanks Dr. A.L. Willingham III my second co-supervisor and ENRECA-LHRP Co-ordinator for his encouragement and support. It is him who made it possible for me to attend quite a number of international workshops and conferences, the latest of which was the World Association for the Advancement of Veterinary Parasitology (WAAVP) conference that was held in August, 2003 in New Orleans, Louisiana, USA. Professor J. Monrad, the ENRECA-LHRP Leader is not forgotten for his invaluable efforts in maintaining the welfare of students and most importantly ensuring that funds for research were readily available.

I am most grateful to the following members of staff of the University of Zambia, School of Veterinary Medicine for their assistance. Mrs. D.S. Banda and M. Chembensofu for their technical assistance and extreme hard work of ensuring that each pig was examined on the tongue and blood collected from it; the drivers,

Mrs. S. Nkhata, P. Nyirenda and M. Masuku for their vast experience especially in driving through the terrible terrain of Gwembe district.

I must also mention the fact that the task of preparing this dissertation was made easier by the continued understanding, care and support of my wife Mainza and my children. I am very grateful to parents for their long term investment in my education.

Lastly but not the least I would like to extend my profound gratitude to the field workers in the various districts visited, the village headmen and the pig-rearing farmers for their understanding and co-operation. I wish I could list all of them by name.

ABSTRACT

The objective of this study was to determine the prevalence and the potential risk factors associated with *T. solium* (Ts) taeniasis/cysticercosis transmission and maintenance in humans and pigs in Southern and Eastern provinces of Zambia. Tongue examination of live pigs and assessment of the presence of circulating parasite antibodies by enzyme-linked-immunosorbent assay (Ab-ELISA) and that of circulating parasite antigen (Ag-ELISA) in serum were used to detect *T. solium* infections in pigs. A questionnaire was administered in households whose pigs were examined in order to obtain information on pig husbandry practices and to study other associated risk factors to *T. solium* infections in man.

A total of 1541 pigs were examined. Of these, 772 and 769 pigs were from Southern and Eastern provinces respectively. Of the total pigs examined 171 (11.1%) were positive after tongue examination. Pigs from Southern province had a significantly higher *T. solium* prevalence of 117 (15.2%); ($p< 0.001$) than those from Eastern province with a prevalence of 54 (7.0%) on tongue examination. Antibody-ELISA analysis gave a total prevalence of 37.2%. There were no differences in *T. solium* prevalence detected between pigs from Southern 287 (37.2%) and Eastern 287 (37.3%) provinces. The Ag-ELISA assay gave a total *T. solium* cysticercosis prevalence of 376 (24.4%). There was significant difference between Southern province with a prevalence of 234 (30.3%); ($p < 0.001$) and Eastern province with 142 (18.5%). The study further showed that the prevalence of *T. solium* on Ag-ELISA in young pigs was 27.3% while adult pigs had 22.8%.

A total of 788 farmers were interviewed out of the 800 households visited. The noted risk factors associated with *T. solium* infections were varied and included; lack of pork inspection at slaughter (96.7%), consumption of pork with cysts (20.1%), selling of pork infected with *T. solium* cysticerci (18.3%), individuals belonging to households with pigs found positive for cysticercosis on Ag-ELISA (37.6%), free-range husbandry system (83.2%) and poor sanitation i.e. allowing pigs access to infected faeces because of absence of toilets (58.0%). However, analysis of the prevalence rates of porcine cysticercosis in pigs raised in households with or without a latrine yielded no statistical significance on Ag-ELISA ($\chi^2 = 1.76$, $p < 0.184$).

This study confirmed a high prevalence of *T. solium* porcine cysticercosis in the surveyed villages and thus suggests the presence of *T. solium* human carriers. The life cycle of *T. solium* is bound to be sustained by pigs having access to infected human faeces because lack of toilets and consumption of cysticercosis-infected pork by villagers. It is evident from this study that *T. solium* infection poses a high public health risk in the study areas. This baseline data on the status of porcine cysticercosis should give the impetus to conduct Taeniosis and cysticercosis prevalence study in humans in these areas.

CHAPTER ONE:

1.0 INTRODUCTION

Infection with *Taenia solium*, the pig tapeworm, is widely prevalent in human and swine hosts in many developing countries of Latin America, Africa and Asia (Sarti *et al.*, 1992a). Data collected during the last decade show that *T. solium* cysticercosis in pigs and man is more widely distributed in sub-Saharan Africa than previously assumed (Geerts *et al.*, 2002; Phiri *et al.*, 2003).

Man is the definitive host while pigs are intermediate hosts. Pigs are therefore, the main source of infection of human taeniasis. Adult intestinal tapeworm infection in man is acquired by eating undercooked pork which is contaminated with cysticerci (*Cysticercus cellulosae*), the larval stage of the cestode. Cysticercosis, on the other hand, is acquired by man who ingests *T. solium* eggs shed in the faeces of a human tapeworm carrier and thus, it may occur in humans who neither eat pork nor directly share environments with pigs (Schantz *et al.*, 1992). Pigs are infected with *T. solium* cysticercosis by ingesting the parasite eggs or proglottids present in human faeces.

Most morbidity and mortality in human cysticercosis occurs when the parasite invades the central nervous system producing cerebral cysticercosis or neurocysticercosis (NCC), which causes epilepsy, chronic headache, seizures, hydrocephalus and other neurological manifestations (Garcia-Garcia *et al.*, 1999). NCC is the frequent cause of neurological disorders in many developing countries and is also increasingly being reported in patients suffering from epilepsy in developed countries like the United States (White 1997). White (1997) reported that

NCC may also cause intracranial hypertension with subsequent death of the infected person and that cysticerci may also develop in the eye with consequent loss of vision.

Diagnosis of cysticercosis in pigs is usually made at meat inspection. However, ante-mortem diagnosis using tongue examination is sometimes possible in living animals, when the vesicles are located on the tongue or certain mucosae like the ocular mucosae (Gonzalez *et al.*, 1990). Meat inspection is the only technique that is routinely used for the detection of infected carcasses; however, serological tests have recently been developed for the detection of specific cysticercosis antibodies or antigens (Geerts *et al.*, 1981, Harrison *et al.*, 1989). 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).

In regions of endemic infections, transmission is clearly related to prevailing low standard of personal hygiene and poor environmental sanitation and control (Toledo *et al.*, 2001). The general lack of sanitary services, especially inadequate disposal of human excrement, and the widespread occurrence of free-roaming pigs permit easy transmission of *T. solium* in most of the endemic regions. Since the epidemiology of *T. solium* infections is closely linked to social, economical and environmental factors, *T. solium* cysticercosis is predominantly found in rural areas of endemic countries (Sanchez *et al.*, 1997). Consequently, many epidemiological studies have been carried out in rural populations.

Pig keeping and pork consumption in Zambia have increased significantly during the past decade (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.

The abattoir survey of pigs at Chibolya slaughter slab in Lusaka, all of which where from Southern province, showed that 10.9% and 20.6% were positive by lingual examination and meat inspection, respectively (Phiri *et al.*, 2002). Phiri *et al.* (2002), in their preliminary field study, reported that 8.2% (n = 98) pigs from some villages in Kalomo district in Southern province and 5.2% (n = 151) pigs from the villages in Sinda area (Katete district) in Eastern province were positive for *T. solium* cysticercosis by tongue examination whereas Ag-ELISA detected positive 20.8% and 9.3% pigs in Southern and Eastern provinces, respectively. This preliminary study clearly showed that *T. solium* was present in these rural areas of Zambia and therefore, the need for more comprehensive study was suggested. It is from this that this study on cysticercosis in pigs was conceived and that the two provinces were thus chosen. These two provinces harbour more than 76% of the total number of pigs

reared under the small scale management system in Zambia (MAFF 1998). Most of these pigs are on free range (Phiri *et al.*, 2002).

The main objectives in this study were therefore:

(a) To establish the prevalence of *T. solium* metacestodes in free-range pigs in Southern and Eastern provinces of Zambia and,

(b) To elucidate the possible risk factors involved in transmission and maintenance of *T. solium* infection in human and porcine cysticercosis in rural Zambia.

CHAPTER TWO:

2. LITERATURE REVIEW

2.1 Background

The scientific study of the taeniid tapeworms of humans can be traced to the late 17th 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 *T. solium* and *T. saginata* were not yet clearly distinguished (Cox, 2002). Although Goeze in 1782 had suspected that *T. solium* and *T. saginata* were different species, it was not until the middle of the 19th century that Kuchenmeister confirmed the differences based on the morphology of the scolex (Cox, 2002). The first indication that intermediate hosts were involved in the life 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).

Taenia solium has caused very devastating effects in man as evidenced by the many names it has acquired, such as "Swiss cheese brain", "you drive me crazy" and "gutless flatworm" (Lightowlers, 1999).

2.2 Morphology of *Taenia solium*

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

The tapeworm inhabits the upper part of small intestine of man and measures 1.8-4.8m long with 800-900 proglottid (Gracey, 1986). The head of *T. solium* is globular and less than 1mm in diameter, while the rosetellum is short and has double crown of 26-28 hooks. The neck is long and slender. *Taenia solium* has no alimentary canal and its segmented body is called the strobila. Each segment is called a proglottid and it is hermaphrodite containing both male and female reproductive organs (Soulsby, 1982). The gravid uterus in a mature proglottid has 7-12 tree-like lateral branches on each side filled with eggs (Soulsby, 1982). *T. solium* eggs (26-34μm in diameter) are embryonated and can only be seen under the microscope as brown coloured and with radiated appearance (Gracey, 1986).

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).

2.3 Life cycle of *T. solium*

The life cycle of *T. solium* includes the human carrier status, free-living eggs and cysticerci in pigs or human. According to Sciutto *et al.* (2000), humans acquire intestinal infection (taeniasis, or tapeworm infection) by ingestion of undercooked pork infected with *T. solium* cysticerci. In the intestine, the protoscolex evaginates and attaches to the intestinal wall by means of suckers and hooks. The adult worm develops in the small intestine of humans by forming proglottids (Figure 2.1) which arise from the caudal end of the scolex (the neck) (Soulsby, 1982; Flisser, 1994). The body formation which is called strobilation is rapid with development of a mature gravid tapeworm (2-7m in length) after 2-3 months post-infection (Flisser, 1994). The earlier proglottids gradually enlarge and mature as they are separated from the scolex by the newly produced proglottids with functional testes and ovaries. The tapeworm produces millions of eggs (Figure 2.1) which are fertilised within the proglottids and are passed to the environment (Toledo *et al.,* 2001). Each terminal gravid proglottid contains about 50,000 eggs which can either be intermittently extruded from the proglottids into the intestine, or the entire gravid proglottids may be passed in the faeces (White, 1997). Most patients are asymptomatic; however, they may note the passage of proglottids in their faeces. Gravid proglottids are opaque, off-white in colour, and about 1-2cm long, 1cm wide, and 2-3mm thick (Pal *et al.*, 2000). Allan *et al.* (1997) reported that since excretion of proglottids is intermittent, stool studies from patients with active tapeworm infection are

commonly negative for parasite ova because eggs are not uniformly distributed in faeces.

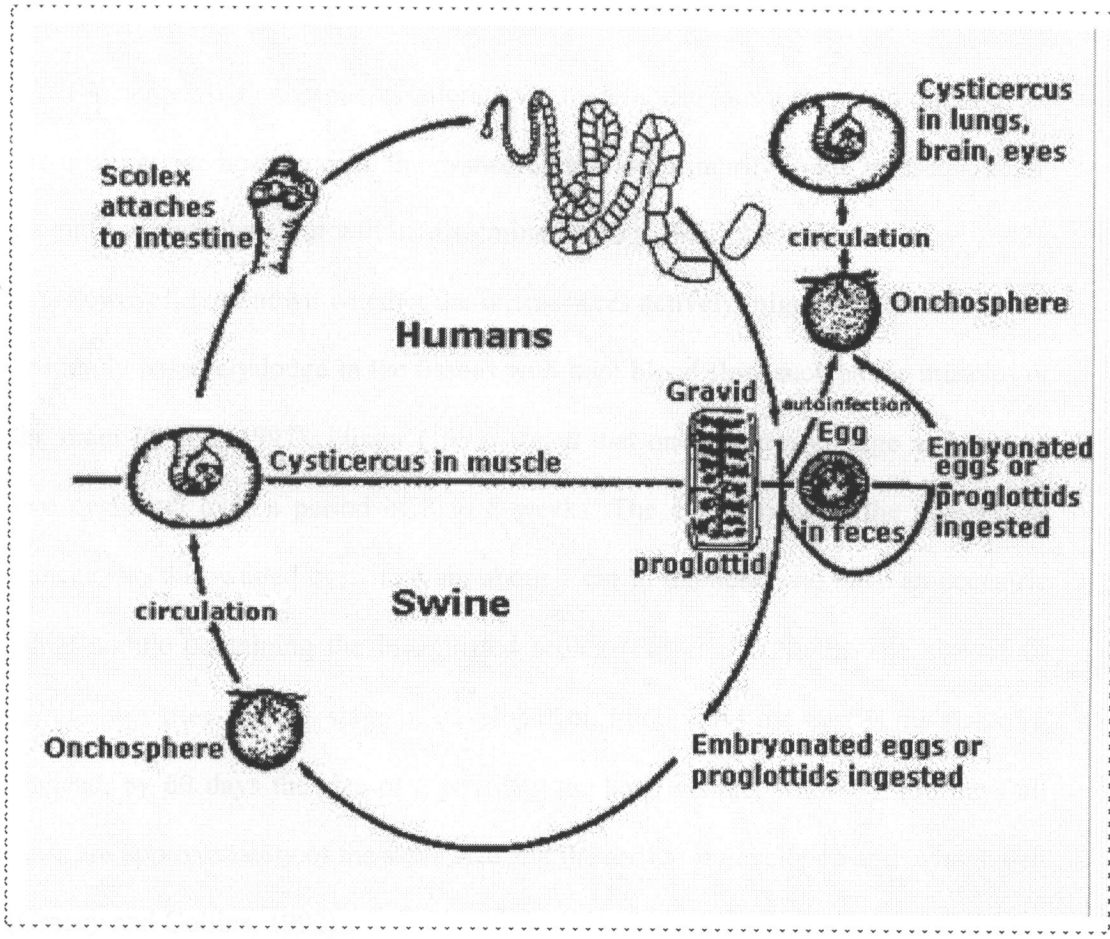


Figure 2.1: The life cycle of *Taenia solium*, showing the infective stages for both man and pig. (Source: www.nlc.net.au/.../ taeniasislifecycle.htm)

Pigs are infected by ingesting parasite eggs or mature proglottids in human faeces (White, 1997). Thus, porcine infection is limited to areas where animal husbandry practices are such that pigs come into contact with human faeces (Toledo *et al.*, 2001). The eggs activated by action of gastric and intestinal fluids are induced to hatch and the hatched larvae, also called oncospheres (Figure 2.1) escape from the

eggs and attach to the intestine via motile hooks, and penetrate the intestinal mucosa and the vessels in the submucosa (Pal *et al.*, 2000). Penetration appears to be facilitated by excretory proteases produced by oncospheres (Flisser, 1994).

After invasion, the oncospheres migrate via the bloodstream throughout the body of the intermediate host. In pigs, the cysticerci develop primarily in the masseter, heart, tongue and shoulders, but will be disseminated throughout the body (Soulsby, 1982). It is however, not known whether the oncospheres actively migrate to specific tissue or merely passively lodge in the tissues with high blood flow such as the muscles or the brain (White, 1997). Flisser (1994) stated that oncospheres enlarge and mature into cysticerci over a period of 3 to 8 weeks. The cyst appear in the muscles as translucent, thin-walled cysts that are about 1 cm in diameter and have an eccentric white nodule containing the invaginated scolex (Pal *et al.*, 2000). 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).

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.

The larval stage also affects humans after the ingestion of *T. solium* eggs present in human faeces leading to a condition called cysticercosis (Flisser, 1994). 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).

2.4 Host range

Taenia solium adult and larval forms are less host-specific. The tapeworm usually occurs singly in intestines of humans but in endemic areas, several tapeworms, up to 25, may be present in an individual (Gracey and Collins, 1992). Man is the natural definitive host for the adult tapeworm, however, Allan *et al.* (1991) reported that *T. solium* taeniasis may be established in Lar gibbon (*Hylobates lar*), Chacma baboon (*Papio urnus*) and golden hamster (*Mesocricetus auratus*). The larval stage of the tapeworm is found most commonly in pigs, though it can also occur in the wild boar, rabbits, hare, monkeys, sheep, man and dogs (Sciutto *et al.*, 2000; Ito *et al.*, 2002). Ito *et al.* (2002) stated that although pigs are the most important intermediate hosts of economic importance, dogs are also highly susceptible and become intermediate hosts

2.5 The importance of *T. solium* taeniasis and cysticercosis

Taenia solium is both an important parasitic disease to pig production as well as human health.

2.5.1 Impact of *T. solium* cysticercosis 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).

2.5.2 Impact of *T. solium* taeniasis and cysticercosis on public health

Man does not only harbour the adult *T. solium* in his small intestines, systemic infection with oncospheres (hatched from tapeworm eggs) does occur causing cysts to establish in the muscular tissue and other organs leading to a condition called human cysticercosis (Flisser, 1994). 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, loses 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 of Preux *et al. (2000) in West Cameroon only 27% of epileptics get married and 39% fail to* enter into any professional activity.

