THE PREVALENCE AND DIFFERENTIATION OF HUMAN *TAENIA SPP.* INFECTIONS IN TWO DISTRICTS OF THE SOUTHERN PROVINCE OF ZAMBIA

BY

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

THE UNIVERSITY OF ZAMBIA

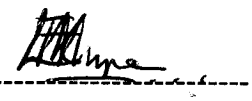
SCHOOL OF VETERINARY MEDICINE

DEPARTMENT OF CLINICAL STUDIES

LUSAKA

DECLARATION

I, **Kabemba Evans Mwape** 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: 16/02/2007

DEDICATIONS

This dissertation is dedicated to my late adopted grand father **Mr Mathias Ngowani** who laid a very good foundation for my life and made me believe that education was the key to success. I also dedicate it to my adopted parents **Mr** (deceased) and **Mrs Musonda Mwape**, my aunt **Florence Chibale** (deceased) for having struggled to enable me get an education and also to my wife **Veronica Nicole Mwape** for her encouragement and support during the research work.

CERTIFICATE OF APPROVAL

This dissertation of **Kabemba Evans Mwape** 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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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
CT	Computer Tomography.
DNA	Deoxyribonucleic Acid
EITB	Enzyme-linked Immunotransfer blot
ELISA	Enzyme-Linked Immunosorbent Assay
Gp	Glycoprotein
HP	Health Post
H_2O_2	Hydrogen peroxide
H_2SO_4	Sulphuric acid
IgG	Immunoglobulin G
M	Molarity
ml	Millilitre
mm	Millimetre
MoAb	Monoclonal antibody
N	Normality
n	Sample size or number examined
NBCS	New born calf serum
NCC	Neurocysticercosis

nm	Nanometre
No.	Number
OD	Optical density
OPD	Orthophenylene diamine
p	Probability
PCR	Polymerase Chain Reaction
PBS	Phosphate buffer saline
PvPP	Polyvinylpolypyrolidone
RFLP	Restriction Fragment Length Polymorphism
RHC	Rural Health Centre
rpm	Revolutions per minute
SPSS	Statistical Programme for Social Sciences
Taq	*Thermus aquaticus*
T20	Tween 20
TCA	Trichloroacetic acid
Ts	*Taenia solium*
TsCF	*Taenia solium* cyst fluid

ACKNOWLEDGEMENTS

I am very grateful to the Flemish Inter-University Council V.L.I.R and the University of Zambia UNZA (VLIR-UNZA IUC) who fully financed this work.

I am greatly indebted to the Samora Machel School of Veterinary Medicine, University of Zambia for giving me the opportunity out of the many others, to conduct this research under the Staff Development Fellowship programme in the Department of Clinical Studies.

I am extremely indebted to my supervisor and Project leader of the veterinary component of the VLIR-UNZA IUC, Dr. Isaac K. Phiri, who has with great care and patience, scrutinized the whole 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.

I especially want to recognize my scientific advisor, Professor Pierre Dorny for the fruitful suggestions and for the provision of monoclonal antibodies for the antigen ELISA and other reagents, without him my work would have been but with difficulty.

I must recognize the contributions of the many researchers on taeniasis/cysticercosis whose works are sited profusely throughout the text.

Without the help of others this dissertation would never have seen the light of day and subsequently, I am most grateful to the following members of staff of the University of Zambia, School of Veterinary Medicine for their assistance; Mr. M. Masuku for the tedious work of processing and examination of human faecal

samples; Mr. M. Chembensofu for his technical assistance in the ELISA work; the driver, Mr. P. Nyirenda for his vast experience especially in driving through the terrible terrain of Gwembe district.

Special thanks go to the members of staff at the Institute of Tropical Medicine, Antwerp, Belgium for helping in the molecular part of this study. Mr Bjorn Victor for his technical support in the PCR-RFLP and for always being there to answer all my questions; Dr Dirk Geysen in whose laboratory the molecular work was done and for his invaluable guidance and comments on the PCR results.

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 Veronica Nicole.

Lastly but not the least I would like to extend my profound gratitude to the health workers in the various health centres visited and indeed the participating individuals for their understanding and co-operation. I wish I could list all of them by name.

ABSTRACT

Taeniosis and cysticercosis are public health problems in many developing countries, with the former being due to the adult worm of both *Taenia solium* (*T. solium*) and *Taenia saginata* (*T. saginata*) while the latter is due to the larval stages of *T. solium.*

Extensive studies in pigs and cattle, which are the intermediate hosts of these important zoonotic parasites, have been carried out in Zambia. However little or no information about the infections in humans is available.

Hence, the objective of this study was to determine the prevalence and the potential risk factors associated with taeniasis/cysticercosis in humans in the districts of Gwembe and Monze of the Southern province of Zambia. We also undertook to differentiate the human *Taenia spp.* found in the study area. The prevalence of human taeniosis was assessed using coproscopic examination and diagnostic PCR. Presence of circulating parasite antigens in urine and serum to detect cysticercosis was assessed by enzyme-linked-immunosorbent assay (Ag-ELISA). Restriction fragment length polymorphism (PCR-RFLP) was used to differentiate the two human *Taenia spp.* A questionnaire was administered to individuals above 12 years of age in order to obtain information on the awareness of the infection by the participants and to study other associated risk factors to taeniosis/cysticercosis infections in man.

A total of 678 faecal samples were examined. Of these, 294 and 384 samples were from Gwembe and Monze districts, respectively. Of the total faecal samples examined 21 (3.1%) were positive for taeniosis after coproscopic examination. PCR

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was conducted on a sub sample of 200 from the 678 faecal samples and 21 (10.5%) were found positive for taeniosis. There was no significant difference in prevalence between the two districts on both tests and perfect agreement (kappa = 0.851) was found between the two tests. Urine Ag-ELISA analysis gave a total cysticercosis prevalence of 13.4% (n = 627). No significant differences in cysticercosis prevalence were detected between individuals from Gwembe 37 (13.8%) and Monze 47 (13.1%) districts. There was no statistical association between gender or age group and positivity on either coproscopic examination or urines Ag-ELISA. Of the 101 serum samples collected from both urine Ag-ELISA positive and negative individuals, 8.9% had cysticercal antigens while 47.5% had antibodies. Comparing the three tests, only slight agreement was found between urine Ag-ELISA and serum Ab–ELISA. Even though the agreement was very slight, it was statistically significant ($p < 0.05$). Upon RFLP, all extracted *Taenia* DNA was confirmed to be that of *T. solium* and no pattern corresponded to that of *T. saginata*.

The following risk factors were noted to be present despite not being statistically significant; lack of pork inspection at slaughter, consumption of pork with cysts, selling of pork infected with *T. solium* cysticerci, free-range pig husbandry system and poor sanitation, thereby allowing pigs access to infected faeces because of absence of toilets.

This study showed a high prevalence of *T. solium* taeniosis/cysticercosis in humans in the surveyed areas, and that all the necessary factors required for the transmission of the parasite are present. The life cycle of *T. solium* is bound to be sustained by pigs being allowed access to infected human faeces because villagers with no toilets defaecated in open bushes. Consumption of uninspected cysticercosis-infected pork

by villagers further enhances this maintenance of the parasite. It is evident from this study that *T. solium* infection poses a high public health risk not only in the study areas but also in urban areas due to migration of tapeworm carriers. The vast baseline data gathered thus far on the status of the diseases in humans should give the impetus to conduct further taeniosis and cysticercosis prevalence study in humans not only in these areas but also in other areas where pigs are raised.

CHAPTER ONE

1.0 INTRODUCTION

Taeniosis/cysticercosis remains a world public health problem not only in developing countries but also increasingly in developed countries (Carpio *et al.*, 1998). Human and porcine cysticercosis is a serious health and economic problem of developing countries (Larralde *et al.*, 1992). Taeniosis is caused by the tapeworms *Taenia saginata* (*T. saginata*) and *Taenia solium* (*T. solium*). *T. Saginata* causes bovine cysticercosis and human taeniosis while *T. solium* causes porcine cysticercosis and human taeniosis/cysticercosis (World Health Organisation, 1983; Yamasaki *et al.*, 2002). Man is not only infected by adult tapeworms but also by metacestodes or larval stages (cysticerci) of *T. solium*. Infection by metacestodes often leads to neurocysticercosis, a major cause of epilepsy associated with considerable morbidity and mortality (Garcia *et al.*, 2003). Cysticerci of *T. saginata,* in contrast, are found exclusively in cattle and do not develop in human (Cruz *et al.*, 1999; Garcia *et al.*, 2003). Human cysticercosis is caused by the pork tapeworm *T. solium* whose life cycle includes pigs as the normal intermediate host harbouring the larva or cysticerci and humans as the definitive host harbouring the adult form of the tapeworm (Taeniosis); however humans can also serve as the intermediate host and suffer from cysticercosis (Garcia *et al.*, 1998).

Adult intestinal tapeworm infection in man is acquired by eating undercooked pork or beef, which is contaminated with the larval stage of the cestodes (Garcia *et al.*, 2003). Cysticercosis is acquired by man through ingestion of *T. solium* eggs shed in the faeces of a human tapeworm carrier (Schantz *et al.*, 1992). Thus, cysticercosis may occur in humans who neither eat pork nor directly share environments with

pigs. Pigs and cattle are infected with *T. solium* and *T. saginata* cysticercosis, respectively, by ingesting the parasite eggs or proglottids 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 taeniosis depends on the detection of parasite material (proglottids or eggs) in the faeces (World Health Organisation, 1983). However, these techniques are known to have both low sensitivity and specificity due to the intermittent nature of egg excretion. Prevalence of taeniosis is, therefore, underestimated. In addition, the eggs of *T. saginata* and *T. solium* appear identical under the light microscope further lowering the diagnostic specificity (Chapman, 1995; World Health Organisation, 1983). Currently immunodiagnosis has advanced the diagnosis of taeniosis/cysticercosis by addressing both sensitivity and specificity (Allan *et al.*, 2003; Dorny *et al.*, 2003; Gonzalez *et al.*, 2000; Chapman *et al.*, 1995).

Diagnosis of cysticercosis in pigs and cattle is usually made at meat inspection. However, ante-mortem diagnosis in pigs using tongue examination is sometimes possible when the vesicles are located on the tongue or certain mucosae like the

ocular mucosa (Gonzalez *et al.*, 1990). Meat inspection is the most common technique that is routinely used for the detection of infected carcasses, although 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). Studies by Dorny *et al.*, 2005 and Phiri *et al.*, 2006, have shown the current routine tests are not effective.

In regions of endemic taeniosis infections, transmission is clearly related to prevailing low standards of personal hygiene and poor environmental sanitation and control (Toledo *et al.*, 2001). The general poor sanitary conditions, especially inadequate disposal of human excreta, and the widespread occurrence of free-roaming pigs facilitates 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 with Eastern province and Southern provinces accounting for a greater part of this increase (Phiri *et al.*, 2003). This has been attributed to 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 (Phiri *et al.*, 2003). 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 cannot afford to confine and feed their pigs. As such the pigs are allowed to roam about (scavenge). This free-range management system exposes pigs to human faeces often contaminated with tapeworm eggs which they eat and become infected. There is lack or absence of meat inspection regimes and disease control in illegal urban livestock markets where many infected pigs are transported to. This has exacerbated public health risk to *T. solium* cysticercosis when infected meat is consumed by unsuspecting people.

The abattoir survey of pigs from Southern province at Chibolya slaughter slab in Lusaka showed that 10.9% and 20.6% were positive by lingual examination and meat inspection, respectively (Phiri *et al.*, 2002). In their preliminary field study, Phiri *et al.*, 2002, 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 20.8% and 9.3% pigs were positive by Ag-ELISA in Southern and Eastern provinces, respectively. In another study, Dorny *et al.*, (2004) used the Bayesian approach to estimate the prevalence of porcine cysticercosis in village pigs slaughtered in Lusaka and found a prevalence of 64.2%. These studies clearly showed that *T. solium* was present in rural areas of Zambia. It was from this that the current study on taeniosis in humans in rural areas was conceived. One of the two provinces (Southern province) was chosen for the current study. The two provinces harbor more than 76% of the total number of pigs reared under the small-scale management system by free ranging (MAFF 1998; Phiri *et al.*, 2002).

Based on the findings of Dorny et al., 2004 and Phiri et al., 2002, a study was designed with the main objective of determining the occurrence of human taeniosis/cysticercosis in the two districts of Monze and Gwembe of the Southern province of Zambia. The specific objectives were:

(a) To establish the prevalence of human taeniosis/cysticercosis in porcine cysticercosis endemic areas of Southern province of Zambia:

(b) To differentiate *Taenia spp.* present in humans in Zambia and;

(c) To study the risk factors associated with human taeniosis/cysticercosis

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Background

The scientific study of the taeniid tapeworms of humans can be traced back 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). 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. Although Goeze had suspected that *T. solium* and *T. saginata* were different species as early as 1782, 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 also 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).

2.2 Morphology of *T. solium* and *T. saginata*

T. solium and *T. saginata* are tapeworms belonging to the phylum of *Platyhelminthes,* in the class *Cestoidea,* order *Cyclophylidea* and the family *Taeniidae* (Soulsby, 1986).

The tapeworms inhabit the upper part of the small intestine of man. The adult worm consists of a head or scolex, bearing attachment organs, a short unsegmented neck and a chain (strobila) of segments (Urquhart *et al.*, 1987). The tapeworms have an elongate flat body and are without a body cavity or alimentary canal. Each segment is called a proglottid and is a hermaphrodite i.e. contains both male and female reproductive organs (Soulsby, 1986). The adult *T.solium* is 1.8-4.8 m long with 800-900 proglottids (Gracey, 1986). The head is globular and less than 1 mm in diameter, while the rostellum is short and has double crown of 26-28 hooks. The neck is long and slender. The gravid uterus in a mature proglottid has 7-12 tree-like lateral branches on each side filled with eggs (Soulsby, 1982). *T. solium* may produce up to 50,000 eggs per proglottid (www.dpd.cdc.gov/dpdx).

The adult *T. saginata* is 4-8 m, rarely up to 25 m, long with about 1000 proglottids. The scolex has four suckers but unlike *T. solium* does not have a rostellum or hooks (Soulsby, 1986). Its mature proglottid has a uterus with 14-32 branches. *T. saginata* may produce up to 100,000 per proglottid (www.dpd.cdc.gov/dpdx). The eggs of *T.*

saginata and *T.solium* are indistinguishable morphologically. The eggs are rounded or subspherical, diameter 31 to 43 μm, with a thick radially striated brown shell. Inside each shell is an embryonated oncosphere (Gracey, 1986; www.dpd.cdc.gov/dpdx).

2.3 Life cycle of *T. saginata* and *T. solium*

The life cycle of taeniid tapeworms involves two hosts and a free-living stage. The adult worm lives in the small intestines of man, the definitive host. The tapeworm produces millions of eggs (Figure 2.1) which are fertilised within the proglottids and passed to the environment (Toledo *et al.*, 2001). Each terminal gravid proglottid contains about 50,000 eggs for *T. solium* and 100,000 eggs for *T. saginata.* The eggs can either be intermittently extruded from the proglottids into the intestine or the entire gravid proglottids may be passed in the faeces (White, 1997). The gravid segments of *T. saginata* are very active and about ten (Soulsby, 1986) escape through the anus, releasing large numbers of eggs in the perianal region or on the ground where they can survive for long periods.

Unlike *T. saginata*, the gravid segments of *T. solium* are less active and usually leave the host with the stools; often several attached proglottids may leave at the same time. Most patients are asymptomatic; but, they may note the passage of proglottids in their faeces. Gravid proglottids of *T. solium* are opaque, off-white in colour, and about 1-2cm long, 1cm wide, and 2-3mm thick (Pal *et al.*, 2000). The passage of these proglottids in faeces is described as being intermittent. 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.

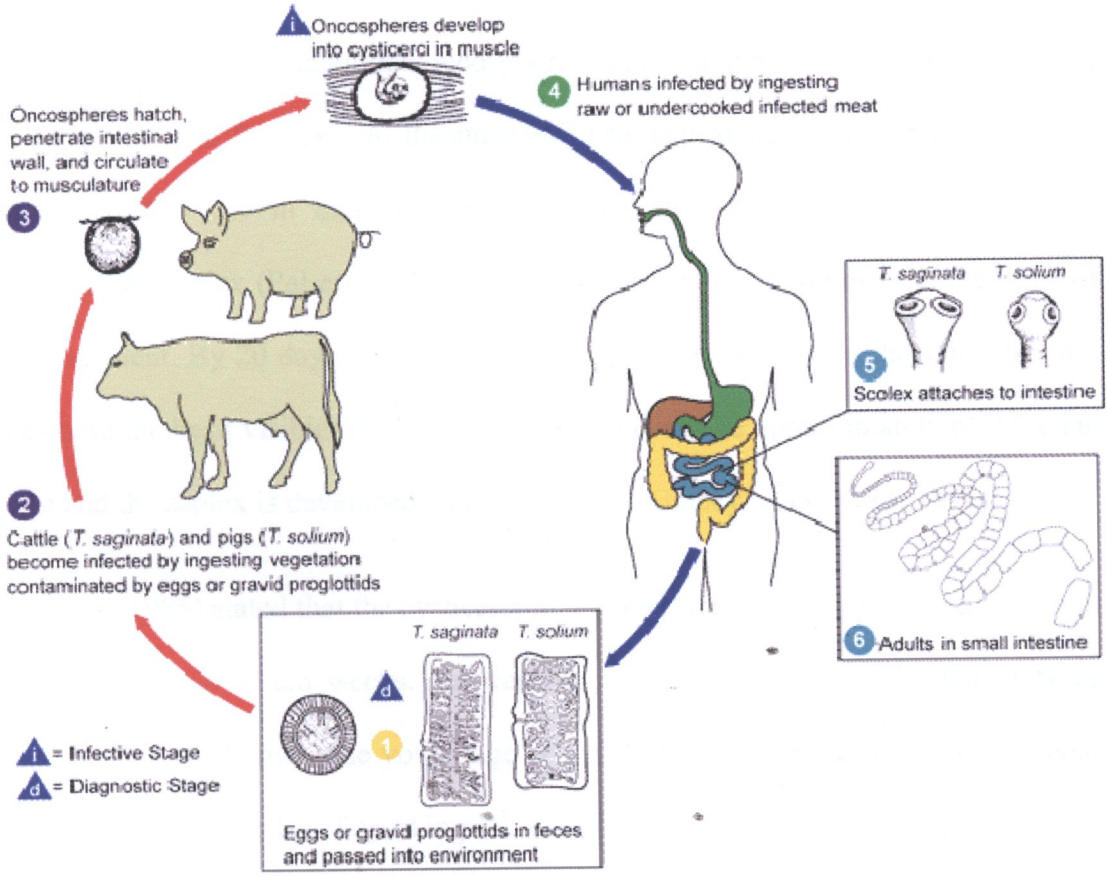

Figure 2.1: The life cycle of *T. solium* and *T. saginata*, showing the infective stages for both man, pig and cattle. (Source: www.dpd.cdc.gov/pdx).

When ingested by pigs or cattle (Figure 2.1), the eggs are activated by action of gastric and intestinal fluids and induced to hatch. Each egg releases an oncosphere which migrates through the intestinal wall and blood vessels to reach striated muscle within which it encysts forming cysticercii (Figure 2.1). Penetration of intestinal mucosa and vessels in the submucosa appears to be facilitated by excretory proteases produced by oncospheres (Flisser, 1994). The cysticerci develop primarily *in the masseter, heart, tongue and shoulders, but will be disseminated throughout the*

body (Soulsby, 1986; Boa *et al.*, 2002; Phiri *et al.*, 2005). 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. In the muscles cysts appear as translucent, thin-walled cysts about 1 cm in diameter with an eccentric white nodule containing the invaginated scolex (Pal *et al.*, 2000). The size of cysticercii varies with the 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 (1986) stated that the cysticerci in the intermediate host are infective to man after about nine to ten weeks. He went on to say that although the longevity of cysticerci is not known, the young age at which pigs, unlike cattle, are slaughtered means that the majority of cysts in pork would be viable.

The larval stage of *T. solium* also affects humans following the ingestion of eggs present in human faeces leading to cysticercosis (Flisser, 1994). Human cysticercosis may occur if eggs are conveyed to the mouth by unclean fingers after defecation or other oral contamination with *T. solium* eggs. Soil, water or vegetation are the most likely vehicles of human infection (Schantz *et al.*, 1992). Flisser (1994) reported that retrograde movement of intestinal contents may cause autoinfection, because the oncospheres are released from the eggs by successive exposure to the stomach acid and alkaline intestinal juices. After infection with eggs, the potentially dangerous larval infection, cysticercosis, is set up in the muscle or any other site. The highest number of cysts establishes in the subcutaneous tissue, then the brain,

but may be found in muscle, particularly of the thigh or calf, or in the heart, liver, lungs and eye (Gracey and Collins, 1992). In pigs, the brain seems to be colonized even if there is a light infection (Phiri *et al.*, 2006).

When inadequately cooked meat containing the cysts is consumed by man, the oncospheres excyst, settle in the small intestines and develop into adult cestodes over the next three months or so (Peters and Pasvol, 2002). The adult worm develops by forming proglottids (Figure 2.1) which arise from the caudal end of the scolex (the neck) (Soulsby, 1982; Flisser, 1994). *T. saginata* is relatively innocuous, since only the intestinal tapeworm phase occurs in man. *T. solium*, on the other hand, has major health effects due to the extra intestinal infection by the larval or cyst phase in the central nervous system (Mayta *et. al.,* 2000). It has been reported that between 5% and 40% of humans who are carriers of the adult *T. solium* develop cysticercosis (Carpio *et al.,* 2002).

2.4 Host range of *T. solium* and *T. saginata*

Man is the natural definitive host for the *Taenia solium* and *Taenia saginata* adult tapeworm, although, Allan *et al.* (1991) reported that the former may be established in Lar gibbon (*Hylobates lar*), Chacma baboon (*Papio urnus*) and Golden hamster (*Mesocricetus auratus*). The larval stage of the *T. solium* 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 intermediate hosts of economic importance, dogs are also highly susceptible and could become intermediate hosts. The larval stage of *T. saginata* is found in cattle although other ruminants such as llama, reindeer, giraffe, wildebeest

and reindeer may serve as intermediate hosts especially where farming of these game animals is undertaken (Soulsby, 1986).

2.5 The importance of taeniasis and cysticercosis

T. solium and *T. saginata* are both important parasitic diseases to pig and cattle production, respectively, and both affect human health. However, as far as human health is concerned *T. solium* is more important because it causes neurocysticercosis, an important cause of late onset epilepsy (Mayta *et al.*, 2000).

2.5.1 Public health importance of *T. solium* taeniasis and cysticercosis

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 tissues and other organs leading to a condition called human cysticercosis (Flisser, 1994).

In his review, White (1997) stated that neurocysticercosis (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.

Zoli *et al.*, (2003) noted that the importance of cysticercosis on human health is rather difficult to estimate because of the highly variable clinical picture of the disease. It ranges from asymptomatic to severe headache, epilepsy and even death. Furthermore, the cost of several visits to the physician, the costs for serology and/or CT-scan, transport and drugs have to be taken into account. Zoli *et al.*, (2003) further stated that although in many African countries, patients are not hospitalised during the treatment of NCC, losses due to the disease are thought to be quite insidiously significant. Djou (2000) estimated the costs of *T. solium* cysticercosis (wage losses not included) for treatment of patient in Cameroon at €260, which is far beyond the reach of the affected resource-poor population. 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 (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).

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 in West Cameroon only 27% of epileptics get married and 39% fail to enter into any professional activity (Preux *et al.*, 2000). Zoli *et al.* (2003) also observed that the overall social burden of human cysticercosis may be higher than is discerned, but this needs considerable further research.

2.5.2 Effects *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).

2.5.3 Effects of *T. saginata* cysticercosis on cattle production and public health

From the public health point of view only the metacestodes of *T. saginata* are important (Brandt *et al.*, 1992). The cysts cause bovine cysticercosis which results in economic losses through condemnation or special treatment of affected carcasses. In humans,

T. saginata taeniosis produces only mild abdominal symptoms. The most striking feature is the passage (active and passive) of proglottids. Occasionally, appendicitis or cholangitis can result from migrating proglottids (www.dpd.cdc.gov/pdx). Unlike those of *T. solium*, the metacestodes of *T. saginata* do not cause cysticercosis in humans. It is for this reason that *T. solium* is a more serious human health problem than *T. saginata*.

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

The transmission dynamics of taeniosis and cysticercosis are poorly understood by most communities that are at risk especially with regard to the relationship between human and porcine infection 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 infection (Garcia *et al.*, 1999).

Sanchez *et al.* (1998) analyzed risk factors in urban residents in Honduras and showed that seropositivity was statistically associated with poor household conditions, raising pigs, poor sanitation, lack of tap water and lack of knowledge about the parasite. Other authors have demonstrated that porcine infection is associated with poverty; lack of latrines, hence free access by scavenging pigs to human faeces and lack of veterinary control provide conditions that sustain the life cycle of *T. solium* (Diaz *et al.*, 1992; Sarti *et al.*, 1992b; Schantz *et al.*, 1992). 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 cities predisposes a higher risk transmission of cysticercosis when there are poor environmental and social conditions.

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

Extensive epidemiological and clinical studies demonstrating the endemicity of *T. solium* have been carried out in many Latin American countries and a few in Asia. These studies involved sampling in both humans and pigs. In Africa, baseline data on the prevalence of *T. solium* has been collected in pigs but very little data exists in humans. Table 2.1 shows prevalences of taeniosis and cysticercosis in some Latin American, Asian and African countries.

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

Country	Human cysticercosis prevalence (%)	Human taeniosis prevalence (%)	Reference
Ecuador	5.0	1.6	Rodriguez *et al.*, (2003)
Mexico	12.0	0.5	Garcia-Garcia *et al.*, (1999)
	10.8	0.3	Sarti *et al.*, (1992b)
Peru	21.0	-	Garcia *et al.*, (1999)
	8.0	-	Diaz *et al.*, (1992)
Bolivia	22.1	-	Carrique-Mas *et al.*, (2001)
Honduras	17.0	2.5	Sanchez *et al.*, (1999)
	15.6	0.6	Sanchez *et al.*, (1998)
China	3-4	-	Rajshekhar *et al.*,(2003)
Vietnam	5-7	-	Rajshekhar *et al.*, (2003)
South Africa	7.4	-	Sacks and Berkowitz,(1990)
Nigeria	-	11.5	Dada *et al.* (1993)
	-	8.6	Onah and Chiejina, (1995)
Mozambique*	20.8	-	Noormohamed *et al.*,(2003)
	12.1		Vilhena *et al.*, (1999)
Cameroon	4.5	0.13	Vondou *et al.*, (2002)
Burundi	31.5	-	Nsengiyumvia *et al.*,(2003)

*Survey done in children aged 0-15 years.

2.8 Diagnosis of taeniosis/cysticercosis

Diagnosis of taeniosis is mainly based on the search for proglottids in the faeces. The coprological diagnosis is based on the demonstration of *Taenia* spp. oncospheres or the demonstration of coproantigens by an ELISA (Enzyme-linked immunosorbent assay) (Allan, *et al.*, 1990).

Cysticercosis in humans may be diagnosed by Magnetic Resonance Imaging (MRI) or on Computerized Axial Tomography that visualise living and calcified cysticerci or the oedematous lesions they cause (Carpio, *et al.*, 1998).

Since the start of the previous century, several immuno-assays have been tried including complement fixation, immunoelectrophoresis and indirect immunofluorescence tests. Indirect hemagglutination reaches levels of sensitivity of 60 % and specificity of 89 % according to Flisser *et al.*, (1994). More recent immunological methods such as EITB (Enzyme-linked immunosorbent transfer blot assay) were described by Tsang (1989). García *et al.*, (1991), Díaz *et al.*, (1992) and Tsang and Wilson (1995) attribute a sensitivity of 95 % and a specificity of 100 % to this test, rendering it very useful for epidemiology. In addition, implementation is easy and it has a low cost. Recently, promising developments on antigen preparation and antibody detection assays were described by Ito *et al.* (1998) and Nunes *et al.* (2000). An ELISA for the detection of circulating secretory and excretory products of *Taenia* spp. metacestode (Ag-ELISA) was described by Harrison *et al.* (1989). Brandt, *et al.*, (1992), modified by Van Kerckhoven, (1998), described an assay based on the use of two different monoclonal antibodies, directed against the secretion and excretion products of *T. saginata* cysticerci allowing to diagnose cysticercosis up to a minimum level of 20 cysticerci (Geerts, 1993).

T. saginata and *T. solium* are difficult to differentiate by parasitological examination because their eggs are indistinguishable. Differentiation of the two human *Taenia* species is based on the number of uterine branches present in well-preserved gravid proglottids or on the absence of hooks in the scolex of the tapeworm. However, obtaining well-preserved gravid proglottids or the scolex after treatment is often very difficult due to the partial destruction of gravid proglottids (Mayta et al., 2000). For these reasons the Polymerase Chain Reaction (PCR) has been found to differentiate parasite from small amount of DNA (Allan *et al.*, 2003).

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

2.8.1 Parasitological methods

2.8.1.1 Coprological examination

Coprological examination allows detection of *Taenia* eggs from stool samples. However, the techniques employed are known to have both low sensitivity and specificity due to the intermittent nature of egg excretion leading to underestimation of the prevalence of taeniosis (Garcia *et al.*, 2003). 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.

If destrobilation has led to a massive discharge of eggs these may be absent from the faeces for up to several weeks thereafter (WHO, 1983). Furthermore *T. saginata* and *T. solium* eggs are identical under the light microscope leading to problems with

diagnostic specificity. This is particularly important given the risks associated with *T. solium* infection (Allan *et al.*, 2003). The formaline-ether concentration technique is the technique that is widely used for the detection of *Taenia* eggs in faeces.

2.8.1.2 Morphological examination

Identification of human adult intestinal taeniids to species level classically relies on the recovery of mature proglottids or scoleces. This recovery has, however, proved difficult due to the disintegration of the proximal end of the worm when modern cestoidal drugs are used (World Health Organisation, 1983). Jeri *et al.*, (2004) improved the treatment method to obtain a recognizable tapeworm, making differentiation between *T. saginata* and *T. solium* easier. Proglottids can be stained with the Semichon's acetocarmine stain method to enable morphological differentiation. The differentiation of the two human *Taenia* species is based on the number of uterine branches present in well-preserved gravid proglottids or on the absence or presence of hooks on the scolex of the tapeworm (Mayta *et al.*, 2000).