White (1997) in his review stated that NCC, being an infection of the central nervous system, by the larval stage of *T. solium,* is the frequent cause of neurological disorders in many developing countries. NCC is also increasingly being reported in patients suffering from epilepsy in the United States which is attributed to the migration of people from *T. solium* endemic regions. White (1997) reported that NCC is the most likely reason for epilepsy and is twice as common in developing as in developed countries of the world. Seizures are the most common clinical symptoms of NCC in 70%-90% of patients (Wadia, 1996; Del Brutto, 1997). Other symptoms include nausea, vomiting, headache, ataxia, confusion, hydrocephalus, vasculitis and stroke (Del Brutto, 1997). Craig *et al.*, (1996) reported that human cysticercosis could be difficult to detect, as symptoms may take years to develop after infection and that in the case of NCC, symptoms appear after irreversible damage to the brain has occurred.

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).

2.6 Epidemiology and risk factors of *T. solium* infection

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.*, 1999). Although the pig is the essential intermediate host for *T. solium*, little attention has been paid to identify and document the risk factors involving human and porcine infection (Garcia *et al.*, 1999).

Widdowson *et al.* (1999) reported that there are only a few studies that have investigated risk factors for porcine infection and that although these are undoubtedly associated with faeco-oral route transmission; specific risk factors for cysticercosis in pigs have not been clearly identified. Sanchez *et al.* (1998) analyzed risk factors for seropositivity in the urban residents in Honduras and showed that seropositivity was statistically associated with poor household conditions, raising pigs, poor sanitation, lack of portable water and lack of knowledge about the parasite. Other authors have also specifically reaffirmed elsewhere that porcine infection is associated with poverty, lack of latrines and free access by scavenging pigs to human faeces and that lack of veterinary control provide the conditions to sustain the life cycle of *T. solium* (Diaz *et al.*, 1992; Sarti *et al.*, 1992b; Schantz *et al.*, 1992). Sarti *et al.* (1997) showed that extensively raised pigs had a higher seroprevalence of cysticercosis than intensively raised pigs.

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 faeco-oral route. Eggs are passed on after direct contact with a tapeworm carrier or by ingesting contaminated food, water, soil or fomites. Thus, eggs are more easily transferred to new victims when carriers unhygienically prepare and serve food (Schantz *et al.,* 1992). Sanchez *et al.* (1997) found that the less the population knew about the existence of the parasite, the greater the risk they had of being seropositive. Schantz *et al.* (1992) also noted that in non-endemic countries, the disease was most likely to be imported or acquired through contact with an immigrant human tapeworm carrier. They further observed that migration of tapeworm carriers from rural areas to the city predisposes a higher transmission risk of cysticercosis when it involves the presence of poor environmental and social conditions.

Pouedet *et al.* (2002), in their study reported no statistical difference in prevalence of cysticercosis in pigs raised in household with or without a latrine. Infection rates were significantly higher in pigs that had access to human faeces than those, which did not have. Rodriquez-Canul *et al.* (1998) also obtained similar results.

Aluja *et al.* (1998) reported that there is evidence that free-range pigs became infected during the initial days after birth. She stated that in rural areas, piglets become infected at the age of 4 weeks that's immediately after weaning and that in tropical areas; infection in piglets is higher during the dry and hot season. Aluja *et al.*

(1998) attributed this to the fact that adult animals are reluctant to move at high temperature while young ones remain active and thus have access to feacal material during the hot season.

Sarti-G *et al.* (1992a) in Mexico found that the prevalence of cysticercosis was slightly higher in male than in female pigs and that it increased with age. Pouedet *et al.* (2002) also found similar results, which demonstrated that adult pigs showed a significantly higher seroprevalence than young ones. However, Rodriquez-Canul *et al.* (1998), in their study reported that there was no significant difference in seroprevalence by sex and that seroprevalence decreased with age.

2.7 Prevalence of *T. solium* taeniasis and cysticercosis in some selected Latin American, Asian and African countries

Most of the studies conducted in Latin America and a few in Asia have involved sampling in both humans and pigs Tables 2.1 and 2.2, respectively. However, in Africa, baseline data on the prevalence of *T. solium* has been collected in pigs but none in humans (Table 2.2).

Table 2.1 Studies on *T. solium* infections in humans conducted in some endemic countries of Latin America and Asia.

Country	Human cysticercosis prevalence (%)	Human taeniasis prevalence (%)	Reference
Mexico	12	0.5	Garcia-Garcia *et al*, (1999)
	10.8	0.3	Sarti *et al*, (1992b)
Peru	21	-	Garcia *et al*, (1999)
	8	-	Diaz *et al*, (1992)
Bolivia	22.1	-	Carrique-Mas *et al*, (2001)
China	3-4	-	Rajshekhar *et al.* (2003)
Vietnam	5-7	-	Rajshekhar *et al.* (2003)

In the Eastern and Southern Africa (ESA) region, a number of surveys have been done. Tongue prevalence of porcine cysticercosis as high as 46.7% in Mbulu district of Tanzania has been reported (Ngowi, 1999).

In Zambia, a postmortem survey was conducted at an unofficial livestock market (Chibolya) in Lusaka, (Table 2.2) which indicated a prevalence of 20.6% to 56.6%

(Phiri *et al.*, 2002). During this survey, it was found that all these pigs originated from Southern province. Phiri *et al.* (2002) suggested that since the pig are subjected to ante-mortem tongue examination at several stages to determine infection status before being brought to urban centres (mostly Lusaka), it is highly possible that the prevalence after lingual examination is much higher in Southern province than found at the Lusaka slaughter slab survey.

Table 2.2 Studies on *T. solium* infections in pigs conducted in some endemic countries of Latin America, Asia and Africa.

Country	Porcine cysticercosis prevalence (%)	Reference
Mexico	4	Sarti *et al*, (1992b)
Peru	61	Garcia *et al*, (1999)
	43	Diaz *et al*, (1992)
Bolivia	37.4	Carrique-Mas *et al*, (2001)
China	5.4 (0.8-40)	Rajshekhar *et al.* (2003)
Vietnam	0.04-0.9	Rajshekhar *et al.* (2003)
Cameroon	11.0	Pouedet *et al.* (2002)
Tanzania	0.04-4.9	Nsengwa (1995)
	3.2-46.7	Ngowi (1999)
Uganda	0-33.7	Kisakye and Masaba (2002)
Zambia	20.6-56.6	Phiri *et al.* (2002)
	8.2-20.8	Phiri *et al.* (2002)

2.8 Diagnosis of *T. solium* infections

2.8.1: 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). This is in contrast with the calcified cysts of *Taenia saginata*, which are comparatively easy to identify at meat inspection because they often form white and fibrotic lesions (Onyango-Abuje *et al.* 1996). Sciutto *et al.* (1998a) also estimated that more than 50% of pigs that harbour metacestodes show them in the tongue, and recommended the tongue as one of the sites for meat inspection in addition to the diaphragm or the shoulder muscles. 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). 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).

Examination of stool or faeces for presence of *Taenia* eggs, scolex or proglottids can be used to diagnose human intestinal infection with *T. solium*. This involves using a sedimentation technique that utilizes formol-ether (Sarti *et al.*, 1992b).

2.8.2 Immunodiagnostic techniques

Although meat inspection is the routine technique for the detection of cysticercosis infected carcasses, 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). Serological diagnostic methods 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 human and pig cysticercosis. Diaz *et al.* (1992), recommended serological studies in both humans and pigs as being useful for determining areas where the disease is endemic and defining and targeting high-risk families to *T. solium* antigen contact as well as for monitoring the success of control programmes by determining the incidence of new cysticercosis infections.

However, the sensitivity of the available serological techniques is low in pigs with low levels of cyst burdens (Sciutto *et al.,* 1998b). Nevertheless, Nguekam *et al.* (2003) were able to detect pigs harbouring a single cyst using Ag-ELISA. When measuring antibodies, antigen exposure is measured rather than actual infection and that interpretation of seropositive results in young pigs may be complicated by the fact that the maternal antibodies transferred by colostrum from a seropositive sow to its piglets may persist for up to 7 months (Gonzalez *et al.*, 1999). In human, the occurrence of a transient antibody response in *T. solium* infection in field conditions was found to be a major contributor to the over estimation of the prevalence of cysticercosis in endemic areas of Peru and Columbia when data from serological surveys demonstrated that about 40% of seropositive people were seronegative when re-sampled after one year (Garcia *et al.*, 2001). Garcia *et al.* (2001) noted that as in humans, the problem of transient antibodies may also have to be considered in pigs which may be exibiting transient antibody response to *T. solium* infection without the establishment of a patent infection. Cross-reactions with *Cysticercus tenuicollis* are rather the rule than the exception in most antibody and antigen detecting tests (Dorny *et al.*, 2003).

2.8.2.1 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 in the 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 ascaridosis have been observed.

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

Several workers including (De Jonge *et al.*, 1987; Harrison *et al.*, 1989; Brandt *et al.*, 1992); Draelants *et al.*, 1995; Onyango-Abuje *et al.*, 1996 and Van Kerckhoven *et al.*, 1998) have contributed to the development of Antigen detecting ELISAs. Harrison *et al.* (1989) developed an antigen detecting ELISA based 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 has been used in sero-epidemiological studies for *T. saginata* and *T. solium* cysticercosis in Zambia (Dorny *et al.*, 2002; Phiri *et al.*, 2002). 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).

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

2.8.2.3 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). The major disadvantage of the test, however, is 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.

2.9 Prevention and control of *T. solium* infection

Sarti-G *et al.* (1992a) recommended that effective and long-lasting control of the transmission of *T. solium* from pigs to humans must include measures to deny pig's access to human faeces. Sarti-G *et al.* (1992a) though, noted that such a change was likely to be resisted because of the traditional and functional aspects of established pig-rearing practices. Widdowson *et al.*, (1999) reported that for an integrated control strategy of cysticercosis to work, there must be recognition of the interrelationship between risk factors for infection, for example husbandry, human defaecation habits and village risk factors including access to clean water or markets with meat certification.

Pal *et al.* (1999) stated that eradication of cysticercosis is possible by removing the disease from either pig or human, or both. They further suggested that reform of animal husbandry techniques, meat inspection procedures and adequate cooking of pork are difficult approaches and of limited relevance in developing countries. Pal *et al.* (1999) further noted that in developing countries, pigs are free roaming and raised by subsistence farmers who can not afford enclosed pens or proper animal feed, and meat is sold off outside the abattoir system.

Carrique-Mas *et al.* (2001) recommended that to reduce the prevalence of human cysticercosis more effective education and vaccination campaigns aimed at preventing both *T. solium* infection and cysticercosis were required. The use of closed clean sites and improved human hygiene are still the most effective methods of preventing pigs from infection.

Though current efforts are centred on the control and potential eradication of the disease, no control strategy has yet been proven effective and sustainable (Cruz *et al.*, 1989; Schantz *et al.*, 1993; Sarti *et al.*, 1997). However, Garcia *et al.* (1999) suggested that the only proven way of eradicating cysticercosis is the improvement of sanitary conditions, potable water and sewage connections as occurred in Europe in the early 1990s. They, however, also noted that the economical and geographical constraints make this impossible in the near future for most developing countries. Garcia *et al.* (1999) further suggested that in the meantime, other intervention strategies such as chemotherapy or porcine vaccination may be useful and that evaluation of these strategies will, however, require the use of appropriate epidemiological indicators of *T. solium* environmental contamination like serological testing of native or sentinel pigs.

Molinari *et al.* (1993) reported that studies on the role of vaccination in pig cysticercosis are comparatively scarce and that they provide information on various antigen preparations inducing some degree of protection in pigs. However, Sciutto *et al.* (1995) reported that vaccination of pigs against *T. solium* cysticercosis should be more explored before being massively applied.

Huerta *et al.* (2000) vaccinated pigs of mixed genetic make-up, and established that there was effective protection to experimental challenge against *T. solium* cysticercosis, since vaccination lowers the number of viable cysticerci capable of developing into tapeworms. He further noted that since the pig is an indispensable intermediate host, lowering the prevalence of pig cysticercosis through effective vaccination could reduce transmission. Scuitto *et al.* (1995) obtained similar results

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

Lightowlers (2003) reported that recent vaccination trials have been able to produce a vaccine called TSOL18 against *T. solium* cysticercosis. This vaccine has been reported to offer 100% protection against *T. solium* cysticercosis infection in pigs. Lightowlers (2003) stated that the future control of *T. solium* infections lies in an integrated approach, as a single control measure is unlikely to achieve effective and long lasting control.

CHAPTER THREE:

3. MATERIALS AND METHODS

3.1 Study areas and animals

3.1.1 Southern province

The Southern province of Zambia lies between latitudes 15° 14'S and longitudes 25° E and 28° E. In this province, the study was done in Gwembe and Monze districts (Figure 3.1). These districts were selected following a preliminary visit that was made which indicated the presence of a lot of free ranging pigs in the area. Monze district is located in the centre of the province, while Gwembe district forms the eastern boundary of the province. Villages in Gwembe district are spread over very large areas with very few isolated households separated by the hilly scrub woodlands. The unimodal rainfall lasts from November to April on the plateau (Monze) and from October to February in the valley (Gwembe). Annual rainfall from 1995 to 2001 ranged from 329mm to 848mm (mean 636mm) in Gwembe and 524mm to 881mm (mean 735mm) in Monze. Mean annual maximum temperatures in the same period ranged from 29.7°C to 31.1°C (mean 30.4°C) in Gwembe and 28.3°C to 30°C (mean 28.9°C) in Monze. The mean annual minimum temperatures ranged from 19.4°C to 21.5°C (mean 20.5°C) in Gwembe with Monze having a range of 13.6°C to 15.2°C (mean 14.2°C). On the plateau where Monze lies, much of the *Brachystegia* "Miombo" and *Acacia* "Munga" woodlands have been cut down to give way to agriculture, while Gwembe in the valley, *Colophospermum mopane* "Mopane" and scrub woodland predominate (Mulofwa, *et al.* 1994).

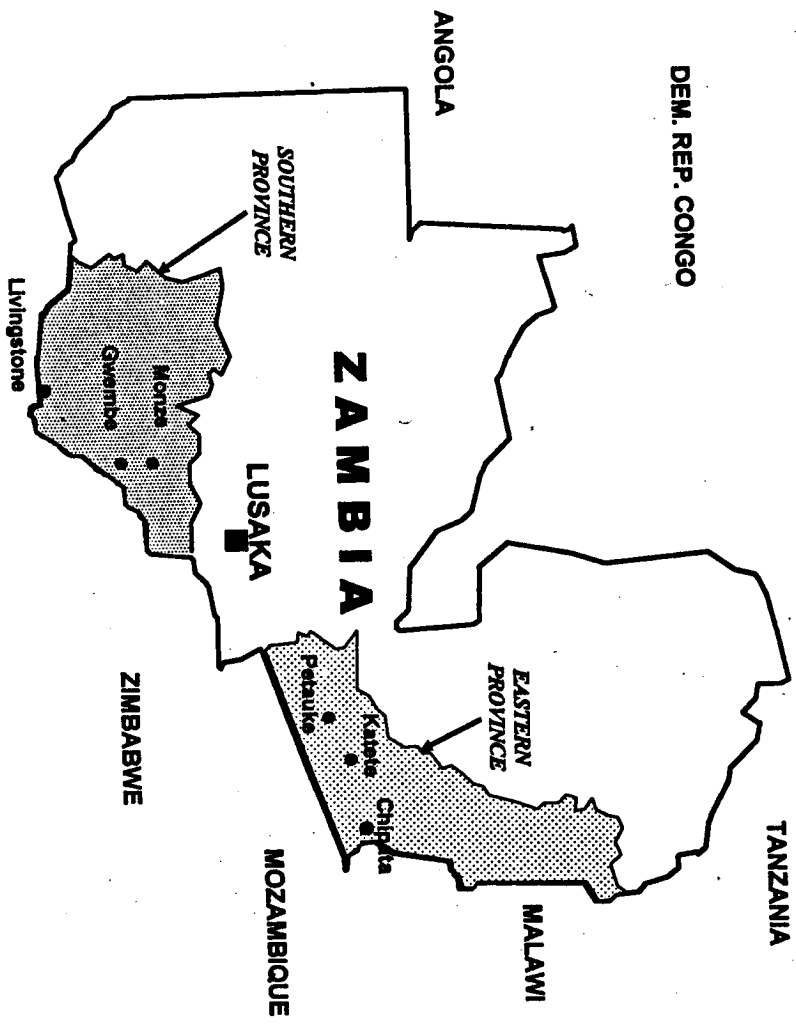


Figure 3.1 Map of Zambia showing the study areas in Katete, Petauke, Gwembe and Monze districts in Eastern and Southern provinces.

A total of 104 villages comprising 60 and 44 villages from Gwembe and Monze districts respectively were visited. A total sample of 772 pigs from 412 households was examined. In Gwembe district 385 pigs were examined from 209 households, while in Monze district 387 pigs were examined from 203 households. All the pigs were crossbreeds between Landrace and Large white breeds (Figure 3.2). The common type of pig house is seen in Figure 3.3. In addition to keeping pigs, the people of Southern province also keep cattle, goats, chickens, guinea fowls and turkeys.

Figure 3.2 The Large white and Landrace cross breed pigs found in the rural areas of Southern province

Figure 3.3 A pig house (pig pen) found in Siang'andu village in Munyumbwe area of Gwembe district in Southern province.

(Note the poor state of the pen which may allow pigs especially young ones to escape).