2.8.2 Immunodiagnostic techniques

The development of improved immunodiagnostic tools has contributed to our knowledge on the importance of taeniosis/cysticercosis by enabling sero-epidemiological surveys and community–based studies to be carried out (Dorny *et al.*, 2003). Immunodiagnostic techniques include detection methods for specific antibodies and for circulating parasite antigens in serum or body fluids such as

cerebrospinal fluid (CSF) and more recently urine. Since pigs are the primary intermediate hosts, prevalence of porcine cysticercosis is a reliable indicator of active transmission zones (Sanchez *et al.,* 1997; Garcia-Garcia *et al.,* 1999). In epidemiological studies, serological tools can be applied to diagnose human and pig cysticercosis. Diaz *et al.* (1992) recommended serological studies in both humans and pigs as being useful for determining areas where the disease is endemic and defining and targeting high-risk families to *T. solium* antigen contact as well as for monitoring the success of control programmes by determining the incidence of new cysticercosis infections. Flisser, (2002) reported that since there are no clinical features specific for cysticercosis, even asymptomatic brain lesions not uncommon, imaging methods unavailable for epidemiological studies; the, definition of cases is based solely on immunodiagnostic methods. In surveys on cysticercosis, immunodiagnostic tools applied on human and pig serum samples are useful in prevalence estimation and identification of risk factors associated with transmission of *T. solium.* A high seroprevalence in a community indicates a "hot spot" where preventive and control measures should be applied (e.g. Garcia-Noval *et al.* 1996; Subahar *et al.*, 2001). Immunodiagnostic tools also offer the possibility of surveillance of the infection during and after control programmes (Garcia *et al.*, 2000; Sarti *et al.*, 2000; Vazguez-Flores *et al.*, 2001).

The occurrence of human transient antibody response in *T. solium* infection in field conditions was found to be a major contributor to the over estimation of cysticercosis prevalence in endemic areas of Peru and Columbia. Data from serological surveys in these areas demonstrated that about 40% of seropositive people were seronegative when re-sampled after one year (Garcia *et al.*, 2001).

However, it has recently been observed that collection of cerebrospinal fluid or blood is an invasive procedure that requires technical expertise and the use of disposable syringes. If the method is not carried out under stringent conditions there is the risk of acquiring blood-borne infections such as hepatitis B virus and human immunodeficiency virus (Parija *et al.*, 2004). Specimens collected using non-invasive methods could therefore be of immense value in the diagnosis and in epidemiological studies of parasitic diseases. Recently there has been much interest in the collection of body fluids including urine, saliva, and tear drops other than serum. Of these, urine is increasingly used as a specimen alternate to blood for the diagnosis of many parasite infections (Parija, 1998).

2.8.2.1 Antibody Detection ELISA

T. solium infection results in a specific antibody response, mainly of the IgG class. Various techniques to detect antibodies to *T. solium* infections in man and pigs have been described such as the complement fixation test, hemaglutination, radioimmunoassay, enzyme linked immunosorbent assay (ELISA), dipstick ELISA, latex agglutination and immunoblot (Ferreira *et al.*, 1997; Garcia and Sortelo, 1991; Ito *et al.*, 1998; Miller *et al.*, 1984; Rocha *et al.*, 2002; Tsang *et al.*, 1989). Serodiagnosis of cysticercosis through detection of anti-parasite antibody has been widely evaluated using several target antigens, ranging from total *T. solium* extracts of the metacestodes (Flisser *et al.*, 1994) to selected preparations, such as cyst fluid, scolex or extracts of external membranes (Larralde *et al.*, 1986). Pinto *et al.* (2000) conducted a study to evaluate antigens of *T. solium* and *T. crassiceps* cysticerci in

the ELISA test for the diagnosis of porcine cysticercosis. Four antigens; (i) vesicular

fluid and (ii) crude *T. crassiceps* antigens and, (iii) scolex and (iv) crude *T. solium* antigen preparations were assayed. The results indicated that though all the antigens showed good performance, the vesicular fluid of *T. crassiceps* was the best followed by crude *T. crassiceps* antigen preparations. A separate study conducted by Nunes *et al.* (2000) also found similar results. According to the study, the use of cyst fluid and crude antigens of *T. crassiceps* metacestodes obtained the best results of overall specificity and sensitivity of 100 and 96.4% respectively.

Garcia *et al.* (2001) noted that antibody detection has an important drawback of failing to distinguish between exposure to infection and an established, viable infection, resulting in transient antibodies. Secondly, antibodies may persist long after the parasite has been eliminated by immune mechanisms and/or drug therapy (Harrison *et al.*, 1989; Garcia *et al.*, 1997). 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. The occurrence of cross-reactions with other diseases such as hydatidosis and ascaridosis has been observed with *T. solium* antigen (Pinto *et al.* 2000).

2.8.2.2 Antibody Detection EITB

The most specific and widely used test developed for the diagnosis of cysticercosis in human and pig serum samples is the EITB, an immunoblot of seven cysticercus glycoproteins, purified by lentil lectin-purified chromatography, which gives close to 100% specificity and a sensitivity varying from 70 to 90% (Tsang *et al.*, 1989).

However, a sensitivity of only 28% has been found in cases with single cysts in the brain (Wilson *et al.*, 1991). The antigen mixture used is not applicable for ELISA because of the presence of non-specific fractions (Dorny *et al.*, 2003). 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, 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. 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.

In developing countries ELISA is preferred because of its better availability, simplicity, and lower cost compared with immunoblot (Rosas *et al.*, 1986).

2.8.2.3 Antigen Detection ELISA

Due to the two drawbacks associated with antibody detection namely production of transient antibodies and persistence of antibody after infection, antigen detection may provide a suitable alternative (Dorny *et al.*, 2003). Several workers (De Jonge *et al.*, 1987; Harrison *et al.*, 1989; Brandt *et al.*, 1992; Draelants *et al.*, 1995; Onyango-Abuje *et al.*, 1996 and Van Kerckhoven *et al.*, 1998) have contributed to the development of antigen detecting ELISAs. Harrison *et al.* (1989) developed an antigen detecting ELISA based on a mouse monoclonal antibody (MoAb) with a repetitive carbohydrate epitope found in lentil-lectin adherent glycoproteins present on the surface and in the secretions of *T. saginata* cysticerci. As the target glycoprotein contains multiple antigenic epitopes 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). The circulating antigen detecting technique offers the advantage over the Ab-ELISA of only demonstrating the presence of live cysts and is reported to give a better correlation between the actual presence of viable infective cysticerci and antigen positive cases (Harrison *et al.* 1989). It is also reported to give fewer cross-reactions with other helminth infections (Dorny *et al.*, 2000).

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

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

The sensitivity of the antigen detecting ELISA is reported to be high. Garcia *et al.* (2000) found a sensitivity of 85%, which is one of the highest recorded at present. However, in their data set only patients who were seropositive on EITB were selected. Erhart *et al.* (2002) found a very good agreement between an ELISA for detecting circulating antigens, computerized tomography (CT) scanning and biopsy examination of subcutaneous cysticerci. Remarkably low levels of cross-reactions have been observed in serum from a wide range of helminth and protozoan infections (Harrison *et al.*, 1989; Erhart *et al.*, 2002).

2.8.2.4 Coproantigen Detection for *T. solium*

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

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

Coproantigen-based immunodiagnostic studies for *Taenia* in dogs and humans have all employed antigen capture ELISA assays using sera from rabbits hyperimmunised with either adult worm somatic or excretory-secretory products (Allan *et al.*, 2003). They have been used to detect antigen in sollubilised faecal samples. Allan *et al.* (2003) further reported that antigen detection is genus specific with *T. saginata* and *T. solium* both reacting in the assays but with no cross reactions with faeces from other infections including *Hymenolepis* cestodes.

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

Whilst these assays have been applied successfully as part of field research programmes in endemic countries, issues such as cost and accessibility remain to be addressed if these tests are to be used routinely in endemic countries (Allan *et al.*, 2003). It is for this reason that the tests are no longer commercially available for diagnosis but only for research.

2.8.3 Molecular approaches

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

Differentiation of human *Taenia spp.* by molecular assays is normally done on proglottids expelled from carriers after treatment (Eom *et al.*, 2002; Rodriguez-Hidalgo *et al.*, 2002; Gonzalez *et al.*, 2002). In recent years, PCR tests for species-specific confirmation of *Taenia spp.* have been developed based on the detection of the parasite DNA in faecal samples (copro-DNA) (Yamasaki *et al.*, 2004), or on cysticercii (Yamasaki et al., 2002; Yamasaki et al., 2004) or eggs present in the faeces (Yamasaki *et al.*, 2004).

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

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

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

2.9 Prevention and control of taeniosis/cysticercosis

Individuals and organisations have, through a lot of research, come up with various recommendations and control strategies for the prevention and control of the taeniosis/cysticercosis disease complex.

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 prevent pigs from accessing human faeces.

Carrique-Mas *et al.* (2001) suggested 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. Health education may play an important role by helping people to change eating habits and to improve the self-diagnosis of *T. saginata* infection. The World Health Organisation (1983) recommends the detection and treatment of tapeworm carriers or treatment of the whole population. In regions where taeniosis is only due to *T. saginata* and human cysticercosis does not occur, praziquantel may be used. Mass treatment of the human population may be performed where *T. solium* is endemic (Allan *et al.*, 1997). For treatment of human taeniosis in *T. solium* endemic areas, niclosamide is preferred because it is highly effective against the intestinal stage of the parasite and has no effect on the cystic stage (WHO, 1983; Miyazaki, 1991; Allan *et al.*, 1997)

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 defecation 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. Sarti-G *et al.* (1992a) however, noted that such a change was likely to be resisted because of the traditional and functional aspects of established pig-rearing practices. Pal *et al.* (1999) further noted that in developing countries, pigs are free roaming and raised by subsistence farmers who cannot afford enclosed pens or proper animal feed, and meat is sold off outside the abattoir system.

The potential of a vaccine for controlling porcine cysticercosis has been described in the past and some promising results were also reported (Gonzalez *et al.*, 2003). A successful vaccine that has the potential of interrupting the cycle should decrease over time the number of infected pigs and humans. Nevertheless, the potential use of a vaccine will depend on its availability and cost (Gilman *et al.*, 1999). Sciutto *et al.* (1995) reported that vaccination of pigs against *T. solium* cysticercosis should be 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 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.

Though current efforts are centred on the control and potential eradication of taeniosis/cysticercosis, no control strategy has yet been proven effective and sustainable (Cruz *et al.*, 1999; Schantz *et al.,* 1993; Sarti *et al.*, 1997). However, Garcia *et al.* (1999) reported that the only proven way of eradicating the disease complex is the improvement of sanitary conditions, tap water and sewage connections as occurred in Europe in the early 1990s. They still noted that the economical and geographical constraints make this impossible in the near future for most developing countries. Suggestions are 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 (Garcia *et al.* 1999).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area

The Southern province of Zambia lies between latitudes 15° 14'S and longitudes 25° E and 28° E and Gwembe and Monze districts (Figure 3.1) were selected as study districts following a preliminary visit and studies (Phiri *et al.*, 2001, 2002; Dorny *et al.*, 2004; Sikasunge 2005) that were made and indicated a high prevalence of porcine cysticercosis and the presence of a lot of free ranging pigs. 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 a very large area and consist of 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 329 mm to 848 mm (mean 636 mm) in Gwembe and 524 mm to 881 mm (mean 735 mm) 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). In Monze , much of the *Brachystegia* "Miombo" and *Acacia* "Munga" woodlands have been cut down to give way to agriculture, while in Gwembe, *Colophospermum mopane* "Mopane" and scrub woodland predominate (Mulofwa, *et al.* 1994).

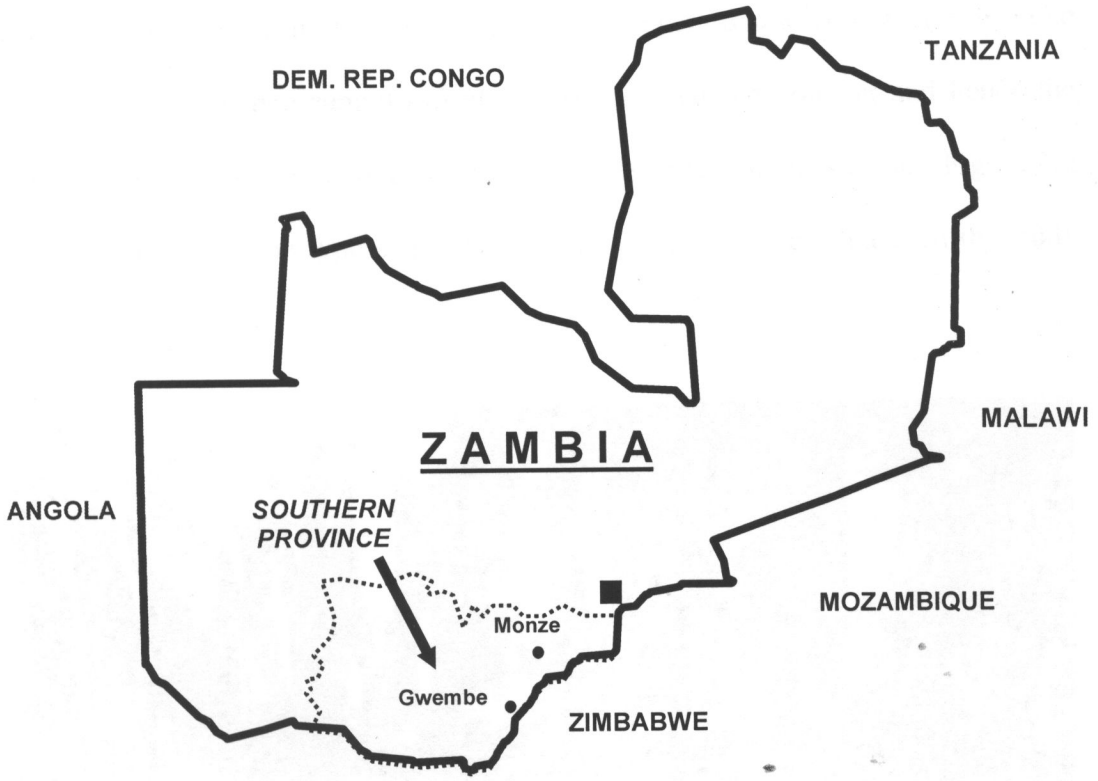

Figure 3.1 Map of Zambia showing the study areas in Gwembe and Monze districts in the Southern province.

Majority of the people in the two districts belong to the Tonga ethnic group which in Zambia has for long been associated with cattle rearing. However, the advent of cattle diseases such as corridor disease, Foot and Mouth disease (FMD) and Contagious Bovine Pleuropneumonia (CBPP) and the lack of grazing lands for ruminants due to poor rainfall have greatly depleted the number of cattle in the area. The farmers have also recognised quicker and higher returns on their investment when they rear pigs. Higher consumer demand for pork has also contributed to an increased interest in raising pigs (Phiri *et al.*, 2003).

The pigs are raised under extensive conditions and are crossbreeds between Landrace and Large white breeds. Most of the villagers that keep pigs are resource poor farmers and hence cannot afford to provide adequate housing and hence the kind of housing provided is one whereby pigs easily find their way out. Figure 3.2 also shows the common type of pig house seen in most of the villages in the study area.

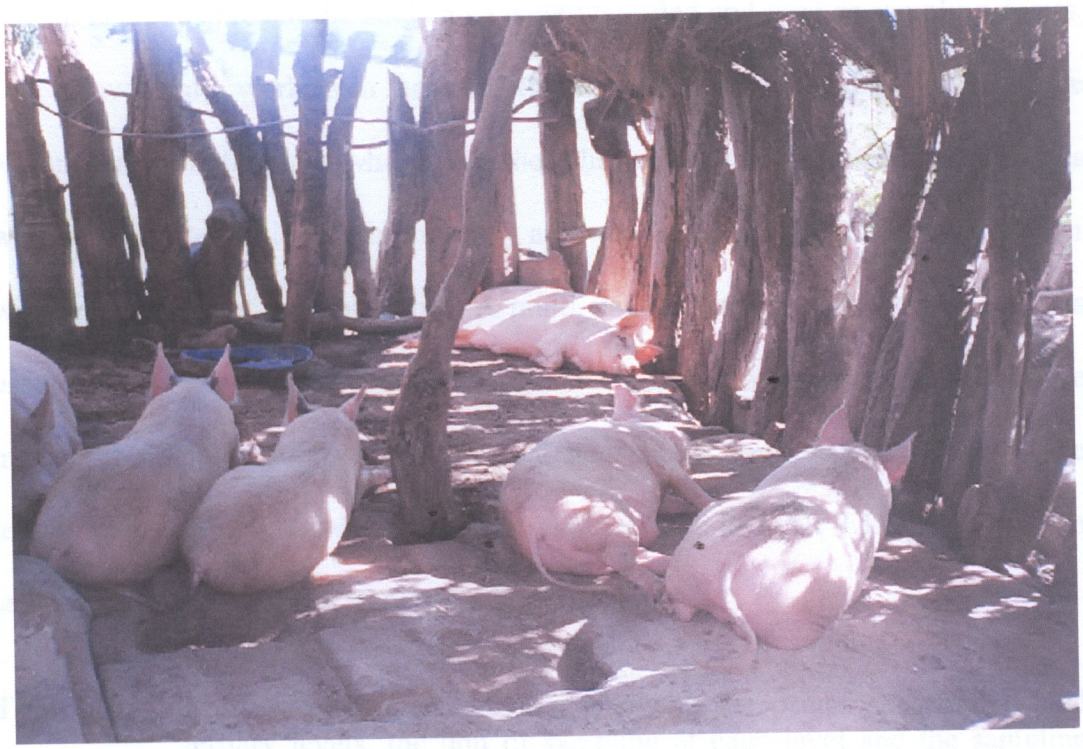

Figure 3.2 The Large white and Landrace cross breed pigs in pens that do not prevent the pigs to escape as commonly found in the rural areas of Southern province

A total of 15 health centres were used as sampling centres in this study. In Monze district, two Mission Hospitals (Monze and Chikuni), four Rural Health Centres (Chisekesi, Nampeyo, Mwanza, and Hamapande) and two Health Posts (St. Mary's and Kaumba) were used. In the Gwembe District, the district general hospital, five rural health centres (Lukonde, Munyumbwe, Bbondo, Luumbo and Chaboboma) and

one rural health post (Chipepo) were used for sampling. All these health centres serve the villages around them. The population served by each of these health centres ranges from 8,000 to 12,000.

3.2 Study design

Fifteen health centres in the study area were identified and used as sampling points. One or two health workers in these institutions were recruited and made part of the research team. Individuals who visited these health centres with unrelated problems were recruited to participate in the study and were asked to bring forward any other willing members of the household. A multi-stage cluster sampling technique was used. Health centres that service villages with households that keep pigs were treated as primary units while individual households were secondary units. The tertiary unit was the individual in the household as seen in Table 3.1. Simple random sampling was done at each stage.

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	Health centres in the study areas	Simple random
Secondary Unit	Households serviced by the health centres	Simple random
Tertiary Units	Individuals in the households	Simple random

The sample size to estimate the prevalence of taeniosis/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 20% to give the maximum sample size. Therefore, $n = 1.96^2 \times 0.2 \times 0.8/0.05^2 = 246$. Thus, at least 246 individuals were examined for presence/absence of taeniosis/cysticercosis in each district. The target was to collect samples from at least fifty percent of the individuals in the household. However, the number of individuals sampled in each household depended on the number of individuals in the household and the willingness of each individual to participate in the study. Thus at least one willing individual from each household was sampled.

3.3 Ethical considerations

As the research involved human subjects, approval was obtained from the University of Zambia Research and Ethic's Committee (Ref.: 006-09-04). Further approval was sought from the District Director of Health of each district and also from the community leaders before commencement of the study. Finally permission was sought from the individual subjects to take part in the study. Subjects were not forced to participate and were free to drop out at any stage of the study. Participation was requested of individuals of all ages i.e. permission to take stool and urine samples, clinical inspection for palpable nodules and collection of data on medical history in relation to presence of adult tapeworm. For individuals below the age of

18, permission was sought from their parents or guardians. For children below the age of 12, parents were asked about their medical history. Participants were divided into six age groups at intervals of twelve years i.e. below 12, 12 – 24, 25 – 36, 37 – 48, 49 – 60 and above 60 years.

3.4 Sample collection

Prior to the commencement of the work, communities were sensitised about the study by the health workers from the selected health centres. A total of 701 individuals from 368 households came forward to participate in the study and were interviewed.

Stool and urine samples were collected from randomly selected individuals after individual informed consent. Those individuals with a history of persistent headaches, seizures, chronic diarrhoea and abdominal pain, nausea, skin nodules and the presence of proglottids in faeces were included in the study. An individual was classified as positive for taeniosis or cysticercosis if he/she was positive on stool coproscopic examination and/or urine Ag-ELISA, respectively.

Recruited individuals were each given two sample bottles, one for urine and the other for stool. They were asked to fill at least half of the sample bottle with specimen and submit the samples to the health centre the following morning. A total of 686 faecal samples and 627 urine samples were obtained from the fifteen health centres. In Monze, 384 faecal and 359 urine samples from 189 households were collected while in Gwembe 294 faecal and 268 urine were collected from 175

households. Blood samples were also collected from 101 randomly selected and willing individuals.

Immediately the samples were submitted, they were placed in a refrigerator or on ice in a cooler box and later refrigerated. The samples were then brought to the laboratory. The stool samples were immediately processed and examined microscopically using the formalin-ether concentration technique. The urine samples were aliquoted in duplicates of about 2 ml each and frozen at about -20°C for later use in the detection of cysticercal antigens using a monoclonal antibody-based sandwich ELISA. The clotted blood was separated by centrifugation at 3000 rpm for 15 min and serum obtained. The supernatant (serum) was dispensed into 2 ml aliquots and stored in labelled vials, which were frozen at -20°C until use. Both Ag and Ab-ELISAs were done on these serum samples.

3.5 Parasitological methods

3.5.1 Coproscopic examination of faecal samples

Presence of helminth ova in stool was done microscopically using the formalin-ether concentration technique as described by Garcia, (2001) and the World Health Organisation (WHO, 1991) with slight modifications to enable use of the available materials. This procedure ensures recovery of most helminth eggs and larvae present in the stool sample.

To prepare 10% formalin-saline solution, 8.5 g of sodium chloride was weighed and added to 1000 ml of distilled water in a conical flask. This was sterilised in an

autoclave for 15 min. 900 ml of the cooled saline solution was obtained and to it was added 100 ml of 40% formalin to give a 10% formal-saline solution.

About two grams of fresh stool was transferred into a centrifuge tube containing 8 mls of 10% formal saline solution, mixed using bamboo skewers and 2 mls of ether added. The tubes were closed with a stopper and shaken vigorously for thorough mixing before being centrifuged at 2500 rpm for 5 min. The supernatant fluid was then decanted out leaving the sediment in the tube. A drop of the sediment was obtained after tapping the tube and placed on a slide. A coverslip was placed and the slide examined under the microscope. Duplicate smears were made for each sample to enhance sensitivity. The slide was systematically scanned using the 10X objective. The entire coverslip area was examined under low power (total magnification, X10). If anything suspicious was seen, the 40X objective was used for a more detailed examination. During examination, the coverslip was tapped when necessary to see objects move and turn over. The researcher and a technician examined two smears per sample.

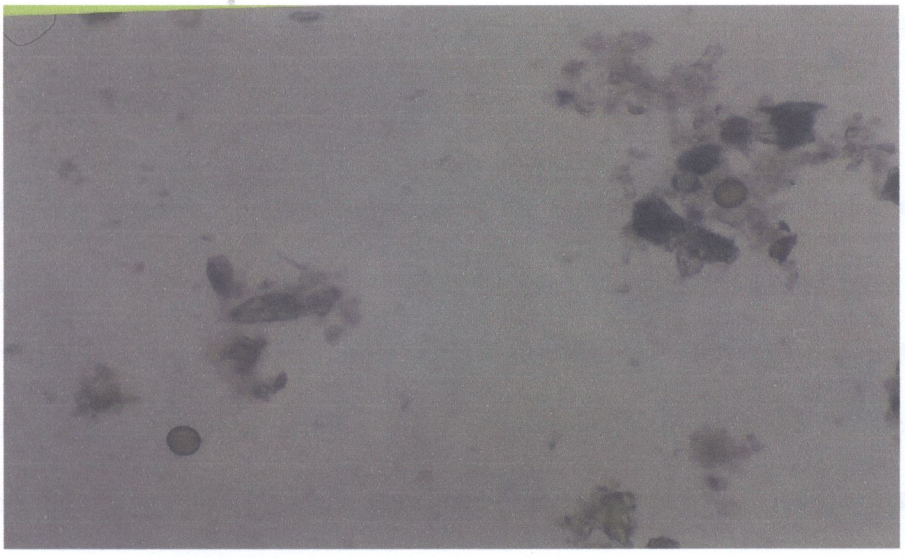

Figure 3.3 Taeniid eggs were seen on a slide for one of the stool samples, these eggs could be for *T. saginata* or *T. solium* as these are indistinguishable morphologically.

3.5.2 Morphological examination of proglottids

Individuals found positive on coproscopic examination were treated with niclosamide (2 g per os for individuals above 6 and 1g for those below 6 years of age). This was followed by a magnesium sulphate purgative (25 g) two hours later. Proglottids were obtained from treated individuals over a period of three days and stored in 70% ethanol at 4°C until use. The collected proglottids were used for morphological identification of the tapeworm.

The proglottids were stained using Schneider's aceto-carmine stain which was prepared by slowly adding 45 ml of glacial acid to 55 ml of distilled water in a flask. Then 5 g of carmine powder was added and the solution boiled for 15 min. To check whether the stain was properly prepared, a few drops of the stain were added to a

small dish of 70% alcohol and if the solution turned moderate to dark pink, then it was well prepared.

The staining procedure began with cutting the tapeworm into parts of about 3 cm each and placing them between two glass slides. The two glass slides were then tied firmly with a string so as to flatten the proglottids. The flattened proglottids were placed in 70% alcohol, overnight. After removing the slides from the alcohol and untying the strings, the flattened specimens were placed in a petri-dish containing aceto-carmine for staining for about 5 minutes. The samples were shaken periodically. The stained specimens were destained in acid-alcohol i.e. 80% alcohol containing about 1% hydrochloric acid. This way, both destaining and dehydration were simultaneously achieved. The degree of destaining was constantly checked with a stereo microscope until it was observed that the superficial tissues were clear but interior organs were well stained. The samples were then left in 95% alcohol overnight and then transferred to a 1:1 solution of 100% alcohol and xylene and left there for 10 minutes. This was followed by placing the samples in xylene for 15 min. Finally, the samples were mounted on slides with Canada balsum, and left to dry.

The slides were checked under the microscope. The number of main uterine branches in gravid proglottids was counted.

3.6 Molecular methods

A sub-sample of 200 was selected from the 678 faecal samples for PCR-RFLP. Of these 14 samples were positive on coproscopic examination. DNA extraction and

PCR-RFLP were carried out at the Institute of Tropical Medicine, Antwerp, Belgium.

3.6.1 Primers

A semi nested primer set (nTAE, ITMF ITMTnR) designed at the ITM were used in the molecular examination of the faecal samples. The primer pair, nTAE/ITMTnR was used in the first round and ITMTnR/ITMF in the semi nested PCR. Primer nTAE and ITMTnR were biotinylated for use in the sequence capture method of DNA extraction as described by Mangiepan *et al.*, (1996).

3.6.2 DNA extraction

DNA was extracted from faecal samples using the Mangiepan technique with slight modifications (Mangiepan *et al.*, 1996). The modifications of the protocol during this study consisted of the use of zirconium beads (Merlin Diagnostics, Cat#. 11079101z; diameter 0.1mm) instead of glass microspheres; the temperature during the capture of sequences being 50°C instead of 60°C and the replacing of the tube during the wash step for a cleaner one to transfer beads (to get rid of remnants of faeces adhering to the tube wall). The process involved three stages, homogenization, digestion and hybridization.

Homogenization involved placing 1-2 g of faeces in a falcon tube and adding 2ml Phosphate Buffered Saline (PBS) + 2% Polyvinylpolypyrolidone (PvPP) (1g PvPP powder in 50 ml PBS). This was heated at 100°C (boiling) for 10 minutes and then centrifuged at 1200 g for 3 min. The supernatant was discarded and the pellet re-

suspended in 4 ml of 2% v/v Tween20 solution in MilliQ water and approximately 8 glass beads (diameter 2 mm) were added. Mixing was done on a vortex for 1 min and the mixture left to stand for 30 min. The resulting supernatant was transferred to a new falcon tube and centrifuged at 1500 g for 30 min. The supernatant was discarded and the pellet re-suspended in 1 ml MilliQ water. This suspension was transferred to an eppendorf tube (1.5 ml) and centrifuged at 1200 g for 5 min and the supernatant discarded.

Digestion was done by adding 500 µl TEN-buffer, 500 µl zirconium beads (0.1 mm) and 50 µl Proteinase K (20 mg/ml). This was mixed on a vortex for 50 sec and incubated overnight while shaking at 1400 rpm and at 50°C. This was followed by mixing on a vortex for 50 sec.

Hybridization involved heating the samples at 100°C for 10 min (boiling) and then cooling on ice (0°C). This was followed by the addition of 200 µl of 3.75 M NaCl (0°C), and 2.5 pmol of each biotinylated primer and then incubated for 3 hours at 50°C and shaking at 800 rpm. The samples were then cooled at room temperature and 10 µl of dynal beads added. Before adding, the dynal beads were washed 5 times with B & W-buffer. The B & W-buffer was prepared by adding 0.5 mls of 1 M Tris stock solution, 1 ml of 0.5 M EDTA stock solution, and 33.3 ml of 3 M NaCl stock solution and 15.2 ml of MilliQ. The beads were then concentrated with a "magnetic block" and washed once with TE-buffer. Then the beads were carefully transferred to a new eppendorf tube and again washed once with TE-buffer. The beads were then suspended in 20 µl of TE-buffer and 5 µl was then used per 25 µl of PCR reaction mixture.