3.1.2 Eastern province

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 carried out in Petauke and Katete districts (Figure 3.1) which are found on the Southern half of the province. The two districts were selected following a preliminary survey that was conducted by Phiri, *et al.,* (2002) which indicated presence of a lot of free ranging pigs in these districts. The rainy season in this province starts in November and ends in April. Annual rainfall from 1995 to 2001 in Petauke and Katete ranged from 740.3mm to 1067.2mm (mean 887.6mm). Mean annual maximum temperatures in the same period ranged from 28.1°C to 29.4°C (mean 28.6°C) in the two districts, while the mean annual minimum temperatures ranged from 16.7°C to 17.5°C (mean 14.2°C). The vegetation in Eastern province includes "Miombo" woodland dominated by *Brachystegia* and *Julbernardia* species, the "Munga" woodland, where the principal tree species are *Acasia, Combretum* and *Terminalia* and the "Mopane" woodland with *Colophospermum mopane* being the dominant tree species (Berkvens *et al.,* 1998).

In Eastern province, the villages were very big with household very close to one another due to village regrouping that was introduced in the first republic (Banda, 2003 personal communication). In many instances, it was very difficult to know the village boundaries. A total of 51 villages from the two districts were visited. These comprised 26 and 25 villages from Petauke and Katete districts respectively. A total of 769 pigs from 388 households were examined. Out of these, 384 pigs were

examined from 168 households in Petauke and 385 pigs were examined from 220 households in Katete. Eastern province is dominated by the indigenous breed called *Nsenga* (Figure 3.4) and all the pigs examined were of this breed. 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. The common type of pig house is seen in Figure 3.5. Like in Southern province, certain farmers in Eastern province also kept other types of livestock such as cattle, goats and chickens.

Figure 3.4 The indigenous black dwarf (*Nsenga*) pigs found in the rural areas of Eastern province.

Figure 3.5 A typical pig house (*kola*) found in a village in Petauke district in Eastern province.

3.2 Study design

A multi-stage cluster sampling technique was used. Villages with pigs were treated as primary units while the pig herds in the individual households were secondary units. The tertiary unit was the individual pig in the herd as seen in Table 3.1. All sampling at each stage was simple random.

Table 3.1 Multi-stage cluster sampling; showing the primary, secondary and tertiary levels, the unit of sampling at each level and the sampling method used.

Levels	Units	Sampling Method
Primary Unit	Villages with pigs	Random
Secondary Unit	Swine Herds in the Households	Random
Tertiary Units	Pigs in the Herds	Random

3.3 Calculation of sample size

The sample size to estimate the prevalence of porcine cysticercosis was calculated according to Martin *et al.* (1987) from the formula:

$$n = \frac{Z^2 \times P \times Q}{L^2}$$

Where: n = required sample size, Z = Z value for a given confidence level, P = known or estimated prevalence, Q = (1-P), and L = allowable error. In this study a 95% confidence level with allowable error of estimation of 0.05 were used. Since there is no known prevalence, P was estimated at 50% to give the maximum sample

size. Therefore, $\mathbf{n} = 1.96^2 \times 0.5 \times 0.5/0.05^2 = 384$. Thus, at least 384 pigs were examined for presence/absence of cysticercosis in each district.

3.3.1 Sampling

A total of 1,541 pigs from 800 households were examined. However, 788 farmers were interviewed out of the 800 households visited.

Sows with advanced pregnancy, those that had recently furrowed and piglets were excluded from the study to avoid stress in sows and false positives due to passive immunity in pigs less than 6 months. The demographic characteristics of the pig population such as breed, sex and age were determined at the time of blood sampling. If the herd size (number of pigs in a household) was less than five all the pigs that met our selection criteria from the randomly selected households had their tongues examined and a blood sample collected. Fifty percent of pigs in the herd per household were examined if the herd size was greater than five. A pig was classified as young if it was less than one year and as an adult if it was one year or older. A household was classified positive if one of the pigs examined was positive on any one of the tests used in the study (i.e. tongue examination, Ag-ELISA and Ab-ELISA).

3.4.1 Tongue examination

Presence of cysticercosis nodules or cysts was done by conducting tongue examination (Figure 3.6). The pig was placed in a left recumbence if the examiner was right handed and right recumbence if left handed. The pig was held by the neck and firmly restrained with the help of three people. The first person held the hind legs while the second person held the fore legs while the third person held the head at

the level of the pig's ears. The fourth person was the examiner. A hard wooden stick was used to open and maintain the mouth open. Using a mutton cloth for grip, the tongue was pulled out, examined and palpated all along its ventral side for the presence of cysticercosis nodules. A cyst was classified as either "Live" (Viable) if it was translucent, thin-walled and fluid-filled cyst containing a visible invaginated whitish protoscolex (Figure 3.6) or "Calcified" if it was degenerated and caseous in appearance.

Figure 3.6 *Taenia solium* cysticerci (cysts) as seen on the ventral aspects of the tongue (arrow) of an infected pig.

(Note that most of the cysts seen here were live).

3.4.2 Blood collection and serum separation

The blood samples were obtained from either the external jugular vein or the cranial vena cava (Figure 3.7) into plain blood collecting tubes and allowed to clot in a cool box. Three persons restrained the pig in dorsal recumbence. The first person held the hind legs while the second held the fore legs with the third person holding the head by the level of the mandible. The fourth person then collected blood using an 18 gauge needle and a 20 ml syringe. The right side of the neck of the pig was preferred for blood collection because on the left side, the left phrenic nerve occupies a more vulnerable position, while the unpaired thoracic duct is also more to the left (Dyce *et al.*, 1996). To obtain serum, the clotted blood was separated by centrifugation at 3000 rpm for 15 min. The supernatant (serum) was dispensed into 2 ml aliquots and stored in labelled vials, which were frozen at -20^{0}C until use.

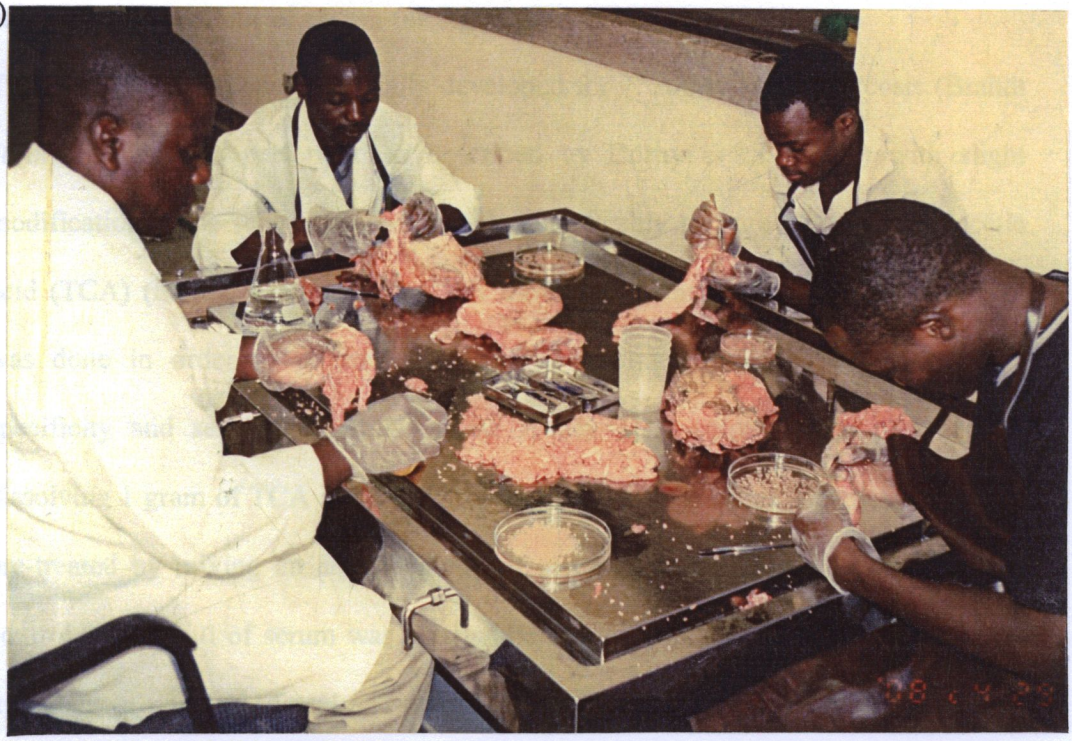
Figure 3.7 Blood collection from the cranial vena cava of a pig.

3.4.3 Cyst fluid collection and determination of its protein concentration for Ab-ELISA

Taenia solium cyst fluid (TsCF) was used as antigen for the Ab-ELISA. TsCF was collected according to the method developed by Ito (1998) with minor changes. Fresh cysticerci were collected by dissection from host tissues of heavily, naturally infected pigs. The cysts were then washed repeatedly in phosphate buffered saline (PBS). The excess PBS was removed by spreading the cysts on Whitman's blotting paper No. 4 (Figure 3.8b). The cysts were then ruptured individually using a sterile scalpel blade on a Petri dish slanted at approximately 30° angle so that the TsCF could be collect at the bottom of the dish. The TsCF was centrifuged at 3200 rpm for 30 min to remove tissue debris from the ruptured cysts and was collect as supernatant. The TsCF supernatant was aliquoted into 2ml vials and stored at -20 °C until used as TsCF antigen in the antibody ELISA.

To determine the protein concentration of the cyst fluid, Phosphate Buffered Saline (PBS) pH 7.2 was used as a reference. Two cuvettes were filled with PBS and the spectrophotometer was set on 280 nm for zero setting. One of the reference cuvettes was left on the spectrophotometer and the other cuvette was replaced with a cuvette filled with the *T. solium* cyst fluid. Readings at OD 280 nm and OD 260 nm were done and the protein content in mg/ml was calculated using the following formula: [(OD280 nm x 1.5) – (OD260 nm x 0.76)] x dilution = mg protein/ml (Dorny, personal communication).

(a)



(b)

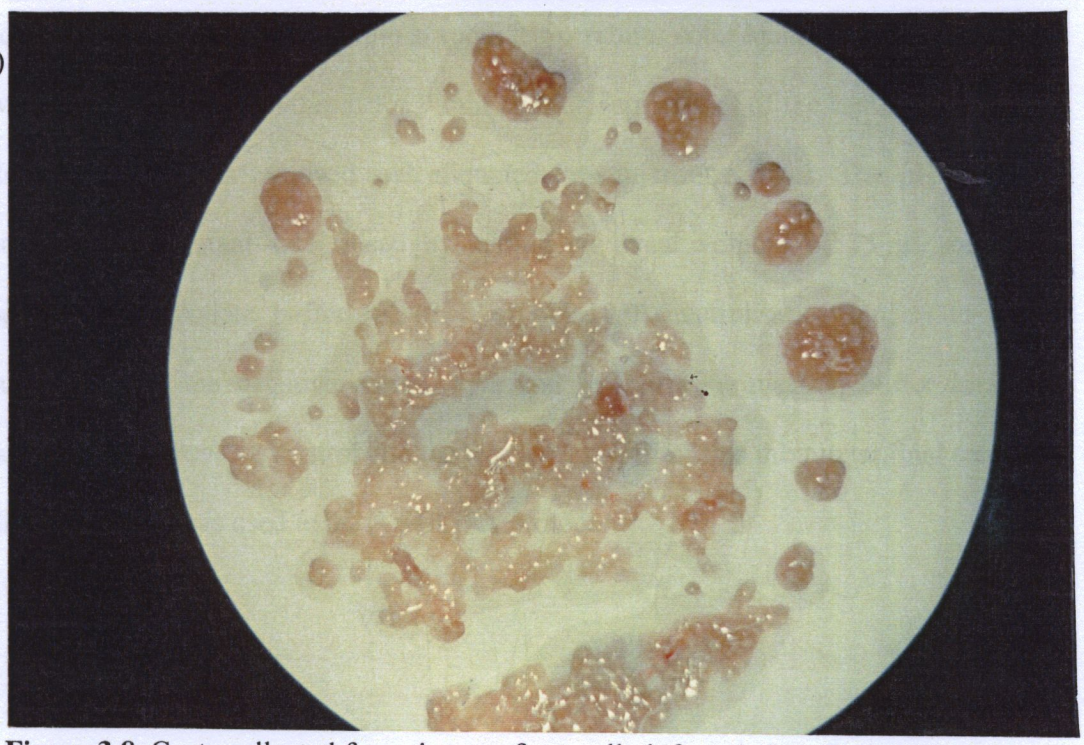


Figure 3.8 Cysts collected from tissues of naturally infected pigs. Shown **(a)** cysts spread on Whitman's blotting paper No. 4 **(b)** during removal of excess PBS prior to being ruptured.

3.4.4 Pre-treatment of serum for Ag-ELISA

The Ag-ELISA, which 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. Pre-treatment was done in order to remove non-specific immune-complexes to increase the specificity and sensitivity of the assay. A 5% TCA solution was prepared 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 was incubated for 20 min at room temperature. After incubation, the mixture was centrifuged at 12,000 rpm for 9 min and the supernatant of the same volume of the added sera 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 (neutralisation buffer) to the supernatant of the negative control sera and 150 µl neutralisation buffer to the supernatant of positive control and the test sera. 100 µl of this mixture at final serum dilution of 1: 4 was used in the Ag-ELISA protocol.

3.4.5 Enzyme-linked-Immunosorbent Assay protocols

Serum samples were examined for circulating *T. solium* cysticercal antibodies (Ab-ELISA) according to Pouedet *et al.* (2002) and for presence of *T. solium* cysticercal antigens using a monoclonal antigen-based double sandwich enzyme-linked immunosorbent assay (Ag-ELISA) as done by Phiri *et al.* (2002).

3.4.5.1 Enzyme-linked immunosorbent assay for the detection of antibodies against *T. solium* cysticerci (Ab-ELISA)

The Ab-ELISA was conducted as described by Pouedet *et al.* (2002) with minor modifications. The optimal dilution of the antigen, serum and conjugate was determined by serial titrations. The assay involved coating polystyrene 96 well ELISA plates (Nunc® Maxisorp) with 100 µl per well of cyst fluid antigen diluted at 1/1000 (5.4 µg/ml) in carbonate buffer (0.06 M, pH 9.6), and incubating at 37 °C on a shaker for 30 min. The plate was washed once with PBS–Tween-20 (phosphate buffered saline + 0.05% T20) and then dried by beating the plate vigorously on blotting paper. Blocking to avoid non specific reactive sites was done by adding 150 µl per well of PBS–T20 + 1% new born calf serum (PBS–T20/1% NBCS), and then the plates were incubated at 37°C on a shaker for 15 min. After incubation, the plates were emptied and dried. Without washing the plate 100 µl of test sera diluted at 1/200 in PBS–T20/1% NBCS was added and incubated on a shaker at 37°C for 15 min. After washing the plate twice, 100 µl of peroxidase conjugate, rabbit anti-pig IgG, (SIGMA) diluted at 1/20,000 in PBS–T20/1% NBCS was added and incubated at 37°C on a shaker for 15 min. The wells were then washed twice after which they were dried. Two tablets of the chromogen/substrate, orthophenylene diamine (OPD) (DAKO, #S2045) were added to 12 ml of distilled water, to which 5 µl of H_2O_2 was

added. 100 µl of this solution was added to the wells following which plate was incubated at room temperature for 15 min in the dark without shaking. The final step involved stopping the reaction by adding 50 µl of 4N H_2SO_4 to each well. The plates were read using an ELISA reader (Labsystem Multiskan RC) at 492 nm.

3.4.5.2 Enzyme-linked-immunosorbent assay for the detection of circulating antigens against *T. solium* cysticerci (Ag-ELISA)

The Ag-ELISA was conducted as described by Phiri *et al.* (2002) with minor modifications. Two monoclonal antibodies (MoAb) were used thus the name double sandwich ELISA as the technique involves trapping the antigen (Ag) between two monoclonal antibodies. The MoAb were obtained from Prince Leopold Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerp, Belgium. The assay involved coating polystyrene 96 well ELISA plates (Nunc® Maxisorp). Monoclonal antibody B158C11A10 was used as first MoAb and a biotinylated MoAb B60H8A4 was used as detector antibody (second MoAb). The plate was coated with 100 µl of MoAb B158C11A10 diluted at 5 µg/ml in carbonate buffer (0.06 M, pH 9.6) and incubation was carried out at 37°C on a shaker for 30 min. After coating, the plates were washed once with PBS–T20 and dried by beating the plate vigorously on blotting paper. Blocking to avoid non-specific reactive sites was done by adding 150 µl per well of PBS-T20/1% NBCS and then the plates were incubated on a shaker for 15min at 37°C. There after plates were emptied and dried. Without washing the plate, 100 µl of pre-treated sera at a dilution of 1/4 was added and incubated at 37°C on a shaker for 15 min. After washing the plate five times it was dried. 100 µl of biotinylated MoAb B60H8A4 diluted at 1.25 µg/ml in PBS–T20/1%NBCS was

added and the plate incubated at 37°C on a shaker for 15 min. After this, the plate was washed five times with PBS–T20 and then dried. 100 µl of streptavidin-horseradish peroxidase (Jackson Immunoresearch Lab, Inc.) diluted at 1/10,000 in PBS–T20/1%NBCS was added to act as conjugate after which the plate was incubated on a shaker at 37°C for 15 min. Two tablets of the chromogen/substrate, orthophenylene diamine (OPD) (DAKO, #S2045) were added to 12 ml of distilled water, to which 5 µl of H_2O_2 was added. 100 µl of this solution was added to the wells and incubation was done at room temperature for 15 min in the dark without shaking. To stop the reaction, 50µl of 4N H_2SO_4 was added to each well. The plates were read using an ELISA reader (Labsystem Multiskan RC) at 492 nm.