3.6.3 PCR

PCR was performed in a total of 25 µl containing 5 µl faecal extract as template and 20 µl of PCR mix. The PCR mix was made by mixing together 1 µl of Yellow sub^{TM} (GENEO Bioproducts, Germany); 3.3 µl of MilliQ water; 12.5 µl buffer (20 mM Tris-HCl pH 8.4; 100 mM KCl; 0.2 % v/v triton X-100; 1.5 mM $MgCl_2$); 100 pmol of a mix of the four deoxynucleotide triphosphate (dNTP final concentration: 0.2 mM); 25 pmol of each primer and 0.4U *Taq* polymerase Silverstar (Eurogentec, Seraing, Belgium). For the semi nested PCR, a final volume of 25 µl, i.e. 0.5µl from the first PCR round plus 24.5 µl PCR mix, as described above except that 0.3U *Taq*-polymerase was used.

The first PCR round and the semi nested PCR were performed in a Biometra Thermal cycler (Westburg, Belgium). The first PCR round, with primers nTAE/ITMTnR, consisted of and initial denaturation step at 92°C for 4 min followed by 40 cycles consisting of 92°C for 30 sec, 56°C for 4 sec and 72°C for 1 min. The semi nested PCR, with primers ITMF/ITMTnR, consisted of an initial denaturation step at 92°C for 4 min, followed by 25 cycles of 92°C for 30 sec, 56°C for 45 sec and 72°C for 45 sec.

Five microlitre of each amplified product together with a gene Ruler marker of 100 bp (MBI Fermentas, GmbH, St. Leon-Rot, Germany) was separated by electrophoresis, using a Mupid-21 system (Eurogentec, Seraing, Belgium) in 2% w/v agarose and 0.04 M Tris-acetate plus 0.002 M EDTA buffer for 20 min at 100 volts. The gel was stained with ethidium bromide (Sigma-Aldrich) for 30 min and the DNA products visualized by ultraviolet light. A picture was then taken using a

digital camera (Nikon, Coolpix 4500). All pictures were edited with "Photostudio" (www.arcsoft.com) on a Mackintosh computer.

3.6.4 Restriction Fragment Length Polymorphism (RFLP)

RFLP was carried out as described by Rodriguez-Hidalgo *et al.*, (2002) using *Dde* I as restriction enzyme. RFLP digestion was performed according to the manufacturer's specifications (Life Technologies) using 1àU *Dde*I/µg DNA, plus 6 µl of amplified DNA in a total of 15 µl. Tubes containing the reaction mixture were incubated overnight at 37°C. Six microlitres of the digested product was mixed with 2 µl of the loading buffer and transferred onto a 10% polyacrylamide gel. A marker of 100 bp was included for size identification of the bands. DNA was separated by a Mighty Small horizontal electrophoresis apparatus (Amercham Pharmacia Biotech, Roosendaal, Netherlands) in TBE (88 mM Tris, 89 mM boric acid, 2 mM EDTA) buffer. The gel was stained using a commercial kit, Plus one (Amercham Pharmacia Biotech), and preserved under plastic foil. A photo was then taken using a digital camera as described for PCR.

3.7 Enzyme-linked-Immunosorbent Assay protocols

3.7.1 Enzyme-linked-Immunosorbent Assay for the detection of *T. solium* cysticerci antigens (Ag-ELISA) in urine

The urine samples were examined for the presence of *T. solium* cysticercal antigens using a monoclonal antigen-based double sandwich Ag-ELISA as done 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 MoAbs 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) with monoclonal antibody B158C11A10 as the first MoAb and was followed by a biotinylated MoAb B60H8A4 as the detector antibody (second MoAb). Pre-treatment of the urine samples involved mixing the urine sample with the second MoAb. For positive control and unknown samples, 220 µl of urine was added to an equal amount of the second MoAb and incubated at 37°C for one hour. Urine from a known highly infected pig (on tongue palpation and post mortem examination) was used as the positive control for the first plate. The plate was coated with 100 µl of MoAb B158C11A10 diluted at 11.8 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% New Born Calf Serum (NBCS). The plates were then incubated on a shaker for 15 min at 37°C. Thereafter, plates were drained as above. Without washing the plate, 100 µl of pre-treated urine was added and incubated at 37°C on a shaker for 15 min. The plates were emptied and without washing, 100 µl of pre-treated urine added. The plates were then incubated overnight at 4°C without shaking. The following day, the plates were emptied and washed five times. Then 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. One tablet of the chromogen/substrate, orthophenylene diamine (OPD) (SIGMA,

#P-8412) was added to 180 ml of distilled water containing phosphate citrate buffer which also contains sodium perborate. Then 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, 50 µl of 4N H_2SO_4 was added to each well. The plates were read using an ELISA reader (Labsystem Multiskan RC) at 492 nm.

3.7.2 Enzyme-linked-Immunosorbent Assay for the detection of circulating *T. solium* cysticerci antigens (Ag-ELISA) in serum

The serum samples were also examined for presence of *T. solium* cysticercal antigens using a monoclonal antigen-based double sandwich Ag-ELISA as described for antigen detection in urine (Section 3.7.1). The same monoclonal antibodies were used namely monoclonal antibody B158C11A10 used as first MoAb and a biotinylated MoAb B60H8A4 used as detector antibody (second MoAb).

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 g of TCA in 20 ml of distilled water. The serum samples were thus, pre-treated by mixing an equal volume of serum and 5% TCA. For the negative control sera, 75 µl of serum was used while 150 µl of serum was used for the pre-treatment of positive control and the test sera. These mixtures of sera and 5% TCA solution were incubated for 20 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 (0.610 M) 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.

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 drained 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 15 min at 37°C. Thereafter plates were drained. 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. The plate was drained and washed five times. 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 drained and washed five times with PBS–T20 as above. 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. One tablet of the chromogen/substrate, orthophenylene diamine (OPD) (SIGMA, #P-8412) was added to 180 ml of distilled water. Then 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, 50 µl of 4N H_2SO_4 was added to each well. The plates were read using an ELISA reader (Labsystem Multiskan RC) at 492 nm.

3.7.3 Enzyme-linked Immunosorbent Assay for the detection of antibodies (Ab-ELISA) against *T. solium* cysticerci in serum

The serum samples were examined for presence of antibodies against cysticercal antigens using *T. solium* cyst fluid (TsCF) as the antigen for the Ab-ELISA. The cyst fluid 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 PBS. The excess PBS was removed by spreading the cysts on Whitman's blotting paper No. 4. 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 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 collected as supernatant. The TsCF supernatant was aliquoted into 2 ml vials and stored at -20 °C until used as TsCF antigen in the antibody ELISA.

The BCA™ Protein Assay Kit (PIERCE) was used to determine the protein concentration of the cyst fluid. This assay is a detergent-compatible formulation based on bicinchoninic acid (BCA) for the calorimetric detection and quantitation of total protein. The assay involves the preparation of a set of protein standards. Albumin (2.0 mg/ml) was used as the standard protein and diluted as shown in Table 3.2.

Table 3.2 Preparation of Diluted Albumin (BSA) Standards (Stock concentration = 2,000µg/ml)

Vial	Volume of diluents	Volume and Source of BSA	Final BSA concentration
A	0	300 µl of stock	2,000 µg/ml
B	125 µl	375 µl of stock	1,500 µg/ml
C	325 µl	325 µl of stock	1,000 µg/ml
D	175 µl	175 µl of vial B dilution	750 µg/ml
E	325 µl	325 µl of vial C dilution	500 µg/ml
F	325 µl	325 µl of vial E dilution	250 µg/ml
G	325 µl	325 µl of vial F dilution	125 µg/ml
H	400 µl	100 µl of vial G dilution	25 µg/ml
I	400 µl	0	0µg/ml

The BCA™ Working Reagent (WR) was prepared by mixing 50 parts of Reagent A (containing sodium carbonate, sodium bicarbonate, bicinchonic acid and sodium tartrate in 0.1 M sodium hydroxide) with 1 part of Reagent B (containing 4% cupric sulphate). Twenty five microlitres each standard or unknown sample replicate was pipetted into a microplate. To each well was added 200 µl of the WR and the plate mixed thoroughly on a shaker for 30 seconds. The plate was then covered and incubated at 37°C for 30 minutes after which the plate was cooled to room temperature. Absorbances were measured using an ELISA reader (Labsystem Multiskan RC) at 492 nm. The average 492 nm absorbance measurements of the Blank standard replicates was subtracted from the 492 nm measurements of all the other individual standard and unknown sample replicates. After this, a standard curve was prepared by plotting the average Blank-corrected 492 nm measurement for each BSA standard versus its concentration in µg/ml. This standard curve was then used to determine the protein concentration of each unknown sample.

Before carrying out the Ab-ELISA's, serial titrations of antigens and test sera were performed to determine the optimum concentrations. A conjugate dilution of

1/20,000 was used as recommended by the manufacturer. Two ELISA plates were divided into four sections A, B, C and D with each section having a different antigen concentration of 24 µg/ml, 12 µg/ml, 6 µg/ml and 3 µg/ml, respectively. The stock antigen concentration was 1200µg/ml and this was diluted 1/50, 1/100, 1/200 and 1/400 to obtain concentrations of 24 µ/ml, 12 µg/ml, 6 µg/ml and 3 µg/ml, respectively.

Table 3.3 ELISA plate layout for the assay for sequential analysis

	1	2	3	4	5	6	7	8	9	10	11	12
A	**B**						**B**					
B	**P_1**						**P_1**					
C												
D	**P_2**						**P_2**					
E												
F												
G	**N**						**N**					
H												

Key

P_1 = Strong positive control sera

P_2 = Weak positive control sera

N = Negative control sera

B = Diluent (Serum blank)

E = Row of wells not used in the ELISA.

The plate layout was as shown in Table 3.3. Ninety six polystyrene ELISA wells were coated with 100 µl of cyst fluid antigen at the three dilutions and incubated at 4°C overnight. The general indirect ELISA as described below in this section was followed. Test sera was diluted 1/50, 1/100 and 1/200. The concentrations that gave the best color reactions were selected for use in the Ab-ELISA.

The Ab-ELISA was conducted as described by Pouedet *et al.* (2002) with minor modifications. Cyst fluid obtained from a highly positive cysticercosis pig was used as the antigen for the assay. The assay involved coating polystyrene 96 well ELISA plates (Nunc® Maxisorp) with 100 µl per well of cyst fluid antigen diluted at 1/100 (12 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 emptied and washed once with PBS–Tween-20 (phosphate buffered saline + 0.05% T20) 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 drained. Without washing the plate, 100 µl of test sera diluted at 1/100 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, AffiniPure Goat Anti-Human IgG, (Jackson Immuno Research Laboratories Inc.) 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 as above. Two capsules of phosphate citrate buffer with sodium perborate (SIGMA) were added to 200 mls of distilled water. 180 ml of this solution was obtained and one tablet of the chromogen/substrate, orthophenylene diamine (OPD) (SIGMA, #P-8412) added. Then 100 µl of this solution was added to the wells following which the 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.

3.8 Assessment of Risk factors by questionnaire

A questionnaire (Appendix 1) was developed and used to collect information on risk factors and other related information from the participants. The questionnaire was administered by the researcher and the health personnel recruited in the RHCs using the native language. Participants were randomly selected as they visited the clinics and recruited depending on the willingness of the individual to participate in the study. They were also asked to bring forward any other member(s) of the family willing to participate. Data was collected from all participants This included keeping of pigs by the household, medical history with particular emphasis on persistent headaches, seizures, skin nodules, chronic diarrhea and passing of proglottids, household's main source of income and the number of household inhabitants and their level of education. Information was also collected from those above 12 years of age on the respondents' knowledge on taeniosis and cysticercosis, feeding habits, with particular attention to pork consumption, source of drinking water and presence and usage of toilets. This is because at or above twelve years of age, a child is most likely to be in secondary education and hence may have heard about or be able to be aware of the infection.

3.9 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 Java Statistics software were used for statistical analysis and included chi-square to assess the association between positivity and the different variables at 95% confidence level. Using the *Kappa* test, the urine and serum ELISA

tests were compared two by two in order to find out the agreement between the tests in the detection of human cysticercosis. Agreement between coproscopic examination and PCR was also assessed using kappa.

CHAPTER FOUR

4.0 RESULTS

4.1 Prevalence of human taeniosis

4.1.1 Coproscopic examination

A total of 678 faecal samples were examined coproscopically as described in chapter three. Of these, 384 were from Monze and 294 from Gwembe districts. Of the total samples examined, 21 (3.1%) were found positive on coproscopic examination (Table 4.1). There was no significant difference in prevalence in the two districts (χ^2 = 2.305, p = 0.129), however, Gwembe district had more people testing positive (4.4%) than Monze district (2.1%).

Table 4.1 Prevalence of taeniosis after coproscopic examination in humans in Gwembe and Monze districts in Southern province of Zambia.

District	No examined	No +ve (%)
Gwembe	294	13(4.4)
Monze	384	8(2.1)
Total	678	21(3.1)

4.1.1.1 Prevalence of taeniosis by health centres

A total of 384 faecal samples were examined covering eight health centres in Monze district. The coproscopic examination results by health centre ranged from 0% for

Hamapande Rural Health Centre, Monze Mission Hospital and St. Mary's Health Post to 6.4% for Chikuni Mission Hospital (Table 4.2).

Table 4.2 Prevalence of taeniosis on coproscopic examination by health centre in Monze district

Health centre	No examined	No +ve (%)
Chisekesi Rural Health Centre	52	1(1.9)
Chikuni Mission Hospital	47	3(6.4)
Hamapande Rural Health Centre	29	0(0.0)
Kaumba Health Post	54	2(3.7)
Mwanza Rural Health Centre	39	1(2.6)
Monze Mission Hospital	49	0(0.0)
Nampeyo Rural Health Centre	86	1(1.2)
St. Mary's Health Post	28	0(0.0)
Total	384	8(2.1)

A total of 294 faecal samples were examined from Gwembe district covering seven health centres. The coproscopic examination results by health centre ranged from 0% for Bbondo Rural Health Centre to 5.3% for Lukonde Rural Health Centre (Table 4.3).

Table 4.3 Prevalence of taeniosis on coproscopic examination by health centre in Gwembe district

Health centre	No examined	No +ve (%)
Bbondo Rural Health Centre	49	0(0)
Chaboboma Rural Health Centre	41	2(4.9)
Chipepo Health Post	24	1(4.2)
Gwembe District Hospital	40	2(5.0)
Lukonde Rural Health Centre	57	3(5.3)
Luumbo Rural Health Centre	32	3(9.4)
Munyumbwe Rural Health Centre	51	2(3.9)
Total	294	13(4.4)

4.1.1.2 Taeniosis prevalence by age

The age range of the 678 stool donors was between 1 and 89 years. When divided into 6 age groups, the taeniosis age group prevalence ranged between 0 in the 49 to 60 and above 60 years age ranges and 4.2% in the below 12 years age group as shown in figure 4.1. When individuals were classified as young (less than 16 years of age) and adults (more than 16 years of age), no significant difference was observed in taeniosis prevalence ($\chi^2 = 1.084$, $p = 0.298$) between the two groups (4.2%, n=265 versus 2.4%, n=413) on coproscopic examination.

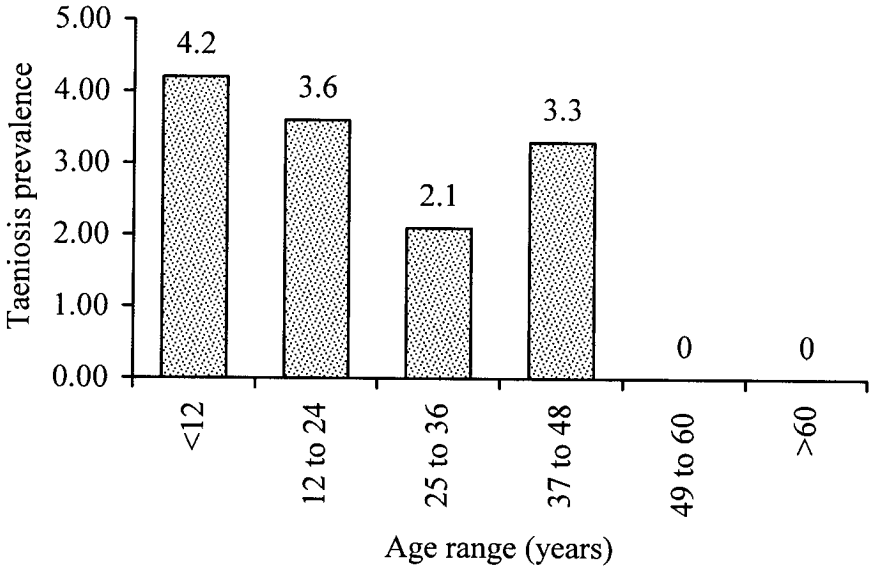

Fig. 4.1 Prevalence of taeniosis on coproscopic examination by age group

4.1.1.3 Taeniosis prevalence by sex

A total of 401 samples from female participants and 277 from males were coproscopically examined giving prevalences by sex of 3.0% for females and 3.2% for males. The results on coproscopic examination did not show any significant difference in prevalence ($\chi^2 = 0.000$, $p = 1.000$) between females and males (Table 4.4). This was also observed when the taeniosis prevalences of females and males were compared within districts (Monze: $\chi^2 = 0.004$, $p = 0.952$; Gwembe: $\chi^2 = 0.000$, $p = 1.000$). Gwembe District Hospital in Gwembe district had the highest prevalence for males with 13.3% while for females the highest recorded was 9.1% at Luumbo Rural Health Centre, which is also in Gwembe district.

Table 4.4 Prevalence of taeniosis according to sex in Gwembe and Monze districts

District	Sex	No examined	No +ve (%)
Gwembe	Female	181	8(4.4)
	Male	113	5(4.4)
Monze	Female	220	4(1.8)
	Male	164	4(2.4)
Total		678	21(3.1)

4.1.2 Polymerase Chain Reaction (PCR)

Amplification of DNA extracts using primer pairs ITMTNR/nTAE and ITMF/ITMTNR in a semi nested PCR was expected to yield amplicons of about ±370 bp. However, almost all samples, negative and positive, gave aspecific bands after analysis on 2% agarose gel and staining with ethidium bromide (Figure 4.2 and 4.3, not all pictures are shown).

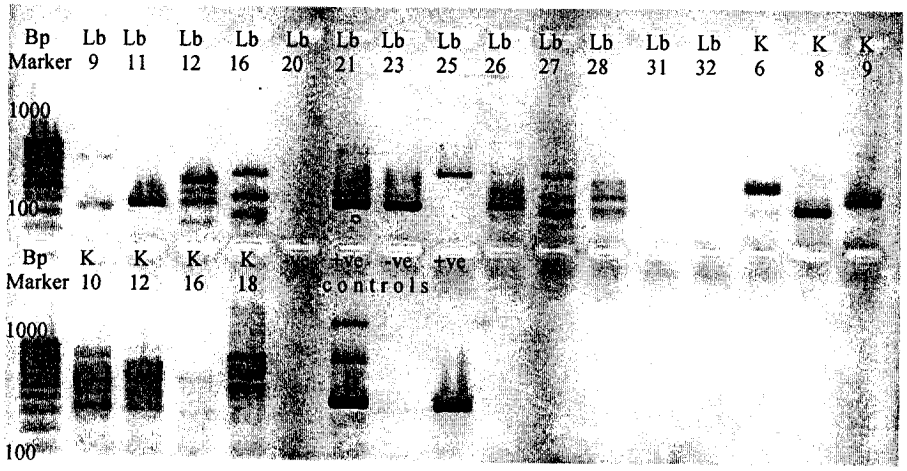

Figure 4.2 PCR results for DNA extracts from faecal samples coded Lb and K. Amplification was done using primers ITMTnR/nTAE (first round) and ITMTnR/ITMF (semi-nested). Positive samples show bands at ± 370bp. Samples Lb11, Lb21, Lb23 and K8 showed bands of ± 370bp and hence positive for *Taenia.*

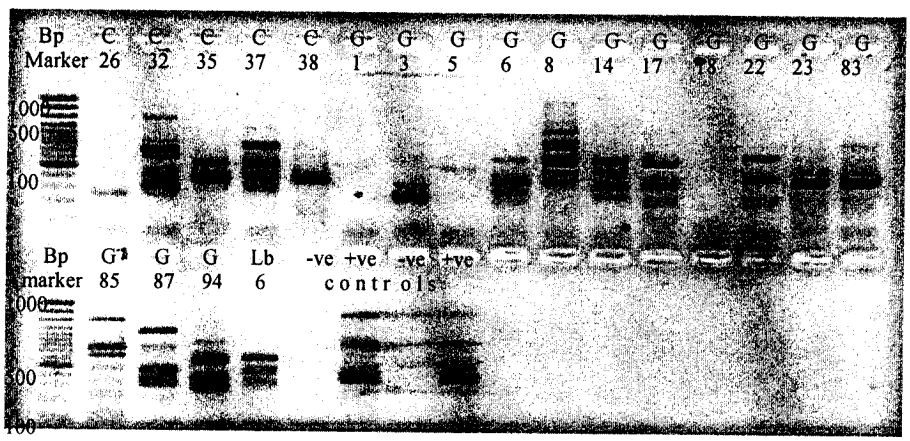

Figure 4.3 PCR results for DNA extracts from faecal samples coded C, G and Lb. Amplification was done using primers ITMTnR/nTAE (first round) and ITMTnR/ITMF (semi-nested). Positive samples show bands at ± 370bp. Samples C35, G22, G23 and G83 showed bands of ± 370bp and hence positive for *Taenia.*

Due to the aspecific bands obtained with the ITMTNR/nTAE and ITMF/ITMTNR set of primers, a new primer was used on those samples that were surely positive and those that were questionable. A new primer pair ITMTNR/TaenF, used in the first round and nTAE/ITMTNR was used on these samples in a semi nested PCR. This set of primers gave amplicons of the size of about 800 bp (Figure 4.4, 4.5 and 4.6).

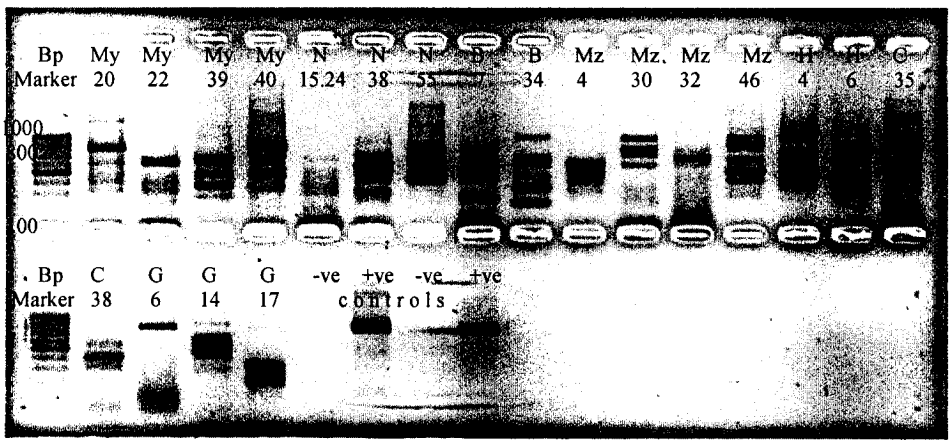

Figure 4.4 PCR results for DNA extracts from faecal samples using primers ITMTnR/ITMF (first round) and ITMTnR/nTAE (semi-nested). Positive samples show bands at ± 800p. Samples My20, My40, N55, Mz30 and G6 showed bands of ± 800bp and hence positive for *Taenia.*

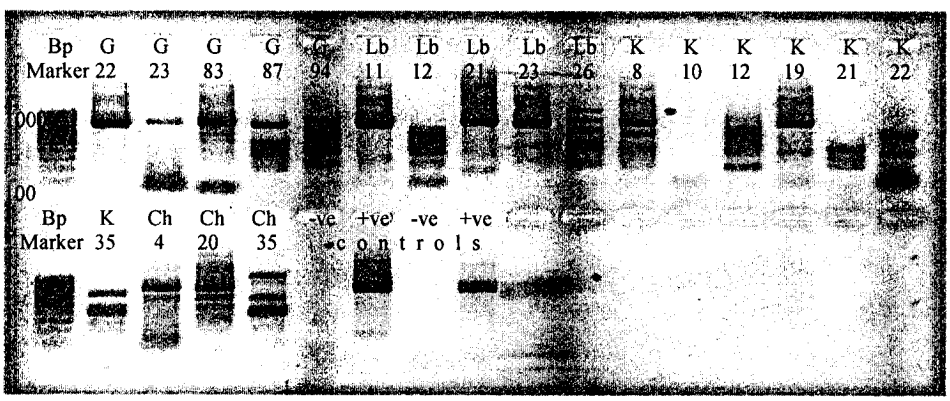

Figure 4.5 PCR results for DNA extracts from faecal samples using primers ITMTnR/ITMF (first round) and ITMTnR/nTAE (semi-nested). Positive samples show bands at ± 800bp. Samples G22, Lb11, Lb21, Lb23, K8, K19, Ch4 and Ch20 showed bands of ± 800bp and hence positive for *Taenia.*

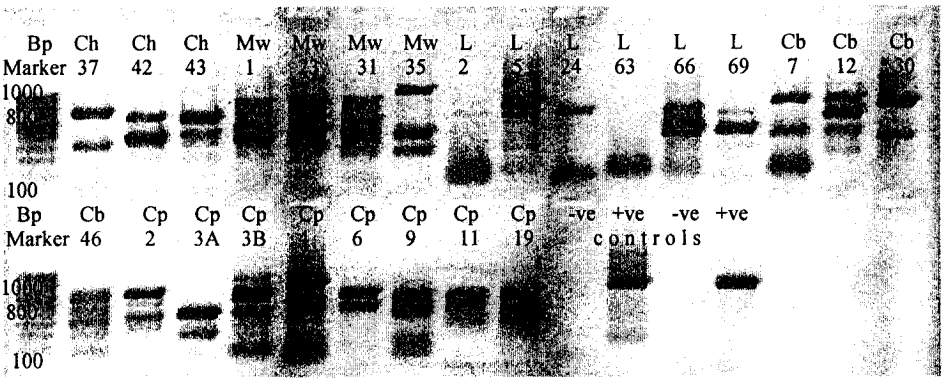

Figure 4.6 PCR results for DNA extracts from faecal samples using primers ITMTnR/ITMF (first round) and ITMTnR/nTAE (semi-nested). Positive samples show bands at ± 800bp. Samples L5, Cb30 and Cp4 showed bands of ± 800bp and hence positive for *Taenia.*

Combining results obtained from the two sets of primer pairs, out of the 200 samples 21 (10.5%) showed that they were positive for *Taenia spp.* thus giving a taeniosis prevalence after PCR of 10.5%. The PCR results correlated well with the coproscopic examination results. The 16 samples positive on coproscopic examination that were included in the sub-sample were also positive on PCR. However, 5 coproscopically negative samples were found positive on PCR.

Gwembe district still had a higher taeniosis prevalence after PCR than Monze district (14.7% versus 5.7%), and this difference was found to be statistically significant

($\chi^2 = 4.369$; $p = 0.037$).

4.1.2.1 Taeniosis prevalence on PCR by health centre

A total of 105 from Monze district and 95 from Gwembe district were examined by PCR. The PCR results by health centre for Monze district ranged from 0% to 16.7% as shown in Table 4.5. For Gwembe district the taeniosis prevalence after PCR ranged from 0% for Bbondo Rural Health Centre to 35.7% for Gwembe District Hospital (Table 4.6)). Similar to coproscopic examination, diagnostic PCR indicated that Gwembe had a higher and wide spread Taenia prevalence as compared to Monze.

Table 4.5 Prevalence of taeniosis on PCR by health centre in Monze district

Health centre	No. examined	No +ve (%)
Chisekesi Rural Health Centre	11	1(9.1)
Chikuni Mission Hospital	12	2(16.7)
Hamapande Rural Health Centre	13	0(0)
Kaumba Health Post	14	2(14.3)
Mwanza Rural Health Centre	14	1(7.1)
Monze Mission Hospital	14	0(0)
Nampeyo Rural Health Centre	13	0(0)
St. Mary's Health Post	14	0(0)
Total	105	6(5.7)

Table 4.6 Prevalence of taeniosis on PCR by health centre in Gwembe district

Health centre	No. examined	No +ve (%)
Bbondo Rural Health Centre	12	0(0)
Chaboboma Rural Health Centre	12	1(7.7)
Chipepo Health Post	13	1(7.1)
Gwembe District Hospital	14	5(35.7)
Lukonde Rural Health Centre	15	2(13.3)
Luumbo Rural Health Centre	14	3(21.4)
Munyumbwe Rural Health Centre	13	3(23.1)
Total	95	15(15.8)

4.1.2.2 Taeniosis prevalence on PCR by age

Of the 200 faecal samples examined by PCR, taeniosis prevalence among the six age groups ranged between 0% for those above 60 years of age and 18.3% for the 49-60 years age group (Figure 4.7). When individuals were classified as young (below 16 years of age) and adults (above 16 of age), no statistical difference was observed in the taeniosis prevalence ($\chi 2 = 1.000$; $p = 0.317$) between the two groups (13.5%, n= 89 versus 8.1%, n=111)

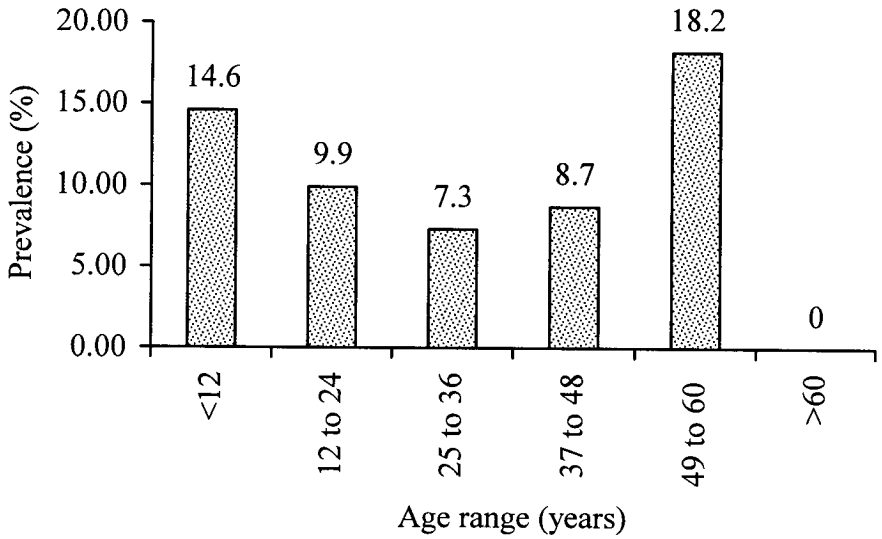

Figure 4.7 The prevalences (%) of human taeniosis using PCR for the different age groups

4.1.2.3 Taeniosis prevalence by sex

A total of 118 faecal samples examined were from female participants while 82 were from males. The taeniosis prevalence after PCR was 7.6% for females as compared to 14.6% for males (Table 4.7). Even though males had a higher taeniosis prevalence than females the difference was found not to be statistically significant ($\chi^2 = 1.837$;

$p = 0.175$).