3.5 Investigation of risk factors

A questionnaire (Appendix 1) was developed and used to collect information on risk factors and other related information from pig farmers. The questionnaire was orally administered by veterinary assistants in charge of the respective areas using the native language. A number of households in each village were randomly selected depending on the willingness of the farmers to participate in the study. Data collected included (i) the number of pigs owned, (ii) general management with particular emphasis on types of husbandry practices, (iii) type of feeds, (iv) the main aim of keeping pigs, (v) the number of household inhabitants and their level of education, (vi) their knowledge on taeniasis and cysticercosis, (vii) peoples feeding habits, with particular attention to pork consumption, (viii) their source of drinking water, (ix) presence and usage of sanitary facilities especially toilets, (x) specific questions

regarding medical history related to the presence of helminthiasis and/or symptoms suggestive of neural disorders in the family and the entire community in the village.

3.6 Statistical analysis

The optical density (OD) of each serum sample was compared with a series of reference negative serum samples (n = 8) at a probability level of p = 0.05 to determine the cut-off using a modified-Student t-test (Sokal and Rohlf, 1981).

The SPSS (Version 11) and Epi-info 2002 software were used for statistical analysis and included chi-square (fisher's exact test and Yates corrected) to assess the association between porcine seropositivity and the different variables at 95% confidence level. Using the *Kappa* test, the tests were compared two by two in order to find out the agreement between the tests in the detection of porcine cysticercosis. The results of each test were also correlated.

CHAPTER FOUR:

4. RESULTS

4.1 Prevalence of porcine cysticercosis

4.1.1 Overall

A total of 1541 pigs were examined as described in chapter three. Of these, 772 and 769 pigs were from Southern and Eastern provinces, respectively. Of the total pigs examined, 171 (11.1%) were found positive on tongue examination, 574 (37.2%) on Ab-ELISA and 376 (24.4%) on Ag-ELISA. Pigs in Southern province had a significantly higher 117 (15.2%) prevalence (χ^2 = 25.01, p< 0.001) than those in Eastern province which had a prevalence of 54 (7.0%) on tongue examination (Figure 4.3). Ab-ELISA analysis gave a total prevalence of 37.2% and, there was no difference observed between Southern and Eastern provinces which had 287 (37.2%) and 287 (37.3%), respectively (Figure 4.1 and Table 4.1).

The Ag-ELISA assay gave a total porcine cysticercosis prevalence of 376 (24.4%). The Ag-ELISA results showed that Southern province with 234 (30.3%) had significantly higher (χ^2 = 28.67, p < 0.001) prevalence than Eastern province which had 142 (18.5%) (Table 4.1 and Figure 4.1). The tongue examination results showed similar trend to the Ag-ELISA giving 15.2% and 7.0% for Southern and Eastern provinces, respectively.

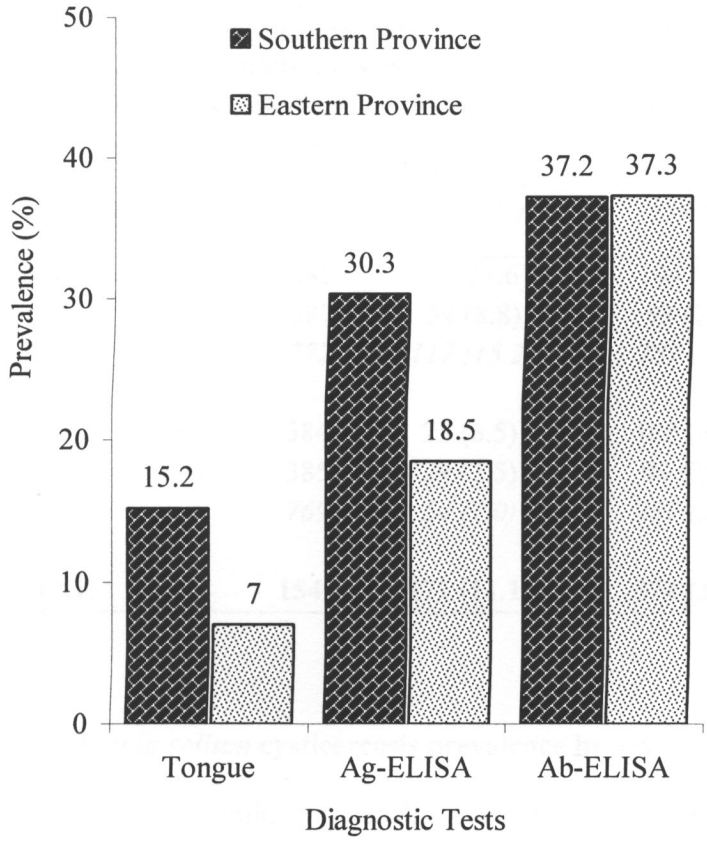


Figure 4.1 Comparative prevalence of *T. solium* cysticercosis in pigs from Southern (n = 772) and Eastern provinces (n = 769) using the three different diagnostic techniques.

Gwembe district had the highest cysticercosis prevalence (36.4%) on Ag-ELISA (Table 4.1), whereas, Petauke district with 16.4% positive pigs was the lowest. Statistical analysis on Ag- ELISA also clearly showed that Gwembe district at 36.4% had a significantly higher prevalence ($\chi^2 = 12.75$, $p < 0.001$) than Monze (24.3%) despite both of them being in Southern province. However, the prevalence in Monze (24.3%) was not significantly higher ($\chi^2 = 1.37$, $p = 0.242$) than that found in Katete (20.5%) in Eastern province.

Table 4.1 Prevalence of *T. solium* cysticercosis in pigs on Tongue examination, Ab-ELISA and Ag-ELISA in Gwembe and Monze districts in Southern and Petauke and Katete districts in Eastern provinces of Zambia.

Province	District	n	Tongue +ve (%)	Ab-ELISA +ve (%)	Ag-ELISA +ve (%)
Southern	Gwembe	385	83 (21.6)	194 (50.4)	140 (36.4)
	Monze	387	34 (8.8)	93 (24.0)	94 (24.3)
Total		*772*	*117 (15.2)*	*287 (37.2)*	*234 (30.3)*
Eastern	Petauke	384	25 (6.5)	188 (49.0)	63 (16.4)
	Katete	385	29 (7.5)	99 (25.7)	79 (20.5)
Total		*769*	*54 (7.0)*	*287 (37.3)*	*142 (18.5)*
TOTAL		**1541**	**171 (11.1)**	**574 (37.2)**	**376 (24.4)**

4.1.2 *Taenia solium* cysticercosis prevalence by sex

Overall tongue examination results showed a significantly higher prevalence in males (13.9%) when compared with females (10.0%) (χ^2 = 4.485, p = 0.034). Ab-ELISA similarly showed a higher prevalence in males 41.5% than females 35.6% (χ^2 = 4.41, p = 0.036). There was also evidence (χ^2 = 3.57, p = 0.059) that male pigs had a higher prevalence 27.8% than females 23.0% on Ag-ELISA results (Figure 4.2). However, there was no significant difference in prevalence on Ag-ELISA in male 23.4% compared to female pigs 16.9% in Eastern province (χ^2 = 3.45; p = 0.06) (Table 4.2).

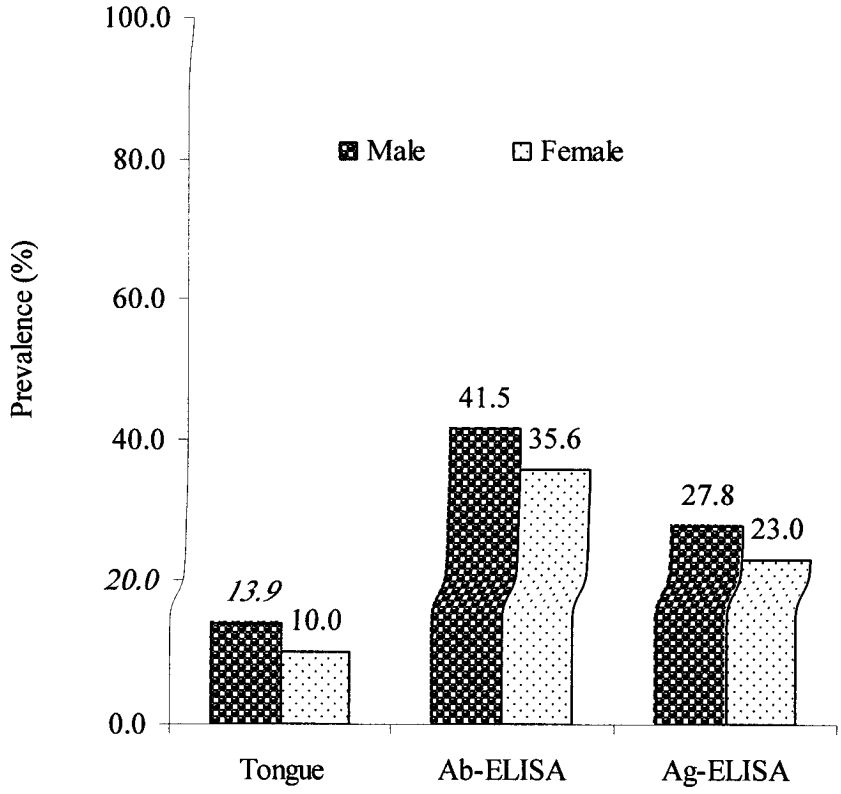


Figure 4.2 Prevalence of *T. solium* cysticercosis in male (n = 439) and female (n = 1,102) pigs on tongue examination, Ab-ELISA and Ag-ELISA.

Table 4.2 Prevalence of *T. solium* porcine cysticercosis according to sex in Southern and Eastern provinces.

Province	Sex	n	Tongue +ve (%)	Ab-ELISA +ve (%)	Ag-ELISA +ve (%)
Southern	Male	255	45 (17.6)	95 (37.3)	79 (31.0)
	Female	517	72 (13.9)	192 (37.2)	155 (30.0)
Total		*772*	*117 (15.2)*	*287 (37.2)*	*234 (30.3)*
Eastern	Male	184	16 (8.7)	87 (47.3)	43 (23.4)
	Female	585	38 (6.5)	200 (34.2)	99 (16.9)
Total		*769*	*54 (7.0)*	*287 (37.3)*	*142 (18.5)*
TOTAL		**1541**	**171 (11.1)**	**574 (37.2)**	**376 (24.4)**

4.1.3 *Taenia solium* cysticercosis prevalence by age

Of the 1271 pigs analysed by age, the prevalence on tongue examination was 11.0% in young and 8.4% in adult pigs (Figure 4.3). There was no significant difference (χ^2 = 2.12, p = 0.146) in prevalence by age on tongue examination. Ab-ELISA result indicated significantly higher (χ^2 = 18.06, p<0.001) prevalence in young pigs (37.5%) than in adult ones (26.2%). The Ab-ELISA detected more positive young pigs 42.5% than adult pigs 23.0% (χ^2 = 24.7, p < 0.001) in Eastern than Southern province (Table 4.3). There was some evidence to suggest that prevalence was significantly higher in young (27.3%) than in adult (22.8%) pigs on Ag-ELISA (χ^2 = 3.24, p = 0.072).

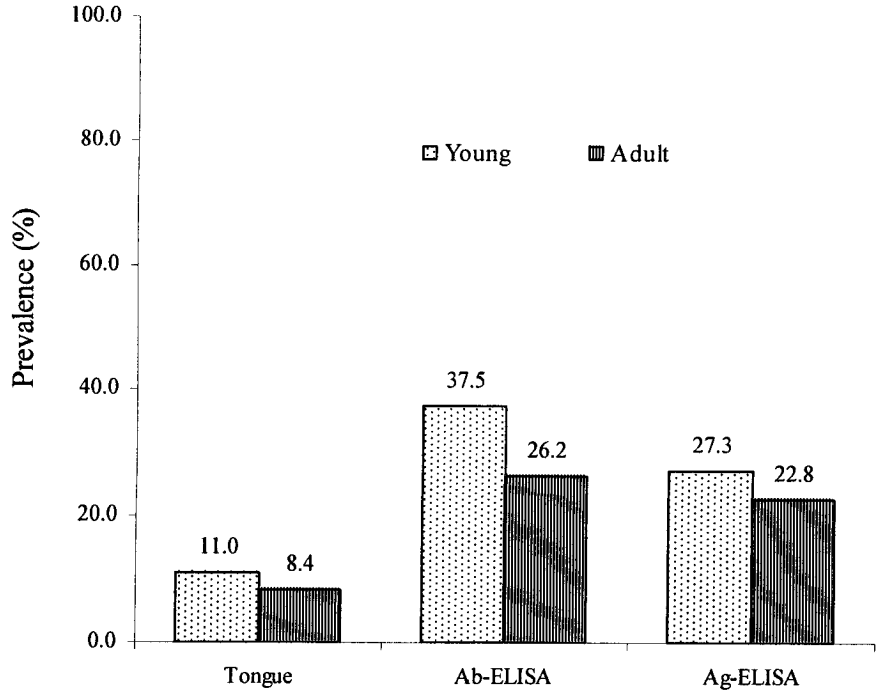


Figure 4.3 Prevalence of *T. solium* cysticercosis in young (n = 608) and adult (n = 663) pigs on tongue examination, Ab-ELISA and Ag-ELISA.

Table 4.3 Prevalence of *T. solium* porcine cysticercosis according to age in Southern and Eastern provinces.

Province	Age	n	Tongue +ve (%)	Ab-ELISA +ve (%)	Ag-ELISA +ve (%)
Southern	Young	389	51 (13.1)	135 (34.7)	121 (31.1)
	Adult	263	32 (12.2)	82 (31.2)	75 (28.5)
Total		*652*	*83 (12.7)*	*217 (33.3)*	*196 (30.0)*
Eastern	Young	219	16 (7.3)	93 (42.5)	45 (20.5)
	Adult	400	24 (6.0)	92 (23.0)	76 (19.0)
Total		*619*	*40 (6.5)*	*185 (29.9)*	*121 (19.5)*
TOTAL		**1271**	**123 (9.7)**	**402 (31.6)**	**317 (24.9)**

4.2 Prevalence of porcine cysticercosis by province

4.2.1 Southern province

A total of 772 pigs were examined in Southern province. Out of these, 385 and 387 pigs were from Gwembe and Monze, respectively. Prevalence on tongue examination results was 21.6% in Gwembe and 8.8% in Monze. On antibody ELISA the prevalence was 50.4% in Gwembe and 24.0% in Monze (Table 4.4).

Table 4.4 Prevalence of *T. solium* cysticercosis in pigs on Tongue examination, Ab-ELISA and Ag-ELISA in Gwembe and Monze districts in Southern province.

Province	District	n	Tongue +ve (%)	Ab-ELISA +ve (%)	Ag-ELISA +ve (%)
Southern	Gwembe	385	83 (21.6)	194 (50.4)	140 (36.4)
	Monze	387	34 (8.8)	93 (24.0)	94 (24.3)
Total		**772**	**117 (15.2)**	**287 (37.2)**	**234 (30.3)**

4.2.2 Eastern province

A total of 769 pigs were examined in Eastern province. Out of these, 384 and 385 pigs were from Petauke and Katete, respectively. Prevalence on tongue examination, Ab and Ag-ELISA results is shown in Table 4.5.

Table 4.5 Prevalence of *T. solium* cysticercosis in pigs on Tongue examination, Ab-ELISA and Ag-ELISA in Petauke and Katete districts in Eastern province.

Province	District	n	Tongue +ve (%)	Ab-ELISA +ve (%)	Ag-ELISA +ve (%)
Eastern	Petauke	384	25 (6.5)	188 (49.0)	63 (16.4)
	Katete	385	29 (7.5)	99 (25.7)	79 (20.5)
Total		**769**	**54 (7.0)**	**287 (37.3)**	**142 (18.5)**

4.3 Comparison of the three diagnostic tests (Tongue examination, Ab-ELISA and Ag-ELISA) used in the survey

Using the *Kappa* test, the tests were compared two by two in order to find out the agreement between the tests in the detection of porcine cysticercosis. The agreement in all the tests compared two by two was statistically significant ($p < 0.001$) (Table 4.6). The rate of agreement between tongue and Ab-ELISA was 67.9% while it was 79.4% between tongue and Ag-ELISA. The Ab-ELISA and Ag-ELISA only agreed in 69.5% of the pigs sampled. A total of 125 pigs had circulating antigens but were negative on tongue examination and antibody ELISA (Tables 4.6). The study also showed that 56 of the 171 tongue positive animals were negative on Ag-ELISA. The findings on cysticercosis prevalence in this study showed that 798 (51.8%) pigs were negative in all the three tests while 102 (6.6%) pigs were positive in all the three tests (Table 4.7). On the other hand, 46 positive pigs on tongue examination had no antibodies against cysticerci but 13 of these showed active infection on Ag-ELISA. However, 33 (2.1%) pigs that had cysts on the tongue had neither circulating antigens nor antibodies against *T. solium* cysticerci.

A total of 376 pigs were positive to Ag-ELISA. Of these 138 (i.e. 125 + 13) were negative on Ab-ELISA (Table 4.7). Twenty three (1.5%) pigs positive on both Ab-ELISA and tongue examination did not show presence of parasite antigens.