Table 4.7 Prevalence of taeniosis on PCR according to sex in Gwembe (n = 95) and Monze (n = 105) districts

District	Sex	No. examined	No. +ve (%)
Gwembe	Female	64	8(12.5)
	Male	31	7(22.6)
Monze	Female	54	1(1.9)
	Male	51	5(9.8)
TOTAL		200	21(10.5)

4.1.3 Comparison of coproscopic examination and PCR diagnostic tests

Comparison of the two tests could only be done on the results based on the 200 faecal on which both coproscopic examination and PCR were conducted. Hence using the *Kappa* test, the two tests were compared two by two in order to find out the agreement between the tests in the detection of human taeniosis. The rate of agreement between coproscopic examination and PCR was 85.1% (Table 4.8) and this agreement was found to be statistically significant ($\chi^2 = 138.069$; $p = 0.000$). A kappa value of 0.851 indicates perfect agreement between the two tests. The findings show that out of the 200 faecal samples, 179 were negative on both tests while 16 were positive on both tests. The study shows that 5 of the 21 samples positive on PCR were negative on coproscopic examination (Figure 4.8).

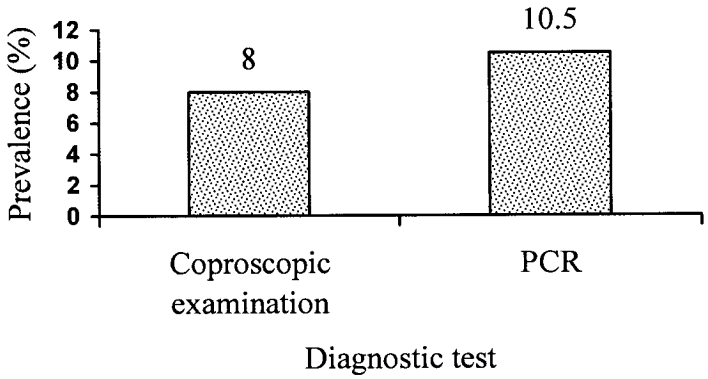

Fig. 4.8 Taeniosis prevalence on coproscopic examination and PCR (n = 200)

Table 4.8 Measure of agreement (Kappa) between coproscopic examination and PCR using the SPSS programme for the detection of human taeniosis (n = 200).

		Serum Ag-ELISA	
		No.+ve	No.-ve
Urine Ag-ELISA	No.+ve	16	0
	No.-ve	5	179
Kappa value (p)		0.851(0.000)	

4.2 Differentiation of the *Taenia spp.*

4.2.1 Morphological Examination

Only three specimens were collected after treatment of taeniosis positive individuals with niclosamide followed by a magnesium sulphate purgative. Two out of these three specimens were identified as *T. solium* because 9-12 uterine branches were counted in the gravid proglottids (Figure 4.9). In the remaining one sample, the uterine branches could not be counted.

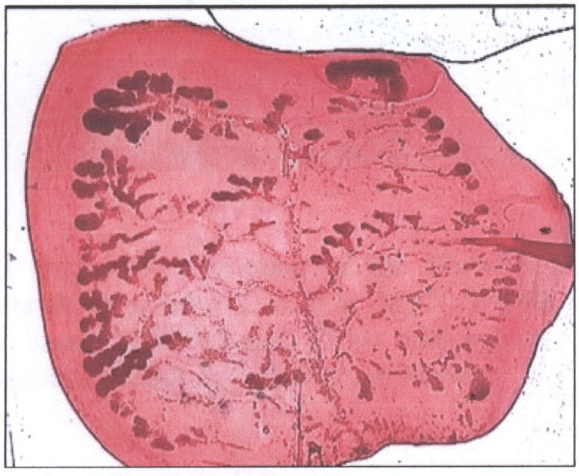

Figure 4.9 Gravid proglottid showing 7-10 uterine branches indicating that the sample came from *Taenia solium*.

4.2.2 Restriction Fragment Length Polymorphism (RFLP)

RFLP was then conducted on the 21 PCR positives and also on all those that were doubtfully positive on PCR so as not to miss out any positives. RFLP was conducted on ITMTNR/nTAE (second round) positives as this gave a clearer picture and corresponded well to the positive controls (See Figure 4.10).

The RFLP revealed that all the *Taenia* positive samples were *T. solium* because they showed two high bands (± 300bp and ± 500bp) which corresponded to *T. solium* profiles (Figure 4.11, 4.12, 4.13 and 4.14).

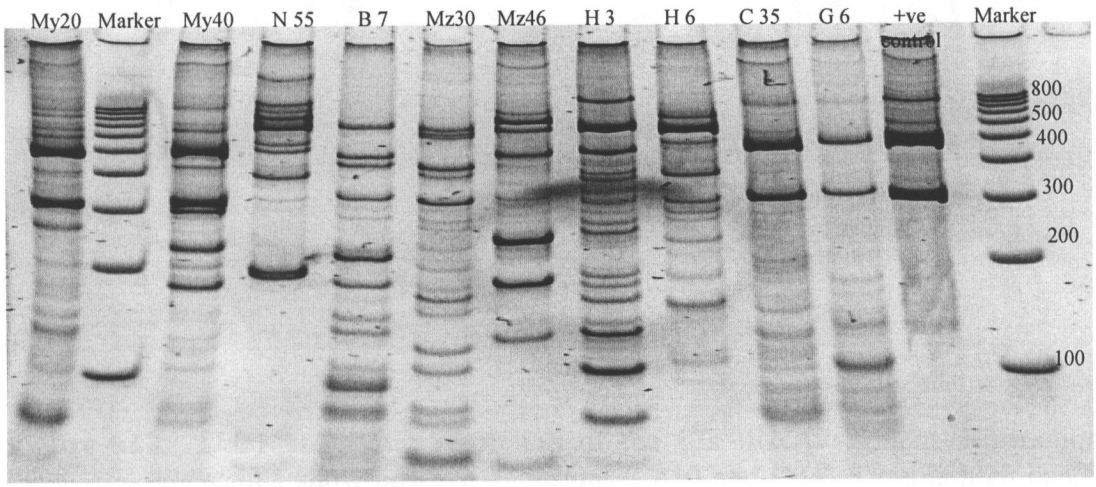

Figure 4.10 D*de*I enzymatic restriction of the 12s rRNA segment. Restriction results show positive *T. solium* (My20, My40, C35 and G6). Double high bands (±300bp and ±500) correspond to *T. solium* profiles. Nonspecific bands are observed in My40.

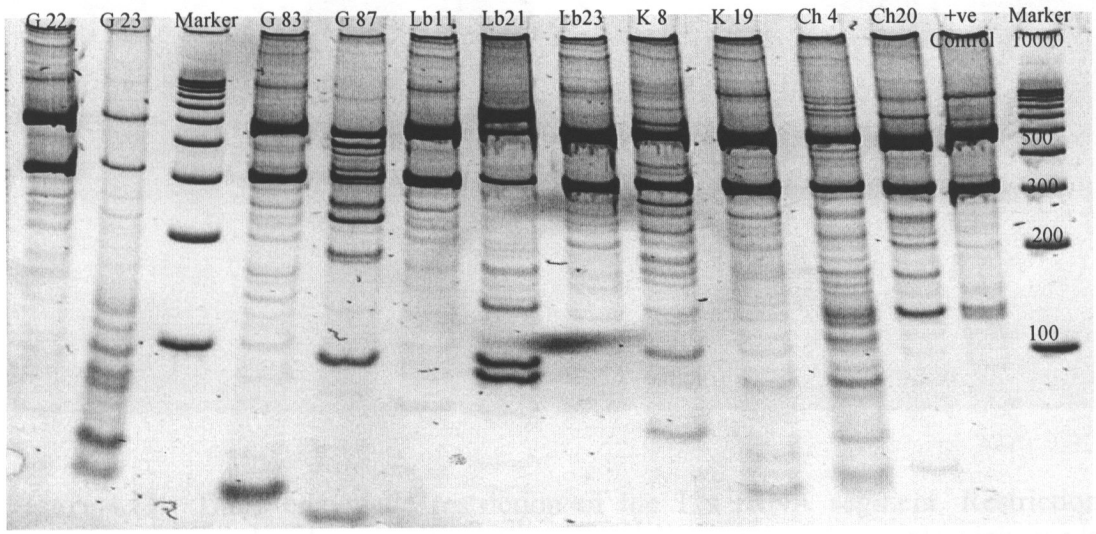

Figure 4.11 D*de*1 enzymatic restriction of the 12s rRNA segment. Restriction results show that all lanes were positive for *T. solium*. Double high bands (±300bp and ±500) correspond to *T. solium* profiles. Nonspecific bands are observed in some lanes.

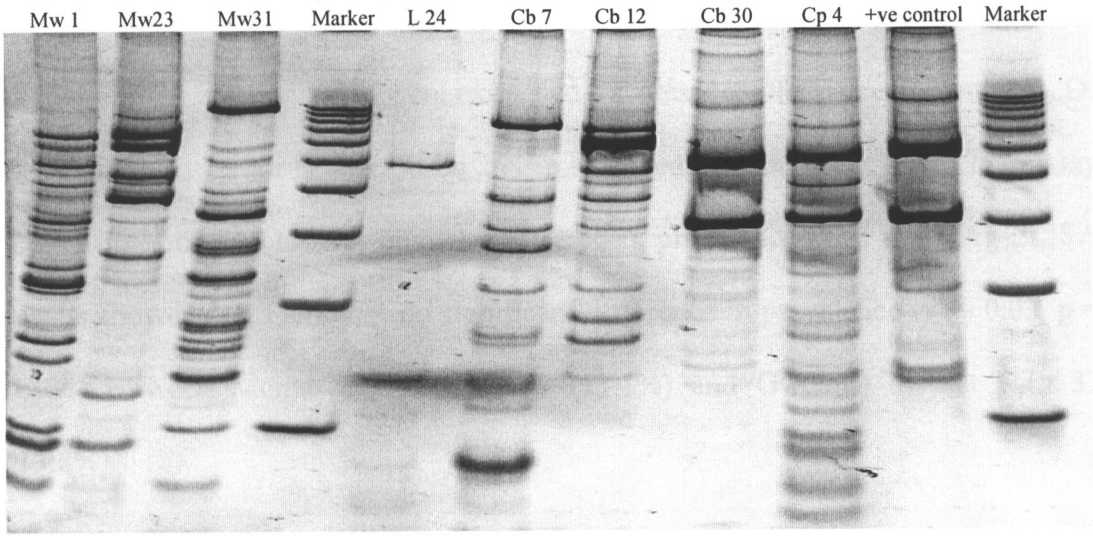

Figure 4.12 D*de*1 enzymatic restriction of the 12s rRNA segment. Restriction results show positive *T. solium* (Cb30 and Cp4). Double high bands (±300bp and ±500) correspond to *T. solium* profiles.

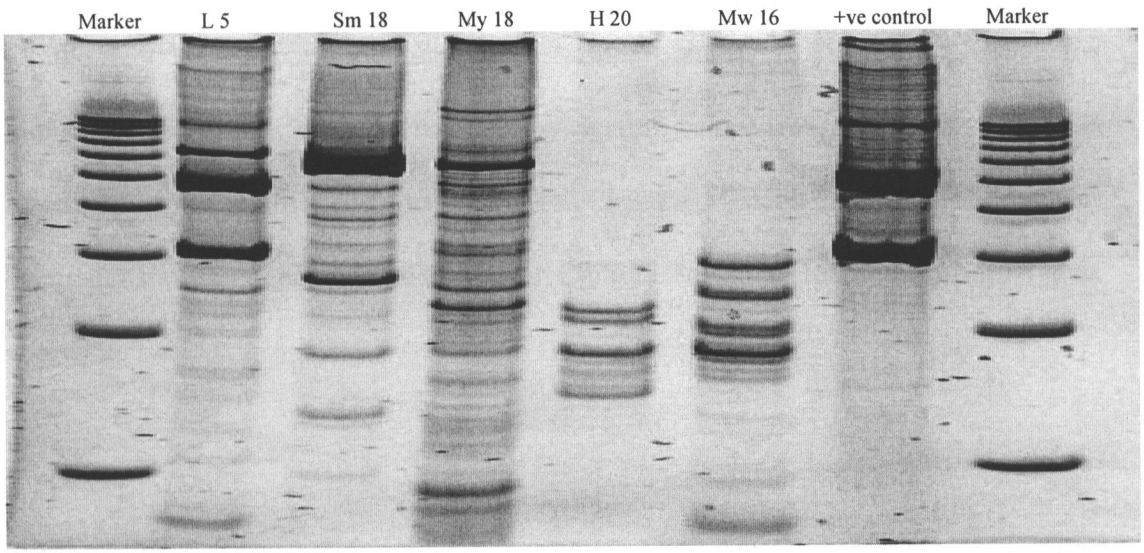

Figure 4.13 D*de*1 enzymatic restriction of the 12s rRNA segment. Restriction results show positive *T. solium* on lane L5 with double high bands (±300bp and ±500) which correspond to *T. solium* profiles.

4.3 Prevalence of human cysticercosis

4.3.1 Urine Ag-ELISA

A total of 627 urine samples were examined for presence of cysticercal antigens. Of these, 359 were from Monze while 268 from Gwembe. The urine Ag-ELISA assay gave a total of 84 (13.4%) human cysticercosis prevalence. The urine Ag-ELISA results showed that there was no significant difference in prevalence ($\chi^2 = 0.02$, $p = 0.888$) between Monze district with 47 (13.1%) and Gwembe district with 37 (13.8%).

However, Gwembe district had slightly higher cysticercosis prevalence (13.8%) on urine Ag-ELISA than Monze district which had 13.1%.

4.3.1.1 Cysticercosis prevalence on urine Ag-ELISA by health centres

Of the 359 urine samples examined in Monze district, 13.1% were positive for human cysticercosis with prevalence by health centre ranging from 2.7% for Mwanza Rural Health Centre to 30.4% for Chikuni Mission Hospital. The highest cysticercosis prevalence recorded was at Chikuni Mission Hospital with 30.4% as shown in Table 4.9.

Table 4.9 The numbers and prevalences (%) of cysticercosis using urine Ag-ELISA for the eight health centres in Monze district (n = 359).

Health centre	No. examined	No. +ve (%)
Chisekesi Rural Health Centre	43	6(14.0)
Chikuni Mission Hospital	46	14(30.4)
Hamapaande Rural Health Centre	29	6(20.7)
Kaumba Health Post	54	2(3.7)
Monze Mission Hospital	49	4(8.2)
Mwanza Rural Health Centre	37	1(2.7)
Nampeyo Rural Health Centre	75	12(16.0)
St. Mary's Health Post	26	2(7.7)
Total	359	47(13.1)

A total of 268 urine samples were examined from Gwembe district. Of the 268 urine samples examined, 13.8% were positive for human cysticercosis with prevalence by health centre ranging from 8.3% for Chipepo Health Post to 32.4% for Gwembe District Hospital. Gwembe District Hospital recorded the highest cysticercosis prevalence of 32.4% in Gwembe district as shown in Table 4.10.