Table 4.6 Measure of agreement (Kappa) between the three different tests; Tongue examination, Ab-ELISA and Ag-ELISA using a two by two table for the detection of cysticercosis in rural pigs (n = 1,541)

	Tongue	Ab-ELISA		Ag-ELISA	
		No. +ve	No. -ve	No. +ve	No. -ve
Tongue	**No. +ve**	125	46	115	56
	No. -ve	449	921	261	1,109
Kappa value (p)		*0.2 (p<0.001)*		*0.32 (p<0.001)*	
Ab-ELISA	**No. +ve**	-	-	240	334
	No. -ve	-	-	136	381
Kappa value (p)				*0.3 (p<0.001)*	

Table 4.7 Correlation of the overall cysticercosis results obtained by Tongue examination, Ab-ELISA and Ag-ELISA

No. of pigs (%)	Tongue	Ag-ELISA	Ab-ELISA
798 (51.8)	-	-	-
311 (20.2)	-	-	+
136 (8.8)	-	+	+
125 (8.1)	-	+	-
33 (2.1)	+	-	-
23 (1.5)	+	-	+
13 (0.8)	+	+	-
102 (6.6)	+	+	+
1541 (100)			

4.4 Viability of cysts and ELISA detection

Of the 171 pigs that were positive by tongue examination, 128 pigs had viable cysticerci while 43 had calcified cysts on the tongue. From the 43 pigs with calcified cysts, 16 were positive to Ag-ELISA while 17 were positive to circulating antibodies against *T. solium* cysticerci (Ab-ELISA). Out of the 128 pigs with live cysticerci on their tongues, 29 were negative to Ag-ELISA while 20 were negative to Ab-ELISA. In Southern province, both the Ab-ELISA and Ag-ELISA detected (39.3%) pigs as having calcified cysts (Figure 4.4).

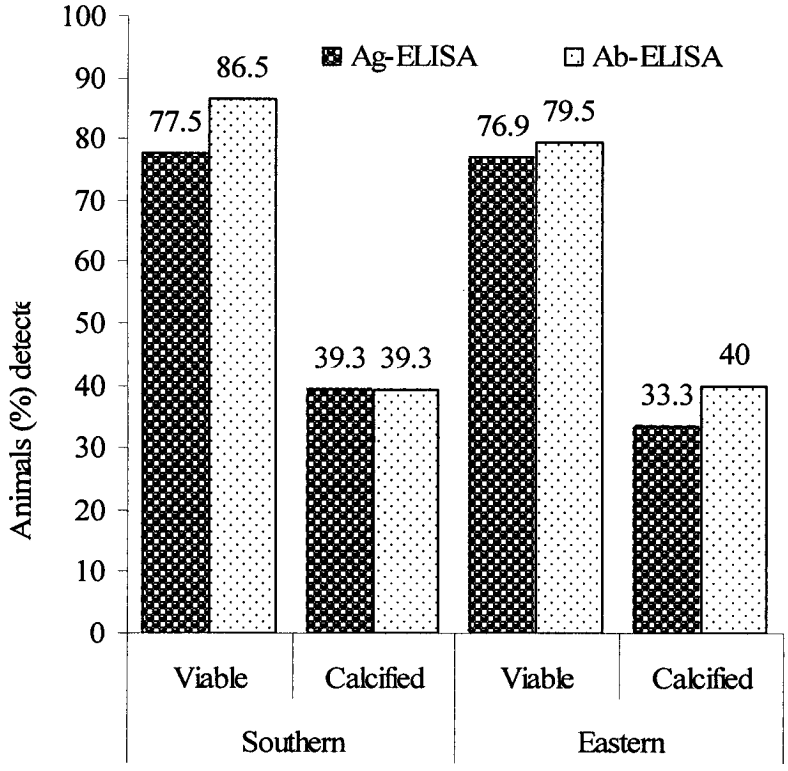


Figure 4.4 Comparison of viable (n = 128) and calcified (n = 43) cysticerci in pigs from Southern and Eastern provinces using Ag-ELISA and Ab-ELISA.

In order to find out the distribution of viable and calcified cysts among young and adult pigs from 1271 pigs, Table 4.8 was developed and used. A total of 123 pigs were positive to tongue examination and of these, 86 had viable and 37 had calcified cysticerci. In Eastern province, 90% of the calcified cysts observed were in adult pigs whereas, in Southern province there were more young pigs (70.4%) than adults (29.6%) which had calcified cysts. In Southern province, all the adult pigs observed with viable cysts were also detected positive on both Ab and Ag-ELISA.

Table 4.8 The distribution of type of cysticerci, viable or calcified found after Tongue examination compared with the results on Ag-ELISA and Ab-ELISA by age.

Province	Cyst type	Age	Tongue	Ab-ELISA	Ag-ELISA
			No. of cysts	No. +ve (%)	No. +ve (%)
Southern	Viable	Young	32	29 (90.6)	31 (96.9)
		Adult	24	24 (100)	24 (100)
	Calcified	Young	19	9 (47.4)	8 (42.1)
		Adult	8	1 (12.5)	2 (25.0)
Eastern	Viable	Young	15	12 (80.0)	13 (86.7)
		Adult	15	12 (80.0)	13 (86.7)
	Calcified	Young	1	0 (0)	0 (0)
		Adult	9	1 (11.1)	3 (33.3)

4.5 Investigation of risk factors

4.5.1 Sample description (Socio-demographics)

A total of 789 households were visited between June 2002 and September, 2003. On average each household had between 6 and 10 members. A total of 175 male respondents and 227 female respondents were interviewed from Southern, whereas 156 male and 231 female respondents were interviewed in Eastern province. The age

and sex distribution of the respondents is shown in Table 4.9. However, information on age was only obtained from 750 respondents. Overall, there were more females interviewed than males.

Table 4.9 The distribution of male and female respondents allotted into five age groups.

Age groups in years	Male Number (%)	Female Number (%)	Total Number (%)
< 20	10 (33.3)	20 (66.7)	30 (100)
20 to 30	92 (45.1)	112 (54.9)	204 (100)
31 to 40	87 (42.2)	119 (57.8)	206 (100)
41 to 50	54 (40.3)	80 (59.7)	134 (100)
> 50	77 (43.7)	99 (56.3)	176 (100)
Total	**320 (42.7)**	**430 (57.3)**	**750 (100)**

The education level attainment of the people in these households is shown in Table 4.10. Most of the respondents in Monze district (59.6%) had reached secondary level of education.

Table 4.10 Comparison of education level (grade 0-4; 5-7; and grade 8 and above) among the respondents in Gwembe, Monze, Petauke and Katete districts.

			Education level		
			Grade 0-4	Grade 5-7	Grade ≥ 8
Province	District	n	Number (%)	Number (%)	Number (%)
Southern	Gwembe	201	46 (22.8)	80 (39.8)	75 (37.3)
	Monze	198	6 (8.8)	74 (37.4)	118 (59.6)
Total		*399*	*52 (13.0)*	*154 (38.6)*	*193 (48.4)*
Eastern	Petauke	162	50 (30.9)	56 (34.6)	56 (34.6)
	Katete	220	69 (31.4)	84 (38.2)	67 (30.5)
Total		*382*	*119 (31.2)*	*140 (36.6)*	*123 (32.2)*
TOTAL		781	171 (21.9)	294 (37.6)	316 (40.5)

4.5.2 General pig management system

From a total of 789 farmers interviewed, 656 (83.2%) kept pigs on free-range, 133 *(16.8%) practiced semi-intensive management system by enclosing pigs during the* rainy season and leaving them to scavenge during the dry season (See Figure 4.5). The majority of those who practiced semi-intensive management system were from Southern province 32.3% while Eastern province had only 0.5%. About 99.5% (n = 388) of the farmers in Eastern province reared pigs on free-range as compared to 67.7% (n = 412) of the farmers in Southern province.

In both provinces, pigs were normally kept in small shelters or Kraals (Figures 3.2 and 3.4) during the rainy season and were left to scavenge during the dry season. The

pigs were often supplementary fed with agricultural by-products such as pumpkins, cucumbers, watermelons and in most cases maize bran.

Figure 4.5 Scavenging pigs on free-range seen at a village ground in Eastern province (Note the background bushes which could easily serve as toilets for the villagers).

4.5.3 Purpose for keeping pigs

Most of the pig farmers in Southern province (86.1%) kept pigs with the sole aim of selling whereas, farmers in Eastern province (98.4%) kept pigs for both sale and home consumption (Table 4.11). Less than 1% of the interviewed farmers kept pigs for home consumption in both Eastern and Southern provinces (Table 4.11).

Table 4.11 Some of the most important reasons advanced by pig farmers for keeping pigs by district and province.

			Reason		
			Sale	Consumption	Sale/Consumption
Province	**District**	**n**	**Number (%)**	**Number (%)**	**Number (%)**
Southern	Gwembe	202	176 (84.2)	2 (1.0)	24 (11.5)
	Monze	200	170 (83.7)	0 (0.0)	30 (14.8)
Total		*402*	*346 (86.1)*	*2 (0.5)*	*54 (13.4)*
Eastern	Petauke	167	2 (1.2)	0 (0.0)	165 (98.8)
	Katete	220	3 (1.4)	1 (*0.5*)	216 (98.2)
Total		*387*	*5 (1.3)*	*1 (0.3)*	*381(98.4)*
TOTAL		**789**	**351 (44.5)**	**3 (0.4)**	**435 (55.1)**

Farmers in Southern province slaughtered their pigs for either home consumption or sale much earlier, at 14.42 (±6.78 S. D) months than the farmers in Eastern province who did not slaughter until 21.57 (±8.43 S. D) months. On average people in Eastern province had kept pigs for 11.59 (±9.77 S. D) years, range 1-66 compared to their counterparts in Southern province who had been keeping pigs for only 6.16 (±6.49 S. D) years with a range of 0.5-40 years. Most of the households (63.2%) in Southern province had a herd size of less than 3 pigs per household (Table 4.12).

Table 4.12 Number of pigs (herd size) per household in Southern and Eastern provinces.

Province	District	n	Herd size				
			< 3 (%)	3 to 5 (%)	6 to 8 (%)	9 to 10 (%)	> 10 (%)
Southern	Gwembe	202	119 (58.9)	50 (24.8)	19 (9.4)	8 (4.0)	6 (3.0)
	Monze	200	135 (67.5)	47 (23.5)	13 (6.5)	1 (0.5)	4 (2.0)
Total		*402*	*254 (63.2)*	*97 24.1)*	*32 (8.0)*	*9 (2.2)*	*10 (2.5)*
Eastern	Petauke	167	36 (21.7)	27 (16.3)	44 (26.5)	17 (10.2)	42 (25.3)
	Katete	220	57 (25.9)	54 (24.5)	44 (20.0)	23 (10.5)	42 (19.1)
Total		*387*	*93 (24.0)*	*81 (20.9)*	*88 (22.7)*	*40 (10.3)*	*84 (21.7)*
TOTAL		**789**	**347 (44.0)**	**178 (22.6)**	**120 (15.2)**	**49 (6.2)**	**94 (11.9)**

4.5.4 Constraints in pig rearing

Farmers expressed diseases and feeding (49.1%) and diseases alone (41.3%) as the most common problems affecting pig rearing in Eastern province. Whereas, feeding (49.5%) was the main problem cited by the pig farmers in Southern province (Figure 4.6). African Swine Fever (A.S.F) was the major disease that the farmers in both Southern and Eastern provinces complained about. In Southern province A.S.F is called *Chigumula* (Tonga) while in the Eastern province it is called *Chigomola* or *Chipumpu,* (Nyanja). Literal translation in both cases simply means something that causes high mortality. The "Other" category of problems farmers cited included lack of market, theft, mischief and none.

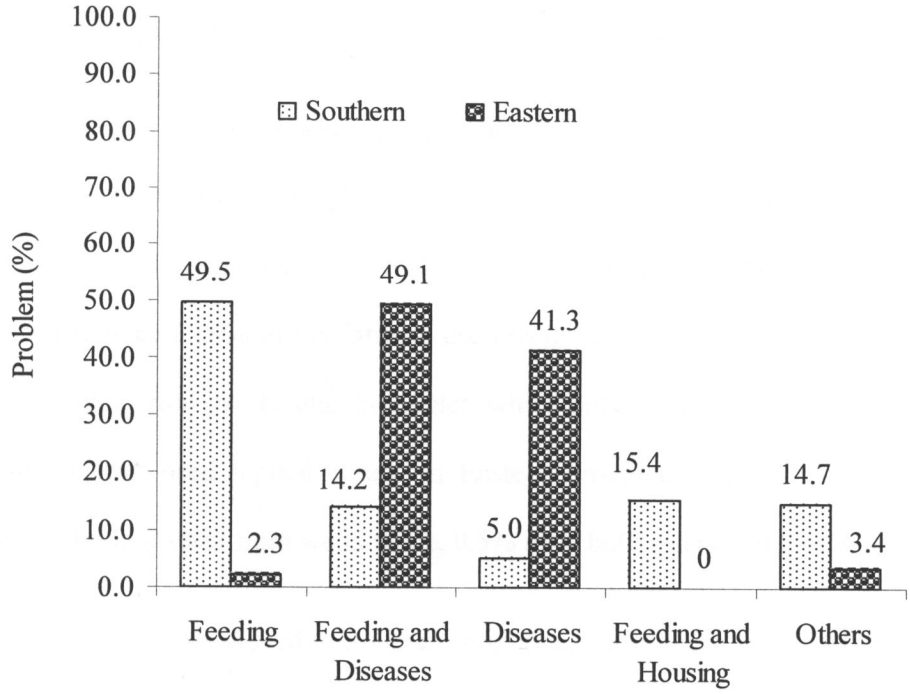


Figure 4.6 Problems encountered by pig farmers in the surveyed areas of Southern and Eastern provinces of Zambia.

4.5.5 Possible transmission risk factors

Most of the farmers in both Eastern and Southern provinces consumed pork (92.3%) and slaughtered pigs at home without inspection (96.7%) (Table 4.13).

It was also found that boreholes were a main source of drinking water to 56.0% of the farmers in Southern and 71.8% in Eastern provinces. Other farmers used rivers as their source of drinking water in Southern (15.9%) and Eastern (9.0%) provinces. In Southern province 22.9% of the farmers used wells, 1.5% used shallow wells, 3.0% obtained water from rivers and boreholes while only 0.8% of the farmers from Gwembe district used tapped water. In Eastern province, 18.6% of the farmers obtained drinking water from wells while, 0.5% used both rivers and boreholes.

Of the 402 households visited in Southern province, 36.9% used toilets while 63.1% did not (Figure 4.7). Out of the 220 households visited in Katete, 49.5% households used toilets with only 33.8% of the respondents in Gwembe that used toilets (Table 4.14).

Seroprevalence based on porcine seropositivity on Ag-ELISA for households without latrines was 38.9%; those that had latrines but did not use them was 35.4% and those that used latrines was 37.0%. Analysis of prevalence of porcine cysticercosis on Ag-ELISA in pigs raised in households with or without a latrine yielded no statistical significance ($\chi^2 = 1.76$, $p < 0.184$).

Table 4.13 The number and percentage of households in Gwembe, Monze, Petauke and Katete districts with respect to pork consumption, home slaughter and status of pork inspection.

Province	District	n	Pork consumption	Home slaughter	Lack of pork inspection
			Number (%)	Number (%)	Number (%)
Southern	Gwembe	202	178 (88.1)	128 (63.4)	193 (95.5)
	Monze	200	171 (85.5)	152 (76.0)	194 (97.0)
Total		*402*	*349 (86.8)*	*280 (69.7)*	*387 (96.3)*
Eastern	Petauke	167	164 (98.2)	156 (93.4)	159 (95.2)
	Katete	220	215 (97.7)	217 (98.6)	217 (98.6)
Total		*387*	*379 (97.9)*	*373 (96.4)*	*376 (97.2)*
TOTAL		789	728 (92.3)	653 (82.8)	763 (96.7)

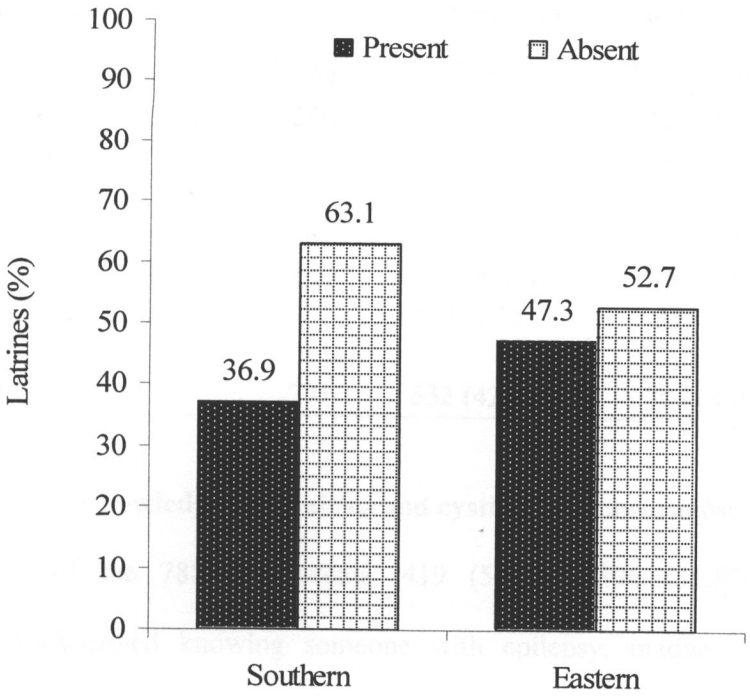


Figure 4.7 Summary presentation of latrine availability (%) and their usage based on the households interviewed in Southern (n = 404) and Eastern (n = 387) provinces.

Table 4.14 The number and percentage (%) of latrine availability and their usage based on the households interviewed in Southern and Eastern provinces

Province	District	n	Latrine is Present and used	Latrine is Present but not used	Latrine is Absent
			Number (%)	Number (%)	Number (%)
Southern	Gwembe	204	69 (33.3)	24 (11.8)	111 (54.4)
	Monze	200	80 (40.0)	22 (11.0)	98 (49.0)
Total		*404*	*149 (36.9)*	*46 (11.4)*	*209 (51.7)*
Eastern	Petauke	167	74 (44.3)	9 (5.4)	84 (50.3)
	Katete	220	109 (49.5)	24 (10.9)	87 (39.5)
Total		*387*	*183 (47.3)*	*33 (8.5)*	*171 (44.2)*
TOTAL		**791**	**332 (42.0)**	**79 (10.0)**	**380 (48.0)**

4.5.6 Knowledge of taeniosis and cysticercosis awareness

Out of the 788 respondents, 419 (53.2%), 306 (38.8%), and 434 (55.1%) acknowledged knowing someone with epilepsy, madness and/or had heard of someone with chronic headache problems, respectively, symptoms associated with taeniasis and cysticercosis infection in their community (Table 4.15). Those who acknowledged hearing of someone suffering from epilepsy and madness were more in Eastern province than Southern province. Only 3.2% of the pig farmers in Southern and 24.3% in Eastern provinces had heard about someone who had suffered from a tapeworm infection. Very few farmers in Southern province (2.7%) admitted having heard of someone complain of tapeworm infection in their communities while in the Eastern province it was 9.8%. Only 2.0% of the farmers in Southern province

and 14.5% in Eastern province knew that a person infected with tapeworm could pass them out in faeces. The knowledge that tapeworm infection in humans was due to eating infected pork was only known to 1.5% and 12.7% of the respondents in Southern and Eastern provinces, respectively.

Table 4.15 Respondents who heard or knew of someone suffering from epilepsy, madness and/or chronic headache from surveyed households in Gwembe, Monze, Petauke and Katete districts.

Province	District	n	Epilepsy	Madness	Headache
			Number (%)	Number (%)	Number (%)
Southern	Gwembe	201	48 (33.9)	12 (6.0)	88 (43.8)
	Monze	200	18 (9.0)	14 (7.0)	30 (15.0)
Total		*401*	*66 (16.5)*	*26 (6.5)*	*118 (29.4)*
Eastern	Petauke	167	143 (85.6)	124 (74.3)	120 (71.6)
	Katete	220	210 (95.5)	156 (70.9)	196 (89.1)
Total		*387*	*353 (91.2)*	*280 (72.4)*	*316 (81.7)*
TOTAL		**788**	**419 (53.2)**	**306 (38.8)**	**434 (55.1)**

This study has revealed that quite a substantial number of respondents (83.3%) had observed cysts in pork (Table 4.16). The Nyanjas of Eastern province called cysts in pork as *mushokwe, mase, masese* or *gaga* meaning maize bran. In Southern province, the cysts in pork were again commonly known as *masese* meaning maize bran or *tukotokoto* meaning nodules.

Of the 659 farmers who observed cysticercosis, 43.8% acknowledged pork measles as just being a pig disease, 6.5% simply referred to it as being husks or maize bran

whereas, 48.3% had completely no idea of what cysts seen in pork were. Only 2.7% of the farmers knew that pig infection with cysticercosis was due to eating human faeces. Interestingly, 20.1% of the respondents admitted eating pork infected with cysts and, 18.3% acknowledged selling pork with cysticercosis (Table 4.16).

Respondents in Petauke and Katete districts of Eastern province (97.4%) admitted seeing porcine cysticercosis relatively more frequently than their Gwembe and Monze colleagues in Southern province (69.8%). A larger proportion of farmers in Katete (41.8%) also admitted to eating infected pork compared with 2.5% in Monze district (Table 4.16).

Table 4.16 *The number (percentage) of farmers that had observed cysts in pork,* and those that ate and sold infected pork in the districts visited; Gwembe, Monze, Petauke and Katete from the households that were interviewed.

			Parameter		
			Observation of cysts in pork	**Ate pork with cysts**	**Sold pork with cysts**
Province	**District**	**n**	**Number (%)**	**Number (%)**	**Number (%)**
Southern	Gwembe	204	136 (65.1)	30 (14.7)	34 (16.7)
	Monze	200	146 (71.9)	5 (2.5)	5 (2.5)
Total		*404*	*282 (69.8)*	*35 (8.7)*	*39 (9.7)*
Eastern	Petauke	167	162 (96.4)	32 (19.2)	33 (19.8)
	Katete	220	215 (97.7)	92 (41.8)	77 (35.0)
Total		*387*	*377 (97.4)*	*124 (32.0)*	*110 (28.4)*
TOTAL		**791**	**659 (83.3)**	**159(20.1)**	**149 (18.3)**

4.5.7 Prevalence of *T. solium* porcine cysticercosis in households

Households with prevalence of *T. solium* in pigs on tongue examination ranged from 12.7% to 32.1 (Table 4.17 and appendices 6 to 9). Ab-ELISA results ranged from 35.0% to 65.1%, with Ag-ELISA having a range of 30% to 51.7% per households. Gwembe had the highest prevalence rates in all the tests employed in this study. The study showed that 37.6% households were positive for porcine cysticercosis having at least one positive pig on Ag-ELISA (Table 4.17).

Table 4.17 Households prevalence of *T. solium* cysticercosis in Gwembe and Monze in Southern and Petauke and Katete in Eastern provinces based on Tongue examination, Ab-ELISA and Ag-ELISA

			Test		
			Tongue	**Ab-ELISA**	**Ag-ELISA**
Province	**District**	**n**	**+ve (%)**	**+ve (%)**	**+ve (%)**
Southern	Gwembe	209	67 (32.1)	136 (65.1)	108 (51.7)
	Monze	203	33 (16.3)	71 (35.0)	76 (37.4)
Total		*412*	*100 (24.3)*	*207 (50.2)*	*184 (44.7)*
Eastern	Petauke	168	22 (13.1)	106 (63.1)	51 (30.4)
	Katete	220	28 (12.7)	81 (36.8)	66 (30.0)
Total		*388*	*50 (12.9)*	*187 (48.2)*	*117 (30.2)*
TOTAL		**800**	**150 (18.8)**	**394 (49.3)**	**301 (37.6)**

CHAPTER FIVE:

5. DISCUSSION

5.1 Prevalence of porcine cysticercosis

In this study a total of 171 (11.1%) pigs examined were positive on tongue examination. Pigs from Southern province had significantly higher prevalence (15.2%) than pigs from Eastern province (7.0%). The Ag-ELISA assay detected a total porcine cysticercosis prevalence of 24.4% thus 376 pigs had circulating antigen. The Ag-ELISA like lingual examination showed that Southern province (30.3%) had significantly higher prevalence than Eastern province (18.5%). These findings confirm the results of a preliminary field survey conducted by Phiri *et al.* (2002) which revealed a similar trend where, 8.2% of pigs from Southern province and 5.2% of pigs from Eastern province were positive for cysticercosis by tongue examination. Similarly, Southern province showed a higher prevalence than Eastern province. Phiri *et al.,* 2002, in the same survey using the Ag-ELISA, found 20.8% and 9.3% of the test pigs positive to cysticercosis in Southern and Eastern provinces, respectively. The prevalence rates obtained in this study, were however, higher than those previously reported by Phiri *et al.* (2002). Probably, this difference could arise from the wider survey coverage and to a larger sample size in the present study compared to Phiri *et al.* (2002). We observed 15.2% positive pigs on tongue examination in Southern province compared to 10.9% which was reported earlier in a tongue examination survey at Chibolya slaughter slab in Lusaka, done on pigs originating from Southern province (Phiri *et al.,* 2002). Our results agree with those of Sarti *et al.* (1992a) and Carrique-Mas *et al.* (2001) who reported that abattoir surveys appear

to under estimate the real prevalence of the disease. This difference in prevalence clearly demonstrates that pig data obtained from abattoirs does not reflect the true situation of the disease in rural communities. This low prevalence rate reported from the abattoir survey could be substantiated by the fact that in Southern province, the tongue examination method of determining cysticercosis in pigs is widely used by certain pig traders. As a result, these pig traders buy only those pigs that pass the test.

Our *T. solium* prevalence results are much higher than those reported from abattoirs studies in the Southern African region. In a retrospective study in Zimbabwe, it was observed that the overall mean prevalence of porcine cysticercosis was only 0.34%, however this biased figure was attributed to use of official abattoir records which included commercial pigs (Matenga *et al.*, 2002). In Mozambique, abattoir records indicated that porcine cysticercosis was present in all provinces of the country (Afonso *et al.*, 2001). A sero-prevalence study using an antibody-detecting ELISA based on purified cyst fluid antigen (fraction 17A) in 11 districts of rural Tete Province showed that 15% (n = 387) pigs were positive (Afonso *et al.*, 2001). These results were again much lower than our total Ab-ELISA results of 37.2% (n = 1,541). An extensive survey conducted in 1937 in 67 slaughterhouses in different areas of South Africa indicated a porcine cysticercosis prevalence of 0.5 to 25.07% (Viljoen, 1937).

The prevalence of *T. solium* observed in this survey is also higher than those reported in a retrospective study conducted at a slaughter slab in Mbulu district of Tanzania from 1985 to 1989 which indicated a prevalence of 0.41% to 4.88% (Nsengwa, 1995). Boa *et al.* (1995) conducted a post-mortem survey in pigs slaughtered at

different slaughter slabs in the northern highlands (Arusha, Moshi and Mbulu districts) of Tanzania, and found a prevalence of porcine cysticercosis of 4.5 - 37.7%, with the majority of the pigs originating from Mbulu district. A more recent field survey by tongue examination of pigs in Mbulu district also revealed an overall district prevalence of 17.4% (Ngowi, 1999).

After comparing prevalence by gender, there was evidence to suggest that a higher prevalence ($\chi^2 = 3.57$, $p = 0.059$) was found in male 27.8% than female 23.0% pigs on Ag-ELISA. This finding agrees with that of Sarti-G *et al.* (1992a) who found that the prevalence of cysticercosis was slightly higher in male than in female pigs and that it increased with age. However, our results disagree with the results obtained by Sarti *et al.* (1992b) who reported that there was no significant difference between male (4.2%) and female (3.9%) pigs after tongue examining 571 pigs. Rodriquez-Canul *et al.* (1998) also found that there was no difference in prevalence by sex ($n = 75$) whether estimated by tongue palpation ($\chi^2 = 0.07$; $p = 0.789$), or by immunoblot ($\chi^2 = 1.42$; $p = 0.233$). Such a result is possible because the number of pigs examined was small compared to our study ($n = 1,541$) and that this difference in results probably could be due to a number of factors including pig breeds, geographical differences and psychosocial factors. One possible explanation for our findings could be that male pigs tend to bully the female counterparts as they scavenge for food. 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.

The Ag-ELISA showed apparently a higher prevalence in young (27.3%) than in adult (22.8%) pigs, though not statistically significant. Rodriquez-Canul *et al.* (1998), also obtained similar results when they found that seroprevalence in pigs decreased with age. This finding is in line with Aluja *et al.* (1998) who reported that in rural areas, piglets become infected at about the age of 4 weeks, immediately after weaning and that in tropical areas; infection in piglets is higher during the dry and hot season. Aluja *et al.* (1998) attributed this to the fact that adult animals are reluctant to move at high temperature while young ones remain active and thus have access to faecal material during the hot season. In contrast, Pouedet *et al.* (2002) found that adult pigs had significantly higher prevalence (15%) than young pigs (8.4%) on Ag-ELISA. Probably the apparent high prevalence in young pigs observed in this study could be due to time of sampling as most of the samples were collected in the dry season.

In our study, the prevalence of porcine cysticercosis on tongue examination was lower than that obtained either with Ag-ELISA or Ab-ELISA. Because tongue examination only offers an estimate of infection levels, not all active infections can therefore be easily detected by this method (Sarti *et al.*, 1992a). In this study, tongue examination could only detect 30.6% (115/376) of those positive on Ag-ELISA and were assumed to have had active infection. However, the tongue examination result in our study was higher than that reported by Viljoen (1937) in South Africa at Bloemfontein abattoir. Viljoen (1937) reported only about 25% of infected pigs had cysticerci visible by tongue examination. Although an experimental study by

Gonzalez *et al.* (1990) in Peru indicated that 70% of infected pigs could be detected at antemortem by tongue examination.

The Ab-ELISA prevalence rates obtained in this study from both Southern and Eastern provinces may be an overestimate on the real situation due to possible cross-reactions with other parasites that could be harboured by the sampled pigs such as ascaridosis and hydatidosis (Pinto *et al.,* 2000). Cross-reactions are also likely to account for the 311 (20.2%) pigs that showed apparent circulating antibodies against *T. solium* cysticerci but had no cysts on the tongue and had no circulating antigens against *T. solium* cysticerci detected. These pigs were therefore, false positives resulting from possible ascaridosis or other helminth infections. However, cross-reactions can be avoided if purified glycoproteins of *T. solium* are used (Ito, 2002). Transient antibodies, especially in young pigs can also result in high Ab-ELISA prevalence results compared with either tongue or Ag-ELISA tests. This implies that presence of maternal antibodies could mean that some of the young pigs that were seropositive on Ab-ELISA had neither active infection nor exposure to *T. solium* infection. A study by Gonzalez *et al.* (1999) showed that maternal antibodies passed from a seropositive sow to the piglets persisted from 7 months to life time of the pig. In humans, the occurrence of a transient antibody response in *T. solium* infection under field conditions was found to be a major contributor to the over estimation of the prevalence of cysticercosis in endemic areas of Peru and Columbia (Garcia *et al.*, 2001). According to Garcia *et al.* (2001), serological surveys demonstrated that about 40% of seropositive people were seronegative when re-sampled after one year. The reliability of the data obtained from the farmers on the age of pigs is also

questionable, because of the lack of stock record keeping. During our study, only those pigs which were equal to and above 6 months of age were sampled to avoid transient antibodies from influencing the Ab-ELISA results. Age estimate becomes complicated when dealing with the dwarf local indigenous breed of Eastern province, where adult pigs could look younger than their actual age.

The 136 (8.8%) found negative on tongue examination but positive to both Ag and Ab-ELISA, was a normal finding because not all positive pigs necessarily have cysts on the tongue. Sarti-G *et al.* (1992a) reported that probably infection intensity could be the most important factor determining whether cysts are discernible by visual inspection of the tongue or not. The 33 (2.1%) pigs positive on tongue examination but negative to both Ag-ELISA and Ab-ELISA could be attributed to the fact that not all the calcified nodules observed were a result of *T. solium* infection. Similar lesions or scars can also be caused by mechanical injuries sustained due to the feeding habits of the pigs, as was observed by Pouedet *et al,* (2002). The cysts observed in 23 (1.5%) pigs found negative to the Ag-ELISA could have been dead or calcified cysts and thus producing no antigen products. Thirteen (0.8%) pigs found positive to both tongue examination and Ag-ELISA but negative to Ab-ELISA could mean that the cysts observed on the tongue were live and released antigen. However, these pigs could have had low cyst burdens and thus, the cysts did not elicit enough antibody response to levels that could be detected by the Ab-ELISA and/or simply represents false positives. This could have been compounded by the fact that the Ab-ELISA, when using crude cyst fluid as antigen has a poor sensitivity (Dorny, personal communication). Sciutto *et al.* (1998b) found that Ag-ELISA, Ab-ELISA or EITB

were not adequate for the diagnosis of porcine cysticercosis in lightly infected village pigs. Sciutto *et al.* (1998b) further noted that pigs with low cyst burden may escape detection by meat inspection thereby maintaining parasite transmission by allowing lightly infected carcasses to remain in the food chain. This may explain the variations in detection by the three tests used in this study.

The 125 (8.1%) pigs found negative to the Ab-ELISA and tongue examination but having circulating antigen against *T. solium* cysticerci could be attributed to cysts located elsewhere in the body other than the tongue, and thus showed positivity to the Ag-ELISA or simply represents false positives. The finding of 137 (125 + 13) pigs (n = 1,541) positive to Ag-ELISA but negative to Ab-ELISA was similar to the finding obtained by Pouedet *et al.* (2002). Pouedet *et al.* (2002) found that 27 pigs (n = 707) were positive to Ag-ELISA and yet were negative to Ab-ELISA. They attributed this to the low sensitivity of the antibody ELISA and that probably there could be some false positive reactions with the Ag-ELISA. The proposition that Ag-ELISA could have false positive reactions was supported by Dorny *et al.* (2004) who found that *T. solium* cysticercosis infection in pigs using the Ag-ELISA cross-reacts with those of *Cysticercus tenuicollis*, the larval stage of *T. hydatigena*. Dorny *et al.* (2004) further stated that the cross reactions observed with the larval stage of *T. hydatigena* were not surprising because the monoclonal antibodies used in this Ag-ELISA for the diagnosis of *T. solium* porcine cysticercosis, were prepared against excretory-secretory antigens of the larval stage of the bovine tapeworm, *T. saginata*. Dorny *et al.* (2004) concluded that the Ag-ELISA is only genus but not species specific. However, Dorny *et al.* (2004) reported that the prevalence of *T. hydatigena*

in small ruminants in Africa and Zambia in particular is very common but rather very negligible in pigs. Unlike Zambia, the prevalence of *T. hydatigena* in pigs in Vietnam is widespread and thus seriously impairs the usefulness of the Ag-ELISA in that country (Dorny *et al.*, 2001; 2004).

The high prevalence *T. solium* infection in pigs found in this study strongly suggests the presence of many human carriers of the pig tapeworm. It also implies that pigs in these rural areas have high exposure to faeces contaminated with *T. solium* eggs.

5.2 Investigation of risk factors

The people of Eastern province have practiced pig rearing for a relatively longer period as seen from the average years of experience in pig keeping than those of Southern province. This may mean that the problem of porcine cysticercosis has equally been in Eastern province for a long period. In Southern province, pig rearing is quite a recent activity. However, the prevalence *T. solium* cysticercosis in pigs was higher in Southern province than Eastern province. This could be that there are more tapeworm carriers in Eastern province and thus more frequent infection in pigs leading to immunity development due to repeated infections. The other reason could be that probably, the small villages and limited use of latrines in Southern province lead to pigs having more access to human faeces than Eastern province. It may be anticipated also that the indigenous pigs that are predominant in Eastern province are more resistant/resilient to the pork tapeworm infection than the exotic breeds in Southern province. The observation that people in Eastern province keep a lot of dogs than in Southern province could also explain why the prevalence is low in Eastern province. Since dogs have a more developed olfactory lobe, it is possible that

they will locate the faeces more easily and faster than pigs. Hence, the relative lower cysticercosis prevalence observed in Eastern province. Our observation is in agreement with that of Ito *et al.* (2002) who reported that although pigs are the most important intermediate hosts of economic importance, dogs are also highly susceptible and become intermediate host and that they may be involved in the completion of the life cycle in areas where dog meat in consumed.

Our study revealed that pigs were of great importance in these rural areas of Zambia economically and constituted a major source of dietary protein to the majority of the pig farmers. It is also evident from this study that farmers venture into the business of rearing pigs so as to get a source of income. Lekule and Kyvsgaard (2003) stated that the pig has functions that are not reflected in a simple economic balance. They further stated that a pig is a source of capital income, which can be realised at times of need, and it can also be used as a way to put aside small amounts of money, which alternatively might be lost. In this study, it was evident that most farmers opt for rearing pigs on free-range because of the problems of lack of resources such as feed and also the labour involved in confining the pigs in houses. This is in agreement with Gilman *et al.* (1999) who 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. These authors also suggested that permitting pigs to range also has a secondary advantage in that pigs are utilised as sanitarians to maintain villages free from garbage, small vermin and animal and human faeces. Rodriquez-Canul *et al.* (1999) noted that the main risk factor for a pig in terms of acquiring cysticercosis appeared to be the free-range type of husbandry system

employed by the owners. Sarti *et al.* (1992b) during their study in a village in Morelos, Mexico observed that pig husbandry practices within the communities appeared to be the main risk factor associated with porcine cysticercosis, as free-ranging pigs, have a much higher access to human faeces in communities with few or no latrines. Pouedet *et al.* (2002) also found that pigs with access to human faeces were more infected than those without access and that some of the households allowed pigs' access to human faecal material.

The average herd size per household found in this study was greater than one. With the problem of lack of feed experienced by these resource poor farmers, feeding more than one pig is indeed a big burden. This study revealed that most of the farmers used maize bran to supplement their pigs. Lekule and Kyvsgaard (2003) reported that though most of these households have some kind of kitchen waste, the amount of waste is limited and only sufficient for the partial feeding of a single pig per household.

This study found that most of the farmers allowed pigs to be on free range. Others were not using toilets and therefore could have been using the nearby bush for defecation. The proposition that people used the nearby bush for defecation is supported by the observation that in Eastern province, most of the households that were located on the periphery of the village had positive pigs on tongue examination. This could also suggest that the pigs in households along the edge of the villages were more exposed to tapeworm eggs than the ones in the interior of the village. The keeping of pigs on free-range and defecating in the bush instead of using the toilets, permitted pigs to have access to human faeces. This could account for the high

prevalence of *T. solium* infection in pigs found in our study. Sarti *et al.* (1997) showed that extensively raised pigs have a higher seroprevalence of cysticercosis than intensively raised pigs. Garcia *et al.* (1999) reported that although the pig is the essential intermediate host for *T. solium*, little attention has been paid to the risk factors involving human and porcine infection and that the transmission dynamics of taeniasis and cysticercosis are poorly understood especially with regard to the relationship of human and porcine infection under field conditions. Analysis of prevalence of porcine cysticercosis in pigs raised in households with or without a latrine on Ag-ELISA yielded no statistical significance. One explanation could be that the households were close to one another and that the pig population is open and homogenous. However, Diaz *et al.* (1992) in their studies in Peru reported a statistically significant association between latrines and households with at least one pig with lingual cysticercosis.

The results also show that the majority of the farmers slaughtered without inspection. Others consumed and sold infected pork and still others did not eat infected pork but sold it to other people within their communities. Besides, some farmers accepted eating infected pork if the carcass was not heavily infected and still others could only eat such pork after drying it (making some kind of "Biltong"). Though most of these farmers admitted eating infected pork, others could have eaten infected pork unknowingly if the carcasses had light (low cyst burden) infections of *T. solium* cysticerci. According to Sarti *et al.* (1994) the risk factors associated with human taeniasis and cysticercosis included eating of infected pork and close proximity to a carrier of *T. solium*. Rodriquez-Canul *et al.* (1999) also reported that eating infected

pork was found to be a high risk of acquisition of infection in an individual who had consumed pork infected with cysticerci.

Poor hygiene and living conditions, allowing pigs access to human faeces, put people at risk of developing cysticercosis (Sarti *et al.,* 1992b; Sanchez *et al.*, 1997). In this study we found that the majority of the households had inhabitants between 6 and 10 implying that these numbers of people per household were at risk of acquiring taeniasis and/or cysticercosis in those households with infected pigs. Sarti *et al.* (1992b) reported that being a member to a household which has a pig infected with cysticercosis may increase the risk of acquiring taeniasis and more so if some members of the household consume pork.

The results of our study show that the people in these surveyed areas did not know porcine cysticercosis *per se* despite giving it many names. The few that knew that tapeworm infection in human was due to eating infected pork were ignorant about the fact that a person with taeniasis could infect another person suggesting that there could be a high prevalence of the disease in humans. Sanchez *et al.* (1997) found that the less the population knew about the existence of the parasite, the greater the risk they had of being seropositive.

The fact that most of the farmers have heard of someone suffering from epilepsy and madness in their communities is suggestive that there could be widespread human cysticercosis and/or neurocysticercosis in these surveyed villages. 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. 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, underrecognized, and undertreated in that population.

In West Cameroon, according to surveys conducted by Preux *et al.* (2000), only 27% of epileptics get married and 39% fail to enter into any professional activity. 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 (Preux *et al.,* 2000).

This study, therefore confirms that there is a high prevalence of porcine cysticercosis in the surveyed villages, which in turn suggests that there are *T. solium* human carriers. It is recommended that a human survey be conducted to verify the human exposure to taeniasis and/or cysticercosis in these surveyed areas. In addition, *T. solium* infection has posed a high public health risk in these rural areas. Though we found a high prevalence of *T. solium* porcine cysticercosis, the farmers' knowledge about how the pig acquires this infection or about the cysts they frequently observed in pork was beyond their scope. Therefore, the life cycle of *T. solium* is sustained because pigs have access to infected faeces, and cysticercosis-infected pork is available for consumption to both pig owners and to those who do not keep pigs. People are ignorant not only about the disease but also about the parasite.

CHAPTER SIX:

6. CONCLUSION AND RECOMMENDATIONS

It is proven that *T. solium* porcine cysticercosis exists and is prevalent in the study areas. Trends also indicate that pig keeping and pork consumption are growing dramatically in Zambia and that pig keeping in Zambia is predominantly of the traditional type, characterised by a free-range management system. Pigs are slaughtered mainly in the backyard or at illegal slaughter slabs where no meat inspection is performed.

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 consequently directly contaminates their environment with eggs containing oncospheres that are infective to both humans and pigs. This in turn suggests that human neurocysticercosis and cysticercosis/taeniasis are most likely a serious health risk in these rural areas as well as urban centres where pigs from rural areas are increasingly sold, slaughtered and consumed. Gilman *et al.* (1999) reported that although the seroprevalence in pigs is usually two to three times that in humans, in a rural community in the coast of Peru, pigs had a lower seroprevalence than did the human population.

The results of our study also suggests that pigs in these surveyed villages have increased exposure to *T. solium* eggs owing either to greater prevalence of human taeniasis, or to free-range systems of keeping pigs. It also appears that villagers do not view eating infected pork as a health risk of getting taeniasis/cysticercosis and as

such it does not influence actions as strongly as does the immediate economic benefit of maintaining pigs in a cheap but unhealthy manner.

This study has therefore, identified some factors contributing to optimal conditions for transmission of this zoonotic pork tapeworm. These included free-roaming management of pigs, the lack of latrines in most households or their use, the absence of pork inspection and the lack of awareness by the farmers of the risks involved in eating pork infected with *T. solium* cysts. Therefore, the life cycle of *T. solium* is sustained because pigs have access to infected faeces, and cysticercosis-infected pork is available for consumption to both pig owners and to those who do not keep pigs.

Pal *et al.* (1999) reviewed that reform of animal husbandry techniques, meat inspection procedures and adequate cooking of pork are difficult approaches and of limited relevance in developing countries, where pigs are free roaming or raised by subsistence farmers who can not afford enclosed pens or proper animal feed, and meat is sold off outside the abattoir system. However, Sarti *et al.* (1997) reported that improved health education has been effective in altering practices associated with parasite transmission.

Though it is commonly thought that the population at highest risk are located in rural areas, where scavenging pigs have access to human faeces and people eat undercooked pork, it is important to note that the transmission of cysticercosis may not be restricted to rural areas alone, due to the migration of people from rural areas to urban communities. It is equally important also to note that the disease may occur in humans who neither eat pork nor share environments with pigs (Schantz *et al.*,

1992). Rajshekhar *et al* (2003), reported that about 95% of the Indian patients with NCC are vegetarians or do not consume pork.

In regions where cysticercosis in pigs is common, human cysticercosis and epilepsy prevalence are also usually high as shown by results from other endemic areas in Latin America (Diaz, 1992; Sciutto *et al.,* 2000). Therefore, there is an urgent need to collect baseline data on human cysticercosis and its possible linkage with epilepsy in Zambia for a better understanding of the local epidemiology and transmission risks.

In conclusion, we would like to say that as for many diseases, prevention is the key to control. We also know that simple changes in social habits and hygiene can prevent infection and disease in humans and animals, even though, changes in habit and custom are not easily accomplished. However, if stakeholders (people in the communities and policy makers at local, regional and national levels) are aware of the consequences of *T. solium* infection, then sustainable and long lasting control programmes can be instituted.

The vast baseline data gathered thus far on the status of the diseases in pigs should give the impetus to conduct taeniasis and cysticercosis prevalence study in humans in these areas, since porcine seroprevalence provides a good indicator of active transmission zones. The assessment of the burden of the disease in humans should then be followed by extension programmes, community (civic) education and other prevention and control initiatives such as monitoring and surveillance may be started while further needed research on cysticercosis and taeniasis is being conducted in humans. This may probably entail the creation of extension services and strong

primary health care networks that would be involved in maintaining hygienic and sanitary conditions in the communities.

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APPENDICES

Appendix 1: Porcine Cysticercosis Research Questionnaire

Date		Persons interviewing:.....
A1. District:		.....
A2. Village:		.....

B. HOUSEHOLD DESCRIPTION

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: .....

C6. Occupation: .....

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

D. PIG MANAGEMENT

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

D2. Breed(s): .....

D3. Husbandry system: (1) indoor (2) semi-intensive (3) Free-range (4) tethering

D4. What do you feed the pigs: (1) pasture (2) other feeds: .....

D5. What is the aim of keeping pigs: (1) sale (2) home consumption

(3) Both 1 and2

D6. What is the mean slaughter age of your pigs: .....

D7. What are the main problems encountered with pig rearing (management /disease):

1. ..... 2. ..... 3.....

Appendix 1 continued

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: Prevalence of *T. solium* porcine cysticercosis in pigs by test in villages in Gwembe district.

Village	n	Tongue		Antigen ELISA		Antibody ELISA	
		No.+ve	% +ve	No.+ve	% +ve	No.+ve	% +ve
Hag'andu	23	8	34.8	5	21.7	11	47.8
Munyumbwe T/ship	2	1	50.0	1	50.0	2	100.0
Hamunjebwa	4	0	0.0	0	0.0	0	0.0
Hanyimbo	9	0	0.0	1	11.1	2	22.2
Hakalinda	1	0	0.0	1	100.0	1	100.0
Hamunali	4	0	0.0	2	50.0	3	75.0
Chisumba	3	1	33.3	1	33.3	1	33.3
Mutantilo	9	1	11.1	3	33.3	4	44.4
Habeene	6	2	33.3	3	50.0	4	66.7
Siang'andu	3	0	0.0	1	33.3	1	33.3
Hameenda	2	0	0.0	2	100.0	2	100.0
Matyola	4	0	0.0	0	0.0	0	0.0
Malambo 2	5	0	0.0	1	20.0	3	60.0
Chisangano	4	1	25.0	1	25.0	2	50.0
Mutinta	1	0	0.0	0	0.0	0	0.0
Kalangwa	3	0	0.0	2	66.7	2	66.7
Hamachila	2	0	0.0	0	0.0	0	0.0
Hamalengwa	12	1	8.3	3	25.0	4	33.3
Hauma	5	1	20.0	2	40.0	3	60.0
Hanjame	1	0	0.0	0	0.0	1	100.0
Sikawale	10	1	10.0	2	20.0	4	40.0
Lusiye	30	9	30.0	8	26.7	18	60.0
Moonga	26	7	26.9	9	34.6	12	46.2
Mundenge	12	3	25.0	6	50.0	5	41.7
Hamasamu	4	0	0.0	1	25.0	3	75.0
Sinaimbwe	12	2	16.7	7	58.3	7	58.3
Hankuna	8	2	25.0	3	37.5	5	62.5
Chasombwa	22	4	18.2	6	27.3	9	40.9
Kanyemba	4	0	0.0	0	0.0	0	0.0
Hamanjanji	9	1	11.1	2	22.2	2	22.2
Chiliga B	11	3	27.3	5	45.5	5	45.5
Chiliga A	22	11	50.0	13	59.1	11	50.0
Hamulelwe	3	1	33.3	3	100.0	1	33.3
Chimbali	8	2	25.0	4	50.0	6	75.0
Chatembwa	5	3	60.0	3	60.0	3	60.0
Munyumbwe	16	1	6.3	6	37.5	8	50.0
Hagwanama	30	6	20.0	14	46.7	14	46.7
Malungo	12	5	41.7	3	25.0	8	66.7
Malambo	9	1	11.1	2	22.2	6	66.7
Tusenke Township	8	2	25.0	4	50.0	5	62.5
Simweene	16	2	12.5	8	50.0	12	75.0
Hajanda	2	0	0.0	0	0.0	1	50.0
Chimuka	3	1	33.3	2	66.7	3	100.0
Total	385	83	21.6	140	36.4	194	50.4

Appendix 3: Prevalence of *T. solium* porcine cysticercosis in pigs by test in villages in Monze district.

Village	n	Tongue		Antigen ELISA		Antibody ELISA	
		No.+ve	% +ve	No.+ve	% +ve	No.+ve	% +ve
Nkonka	4	0	0.0	1	25.0	0	0.0
Munankopa	2	0	0.0	0	0.0	0	0.0
Sikabali	7	0	0.0	1	14.3	1	14.3
Maimbo	7	0	0.0	0	0.0	0	0.0
Chobana	5	0	0.0	0	0.0	0	0.0
Makwembo	9	1	11.1	1	11.1	4	44.4
Malambo	17	0	0.0	1	5.9	1	5.9
Kalulu	12	1	8.3	2	16.7	3	25.0
Misozi	1	0	0.0	0	0.0	0	0.0
Bbole	1	0	0.0	0	0.0	0	0.0
Hanzila	2	0	0.0	0	0.0	1	50.0
Liso	7	1	14.3	4	57.1	4	57.1
Hanamoonga	10	0	0.0	0	0.0	0	0.0
Choona	29	4	13.8	11	37.9	6	20.7
Ngalizya	20	0	0.0	4	20.0	1	5.0
Choobana	15	2	13.3	2	13.3	3	20.0
Sikabenga	16	3	18.8	3	18.8	3	18.8
Kabimba	2	0	0.0	0	0.0	0	0.0
Mayoba	3	1	33.3	1	33.3	1	33.3
Hamunyanga	2	0	0.0	0	0.0	1	50.0
Halwindi	11	0	0.0	4	36.4	1	9.1
Makondo	1	0	0.0	0	0.0	0	0.0
Suntwe chimuka	5	1	20.0	1	20.0	1	20.0
Namakube	5	1	20.0	1	20.0	1	20.0
Munene	2	0	0.0	0	0.0	0	0.0
Mainga	7	2	28.6	3	42.9	5	71.4
Chuuka	1	0	0.0	1	100.0	1	100.0
Mapafula	1	0	0.0	1	100.0	0	0.0
Lumamba	2	0	0.0	1	50.0	2	100.0

Appendix 3 continued

Village	n	Tongue		Antigen ELISA		Antibody ELISA	
		No.+ve	% +ve	No.+ve	% +ve	No.+ve	% +ve
Simuyandi	11	0	0.0	2	18.2	3	27.3
Mutwa	2	0	0.0	1	50.0	2	100.0
Macheba	1	0	0.0	0	0.0	0	0.0
Hachani	3	2	66.7	3	100.0	2	66.7
Ndeleki	4	2	50.0	3	75.0	3	75.0
Sikaula	3	0	0.0	0	0.0	2	66.7
Simoonga	2	1	50.0	2	100.0	2	100.0
Chilunga	3	0	0.0	2	66.7	3	100.0
Hikaloze	2	1	50.0	2	100.0	1	50.0
Namukamba	3	0	0.0	2	66.7	0	0.0
Mwenechepa	4	0	0.0	1	25.0	0	0.0
Sinyendende	13	1	7.7	6	46.2	5	38.5
Hamoonga	5	1	20.0	2	40.0	2	40.0
Chilapula	8	0	0.0	0	0.0	0	0.0
Chinjila	12	1	8.3	2	16.7	1	8.3
Liso	1	0	0.0	0	0.0	0	0.0
Mwangani	21	0	0.0	7	33.3	7	33.3
Chikwala	12	1	8.3	3	25.0	5	41.7
Hakasenke	4	1	25.0	1	25.0	1	25.0
Simwelezya	8	1	12.5	4	50.0	4	50.0
Gaali	2	0	0.0	1	50.0	1	50.0
Havuka	2	0	0.0	1	50.0	1	50.0
Chikwangala	5	1	20.0	3	60.0	1	20.0
Hachikonga	4	0	0.0	0	0.0	0	0.0
Munamuzongwe	3	2	66.7	2	66.7	1	33.3
Muunga	1	0	0.0	0	0.0	0	0.0
Chiile	14	1	7.1	0	0.0	0	0.0
Simapani	2	0	0.0	0	0.0	0	0.0
Tebele	13	0	0.0	1	7.7	2	15.4
Namuwa	4	0	0.0	0	0.0	1	25.0
Munamwala	9	1	11.1	0	0.0	3	33.3
Total	387	34	8.8	94	24.3	93	24.0

Appendix 4: Prevalence of *T. solium* porcine cysticercosis in pigs by test in villages in Petauke district

Village	n	Tongue		Antigen ELISA		Antibody ELISA	
		No.+ve	% +ve	No.+ve	% +ve	No.+ve	% +ve
Kapelele	22	0	0.0	1	4.5	13	59.1
Pandala	21	2	9.5	4	19.0	15	71.4
Mtyola	5	0	0.0	3	60.0	5	100.0
Kalililo	12	0	0.0	1	8.3	10	83.3
Kalichelo	12	1	8.3	0	0.0	9	75.0
Kakwiya	23	0	0.0	0	0.0	9	39.1
Sikalinda	9	0	0.0	4	44.4	1	11.1
Wonzi	1	0	0.0	0	0.0	0	0.0
Nyakachonkho	17	1	5.9	1	5.9	5	29.4
Kasanila	2	0	0.0	1	50.0	1	50.0
Nyamphande	30	1	3.3	3	10.0	15	50.0
Nyatuwondo	19	2	10.5	4	21.1	8	42.1
Kalito	2	0	0.0	0	0.0	1	50.0
Mphamba	45	3	6.7	5	11.1	19	42.2
Chimtowe	21	0	0.0	4	19.0	6	28.6
Nyanje	27	4	14.8	7	25.9	15	55.6
Zuze	14	0	0.0	0	0.0	3	21.4
Kalindawalo	13	1	7.7	2	15.4	6	46.2
Manchichi	22	2	9.1	7	31.8	9	40.9
Dube	4	0	0.0	1	25.0	0	0.0
Mtuta	12	1	8.3	10	83.3	3	25.0
Yelesani	5	0	0.0	0	0.0	3	60.0
Kalowa	7	0	0.0	2	28.6	4	57.1
Chandiyo	1	0	0.0	0	0.0	0	0.0
Mpaso	10	1	10.0	0	0.0	6	60.0
Chataika	28	6	21.4	3	10.7	22	78.6
Total	384	25	6.5	63	16.4	188	49.0

Appendix 5: Prevalence of *T. solium* porcine cysticercosis in pigs by test in villages in Katete district

Village	n	Tongue		Antigen ELISA		Antibody ELISA	
		No.+ve	% +ve	No.+ve	% +ve	No.+ve	% +ve
Chilembwe	49	1	2.0	6	12.2	7	14.3
Guma	3	0	0.0	0	0.0	0	0.0
Kamphodza	36	2	5.6	5	13.9	8	22.2
Ngwangwa	7	0	0.0	1	14.3	0	0.0
Mugwentu	8	0	0.0	1	12.5	1	12.5
Chandiyo-KT	2	0	0.0	1	50.0	0	0.0
Mwanje Farm	7	1	14.3	2	28.6	1	14.3
Njilatenga	13	2	15.4	2	15.4	1	7.7
Chiluzi	27	2	7.4	4	14.8	4	14.8
Chibbala	23	2	8.7	7	30.4	7	30.4
Mwasekela	1	1	100.0	1	100.0	0	0.0
Chikwela	2	1	50.0	1	50.0	1	50.0
Chaopa	12	1	8.3	3	25.0	3	25.0
Chinkhungu	14	0	0.0	2	14.3	3	21.4
Kabbula	23	0	0.0	4	17.4	6	26.1
Kaluwamba	2	0	0.0	0	0.0	1	50.0
Malata	4	0	0.0	0	0.0	0	0.0
Chasaba	4	2	50.0	2	50.0	3	75.0
Kawanje	30	7	23.3	14	46.7	9	30.0
Chapita	25	4	0.0	8	32.0	14	56.0
Mtika	3	0	0.0	0	0.0	1	33.3
Mshulu	36	1	2.8	2	5.6	10	27.8
Maonde	7	0	0.0	2	28.6	3	42.9
Tolani	16	1	6.3	5	31.3	6	37.5
Joel	31	1	3.2	6	19.4	10	32.3
Total	385	29	7.5	79	20.5	99	25.7

Appendix 6: Prevalence of *T. solium* porcine cysticercosis by test in households in the villages in Gwembe district

Village	n	Tongue		Antigen ELISA		Antibody ELISA	
		No.+ve	% +ve	No.+ve	% +ve	No.+ve	% +ve
Hag'andu	16	8	50.0	5	31.3	8	50.0
Munyumbwe T/ship	1	1	100.0	1	100.0	1	100.0
Hamunjebwa	3	0	0.0	0	0.0	3	100.0
Hanyimbo	2	0	0.0	1	50.0	2	100.0
Hakalinda	1	0	0.0	1	100.0	1	100.0
Hamunali	2	0	0.0	1	50.0	2	100.0
Chisumba	2	1	50.0	1	50.0	1	50.0
Mutantilo	5	1	20.0	3	60.0	3	60.0
Habeene	3	1	33.3	2	66.7	3	100.0
Sing'andu	3	0	0.0	1	33.3	1	33.3
Hameenda	1	0	0.0	1	100.0	1	100.0
Matyola	2	0	0.0	0	0.0	0	0.0
Malambo 2	3	1	33.3	1	33.3	2	66.7
Chisangano	4	1	25.0	1	25.0	2	50.0
Mutinta	1	0	0.0	0	0.0	0	0.0
Kalangwa	2	0	0.0	2	100.0	2	100.0
Hamachila	1	0	0.0	0	0.0	0	0.0
Hamalengwa	9	1	11.1	3	33.3	4	44.4
Hauma	3	1	33.3	1	33.3	1	33.3
Hanjame	1	0	0.0	0	0.0	1	100.0
Sikawala	5	1	20.0	2	40.0	3	60.0
Lusiye	13	5	38.5	6	46.2	10	76.9
Moonga	12	5	41.7	8	66.7	7	58.3
Mundenge	6	2	33.3	5	83.3	4	66.7
Hamasamu	4	0	0.0	1	25.0	3	75.0
Sinaimbwe	6	2	33.3	6	100.0	4	66.7
Hankuna	5	2	40.0	3	60.0	4	80.0
Chasombwa	8	1	12.5	3	37.5	4	50.0
Kanyemba	3	0	0.0	0	0.0	0	0.0
Hamanjanji	1	1	100.0	1	100.0	1	100.0
Chiliga B	5	3	60.0	3	60.0	4	80.0
Chiliga A	11	8	72.7	8	72.7	7	63.6
Hamulelwe	3	1	33.3	3	100.0	1	33.3
Chimbali	5	2	40.0	4	80.0	4	80.0%
Chatembwa	3	2	66.7	2	66.7	2	66.7
Munyumbwe	7	1	14.3	3	42.9	5	71.4
Hagwanama	16	5	31.3	10	62.5	10	62.5
Malungo	6	4	66.7	2	33.3	5	83.3
Malambo	8	1	12.5	2	25.0	6	75.0
Tusenke township	6	2	33.3	3	50.0	4	66.7

Appendix 6 Continued

Village	n	Tongue		Antigen ELISA		Antibody ELISA	
		No.+ve	% +ve	No.+ve	% +ve	No.+ve	% +ve
Simweene	8	2	25.0	7	87.5	8	100.0
Hajanda	2	0	0.0	0	0.0	1	50.0
Chimuka	1	1	100.0	1	100.0	1	100.0
Total	209	67	32.1	108	51.7	136	65.1

Appendix 7: Prevalence of *T. solium* porcine cysticercosis by test in households in the villages in Monze district.

Village	n	Tongue		Antigen ELISA		Antibody ELISA	
		No. +ve	% +ve	No. +ve	% +ve	No. +ve	% +ve
Nkonka	1	0	0.0	1	100.0	0	0.0
Munankopa	1	0	0.0	0	0.0	0	0.0
Sikabali	3	0	0.0	1	33.3	1	33.3
Maimbo	3	0	0.0	0	0.0	0	0.0
Chobana	1	0	0.0	0	0.0	0	0.0
Makwembo	3	1	33.3	1	33.3	2	66.7
Malambo	4	0	0.0	1	25.0	1	25.0
Kalulu	7	1	14.3	1	14.3	2	28.6
Misozi	1	0	0.0	0	0.0	0	0.0
Bbole	1	0	0.0	0	0.0	0	0.0
Hanzila	2	0	0.0	0	0.0	1	50.0
Liso	3	1	33.3	2	66.7	2	66.7
Hanamoonga	5	0	0.0	0	0.0	0	0.0
Choona	17	4	23.5	8	47.1	5	29.4
Ngalizya	7	0	0.0	4	57.1	1	14.3
Choobana	9	2	22.2	2	22.2	2	22.2
Sikabenga	12	3	25.0	3	25.0	3	25.0
Kabimba	1	0	0.0	0	0.0	0	0.0
Mayoba	3	1	33.3	1	33.3	1	33.3
Hamunyanga	1	0	0.0	0	0.0	1	100.0
Halwindi	9	0	0.0	4	44.4	1	11.1
Makondo	1	0	0.0	0	0.0	0	0.0
Suntwe chimuka	3	1	33.3	1	33.3	1	33.3
Namakube	3	1	33.3	1	33.3	1	33.3
Munene	1	0	0.0	0	0.0	0	0.0
Mainga	3	2	66.7	2	66.7	3	100.0
Chuuka	1	0	0.0	1	100.0	1	100.0
Mapafula	1	0	0.0	1	100.0	0	0.0
Lumamba	2	0	0.0	1	50.0	2	100.0
Simuyandi	6	0	0.0	2	33.3	3	50.0

Appendix 7 continued

Village	n	Tongue		Antigen ELISA		Antibody ELISA	
		No. +ve	% +ve	No. +ve	% +ve	No. +ve	% +ve
Mutwa	1	0	0.0	1	100.0	1	100.0
Macheba	1	0	0.0	0	0.0	0	0.0
Hachani	2	2	100.0	2	100.0	2	100.0
Ndeleki	1	1	100.0	1	100.0	1	100.0
Sikaula	2	0	0.0	0	0.0	1	50.0
Simoonga	1	1	100.0	1	100.0	1	100.0
Chilunga	1	0	0.0	1	100.0	1	100.0
Hikaloze	1	1	100.0	1	100.0	1	100.0
Namukamba	1	0	0.0	1	100.0	0	0.0
Mwenechepa	1	0	0.0	1	100.0	0	0.0
Sinyendende	7	1	14.3	4	57.1	3	42.9
Hamoonga	2	1	50.0	1	50.0	1	50.0
Chilapula	3	0	0.0	0	0.0	0	0.0
Chinjila	8	1	12.5	2	25.0	1	12.5
Liso	1	0	0.0	0	0.0	0	0.0
Mwangani	12	0	0.0	7	58.3	7	58.3
Chikwala	7	1	14.3	3	42.9	4	57.1
Hakasenke	4	1	25.0	1	25.0	1	25.0
Simwelezya	4	1	25.0	3	75.0	4	100.0
Gaali	2	0	0.0	1	50.0	1	50.0
Havuka	2	0	0.0	1	50.0	1	50.0
Chikwangala	3	1	33.3	3	100.0	1	33.3
Hachikonga	1	0	0.0	0	0.0	0	0.0
Munamuzongwe	2	2	100.0	2	100.0	1	50.0
Muunga	1	0	0.0	0	0.0	0	0.0
Chile	6	1	16.7	0	0.0	0	0.0
Simapani	2	0	0.0	0	0.0	0	0.0
Tebele	5	0	0.0	1	20.0	1	20.0
Namuwa	2	0	0.0	0	0.0	1	50.0
Munamwala	2	1	50.0	0	0.0	2	100.0
Total	203	33	16.3	76	37.4	71	35.0

Appendix 8: Prevalence of *T. solium* porcine cysticercosis by test in households in the villages in Petauke district

Village	n	Tongue		Antigen ELISA		Antibody ELISA	
		No.+ve	% +ve	No.+ve	% +ve	No.+ve	% +ve
Kapelele	11	0	0.0	1	9.1	10	90.9
Pandala	13	1	7.7	3	23.1	11	84.6
Mtyola	1	0	0.0	1	100.0	1	100.0
Kalililo	5	0	0.0	1	20.0	5	100.0
Kalichelo	5	1	20.0	0	0.0	5	100.0
Kakwiya	14	0	0.0	0	0.0	6	42.9
Sikalinda	5	0	0.0	4	80.0	1	20.0
Wonzi	1	0	0.0	0	0.0	0	0.0
Nyakachonkho	8	1	12.5	1	12.5	3	37.5
Kasanila	2	0	0.0	1	50.0	1	50.0
Nyamphande	9	1	11.1	3	33.3	7	77.8
Nyatuwondo	8	2	25.0	3	37.5	2	25.0
Kalito	1	0	0.0	0	0.0	1	100.0
Mphamba	16	3	18.8	4	25.0	10	62.5
Chimtowe	8	0	0.0	2	25.0	4	50.0
Nyanje	14	3	21.4	7	50.0	10	71.4
Zuze	5	0	0.0	0	0.0	3	60.0
Kalindawalo	4	1	25.0	2	50.0	4	100.0
Manchichi	10	2	20.0	6	60.0	7	70.0
Dube	1	0	0.0	1	100.0	0	0.0
Mtuta	7	1	14.3	6	85.7	2	28.6
Yelesani	1	0	0.0	0	0.0	1	100.0
Kalowa	4	0	0.0	2	50.0	1	25.0
Chandiyo	1	0	0.0	0	0.0	0	0.0
Mpaso	2	1	50.0	0	0.0	2	100.0
Chataika	12	5	41.7	3	25.0	9	75.0
Total	168	22	13.1	51	30.4	106	63.1

Appendix 9: Prevalence of *T. solium* porcine cysticercosis by test in households in the villages in Katete district

Village	n	Tongue		Antigen ELISA		Antibody ELISA	
		No.+ve	% +ve	No.+ve	% +ve	No.+ve	% +ve
Chilembwe	18	1	5.6	5	27.8	5	27.8
Guma	3	0	0.0	0	0.0	0	0.0
Kamphodza	15	1	6.7	3	20.0	6	40.0
Ngwangwa	4	0	0.0	1	25.0	0	0.0
Mugwentu	5	0	0.0	1	20.0	1	20.0
Chandiyo	2	0	0.0	1	50.0	0	0.0
Mwanje Farm	1	1	100.0	1	100.0	1	100.0
Njilatenga	7	2	28.6	2	28.6	1	14.3
Chiluzi	20	3	15.0	4	20.0	4	20.0
Chibbala	11	2	18.2	4	36.4	4	36.4
Mwasekela	1	0	0.0	1	100.0	0	0.0
Chikwela	2	1	50.0	1	50.0	1	50.0
Chaopa	8	1	12.5	3	37.5	3	37.5
Chinkhungu	8	0	0.0	2	25.0	3	37.5
Kabbula	14	1	7.1	4	28.6	6	42.9
Kaluwamba	2	0	0.0	0	0.0	1	50.0
Malata	2	0	0.0	0	0.0	0	0.0
Chasaba	3	2	66.7	2	66.7	2	66.7
Kawanje	18	6	33.3	11	61.1	9	50.0
Chapita	12	4	33.3	7	58.3	8	66.7
Mtika	3	0	0.0	0	0.0	1	33.3
Mshulu	21	1	4.8	2	9.5	8	38.1
Maonde	6	0	0.0	2	33.3	3	50.0
Tolani	10	1	10.0	4	40.0	5	50.0
Joel	24	1	4.2	5	20.8	9	37.5
Total	220	28	12.7	66	30.0	81	36.8