Table 4.10 The numbers and prevalences (%) of cysticercosis using urine Ag-ELISA for the seven health centres in Gwembe district (n = 268).

Health centre	No. examined	No. +ve (%)
Bbondo Rural Health Centre	44	4(9.1)
Chaboboma Rural Health Centre	34	3(8.8)
Chipepo Health Post	24	2(8.3)
Gwembe District Hospital	34	11(32.4)
Lukonde Rural Health Centre	55	5(9.1)
Luumbo Rural Health Centre	32	4(12.5)
Munyumbwe Rural Health Centre	45	8(17.8)
Total	268	37(13.8)

4.3.1.2 Cysticercosis prevalence on urine Ag-ELISA by age

The urine Ag-ELISA result showed cysticercosis prevalence rates ranging between 9.1 to 24.2% for the > 60 and 49-60 years age groups, respectively. As for taeniosis, there was no significant difference in cysticercosis prevalence (χ^2 = 5.970, p = 0.309) by age (Figure 4.14).

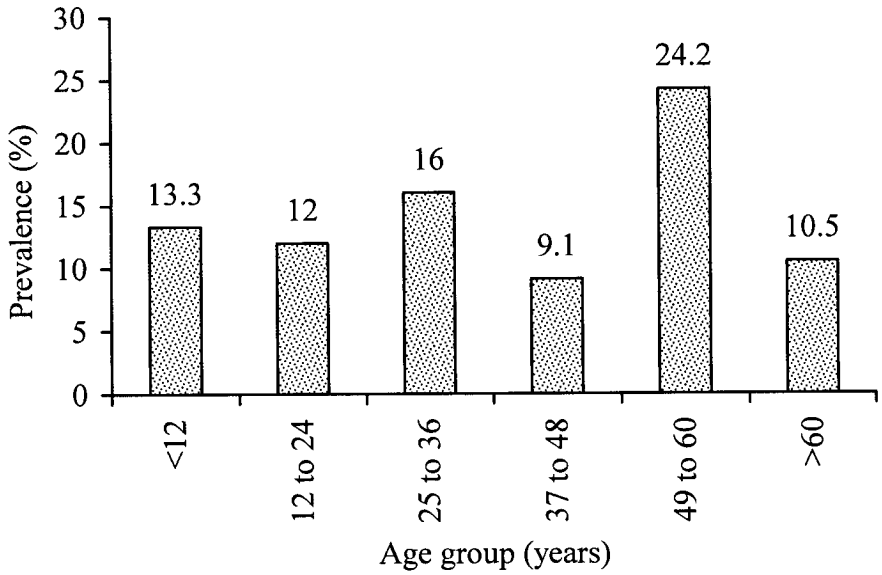

Figure 4.14 The prevalence (%) of cysticercosis on urine Ag-ELISA for the six different age groups.

4.3.1.3 Cysticercosis prevalence on urine Ag-ELISA by sex

A total of 372 urine samples from female participants and 255 from males were examined giving cysticercosis prevalence by sex of 14.8% (55/372) for females and 11.4% (29/255) for males. As for taeniosis, there was also no significant difference in cysticercosis prevalence on urine Ag-ELISA in males (11.4%) compared to females (14.8%) in the two districts ($\chi^2 = 1.239$; $p = 0.266$).

Within districts, there was no significant difference ($\chi^2 = 0.011$, $p = 0.915$) in cysticercosis prevalence on urine Ag-ELISA between females (12.7%) and males (13.6%) in Monze district. However, a significantly higher cysticercosis prevalence ($\chi^2 = 3.957$, $p = 0.047$) was observed in females (17.4%) as compared to males (7.9%) in Gwembe on urine Ag-ELISA.

4.3.2 Serum Ab -ELISA

4.3.2.1 Cyst fluid protein concentration

The cyst fluid protein concentration was measured by subtracting the absorbances at 492 nm of the Blank replicates from the individual standards and unknown samples, X – Blank (Table 4.11).

Table 4.11 Mean absorbances at 492 nm of the blank standards and unknown sample (X) and also the difference between the individual standards and unknown sample and the blank (X-Blank) used to draw a standard curve for determination of cyst fluid protein concentration.

Vial	Mean absorbance(X)	X - Blank
Blank	0.062	0
A	0.968	0.902
B	0.752	0.690
C	0.553	0.491
D	0.439	0.377
E	0.334	0.272
F	0.206	0.144
G	0.136	0.074
H	0.077	0.015
Unknown sample	0.671	0.609

A standard curve was prepared by plotting the average Blank-corrected 492 nm measurement for each bicinchoninic acid (BCA) standard versus its concentration in µg/ml. This standard curve was then used to determine the protein concentration of the unknown sample (Figure 4.15).

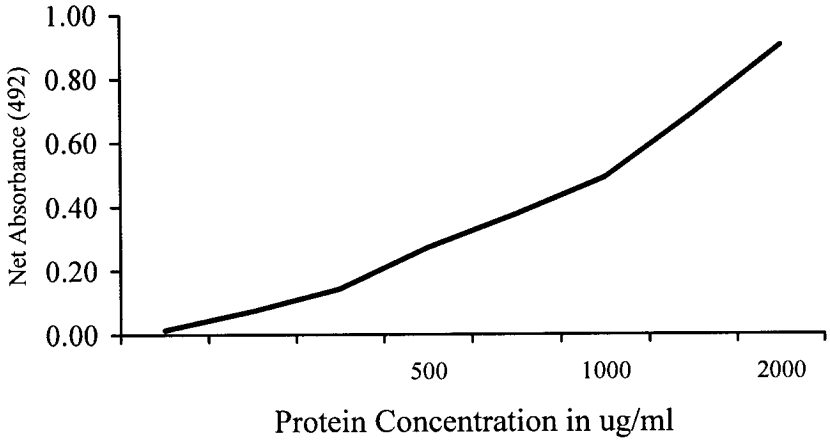

Fig. 4.15 Colour response curve for bicinchoninic acid (BCA) standards used to determine the protein concentration of the cyst fluid.

The average absorbance at 492 nm for the unknown sample (cyst fluid) was 0.609 and by extrapolation on the curve, the diluted cyst fluid protein concentration was determined to be 1200 µg/ml. Since the cyst fluid was diluted 1 in 3, it follows therefore that the protein concentration of the crude cyst fluid was 3600 µg/ml.

4.3.2.2 Titration for Antibody Enzyme Linked Immunosorbent Assay

The optimum assay conditions were determined by titration of antigen (cyst fluid) and serum for the monoclonal detection system. Conjugate dilution of 1:20,000 was used as per manufacturer's recommendations. The mean optical density values were calculated (Appendix 2. 1-4). Figure 4.16 shows representative titrations for the antigen and serum. The optimal dilutions of 1/100 for test sera, 12 µg/ml (1:100) and 1:20,000 for conjugate were selected for analysis of the human serum samples.

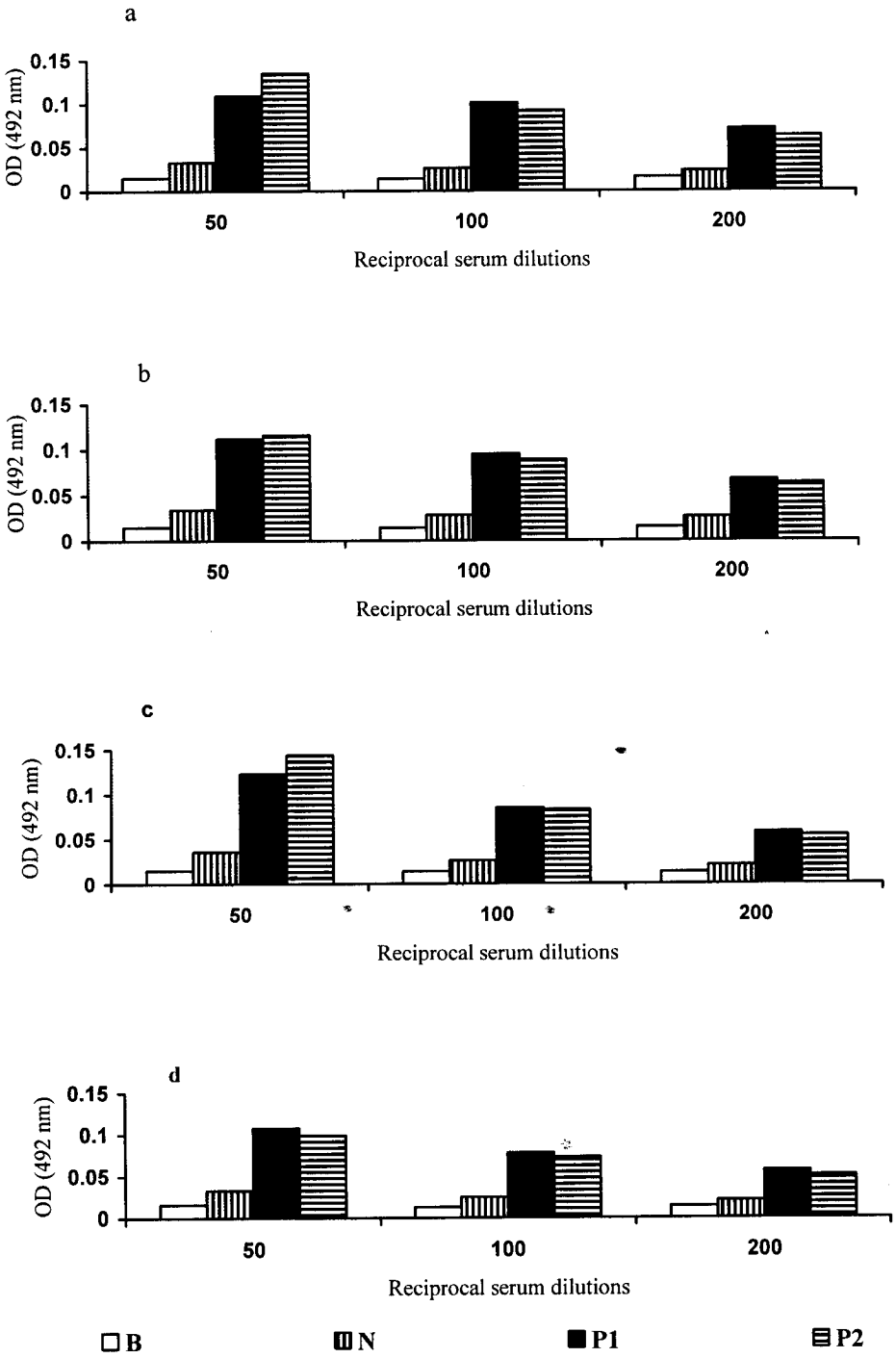

Fig. 4.16: Serum titrations showing the mean ELISA (492nm) values obtained for diluent (B), negative (N), positive (P1) and positive (P2) at different antigen concentrations 24µg/ml (a), 12µg/ml (b), 6µg/ml (c) and 3µg/ml (d). Conjugate dilution was maintained at 1:20000.

Out of the 101 serum samples collected from the two districts, 45 were from Gwembe and 56 from Monze. On Ab-ELISA, 48 (47.5%) were positive for circulating cysticercal antibodies. There was no statistical difference in cysticercosis prevalence ($\chi^2 = 2.720$, $p = 0.099$) between Monze with 22 (39.3%) and Gwembe with 26 (57.8%) by Ab-ELISA.

In Monze district, Kaumba Health Post recorded the highest cysticercosis prevalence of 80.0% on Ab-ELISA with Chisekesi Rural Health Centre recording nothing (Table 4.12). Gwembe district had Chaboboma Rural Health Centre recording the highest cysticercosis prevalence of 83.3% with Chipepo Health post recording 25.0% (Table 4.13).

Table 4.12 The numbers and prevalences (%) of cysticercosis using serum Ab-ELISA for the eight health centres in Monze district (n = 56).

Health centre	No. examined	No. +ve (%)
Chisekesi Rural Health Centre	3	0(0)
Chikuni Mission Hospital	15	2(13.3)
Hamapande Rural Health Centre	9	7(77.8)
Kaumba Health Post	5	4(80.0)
Monze Mission Hospital	0	0(0)
Mwanza Rural Health Centre	2	1(50.0)
Nampeyo Rural Health Centre	18	6(33.3)
St. Mary's Health Post	4	2(50.0)
Total	56	22(39.3)

Table 4.13 The numbers and prevalences (%) of cysticercosis using urine Ab-ELISA for the seven health centres in Gwembe district (n = 48).

Health centre	No. examined	No. +ve (%)
Bbondo Rural Health Centre	7	5(71.4)
Chaboboma Rural Health Centre	6	5(83.3)
Chipepo Health Post	4	1(25.0)
Gwembe District Hospital	7	2(28.6)
Lukonde Rural Health Centre	9	5(55.6)
Luumbo Rural Health Centre	7	5(71.4)
Munyumbwe Rural Health Centre	5	3(60.0)
Total	48	26(57.8)

On serum Ab-ELISA, cysticercosis prevalence by age ranged between 27.3% for the 37-48 years age group and 100% for those above 60 years of age (Figure 4.17).

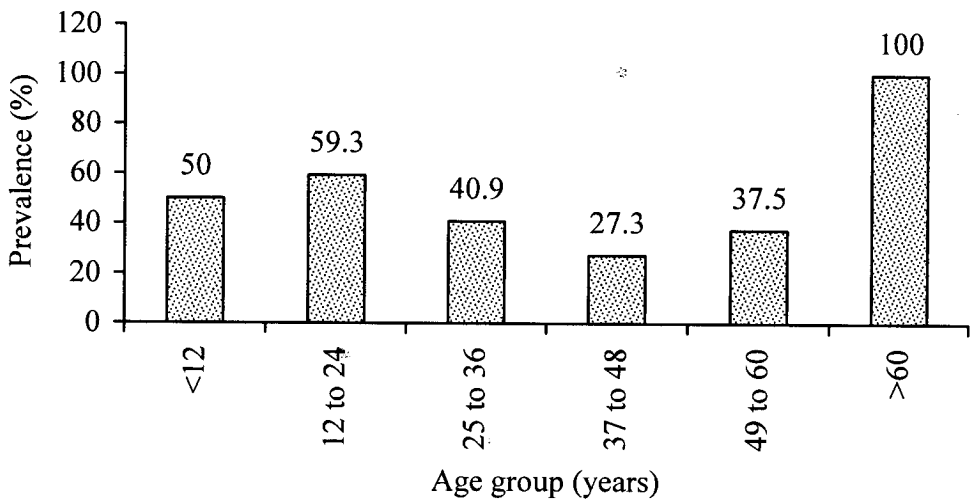

Fig. 4.17 The prevalences of *T. solium* cysticercosis on serum Ab-ELISA for the six different age groups (n = 101).

Comparing cysticercosis prevalence by gender, females had a higher prevalence rate (50.0%) than males (43.6%). However, there was no significant statistical difference in cysticercosis prevalence in the two sexes in the two districts ($\chi 2 = 0.000$; $p = 1.000$: Gwembe: $\chi 2 = 0.087$; $p = 0.767$). See figure 4.18 below.

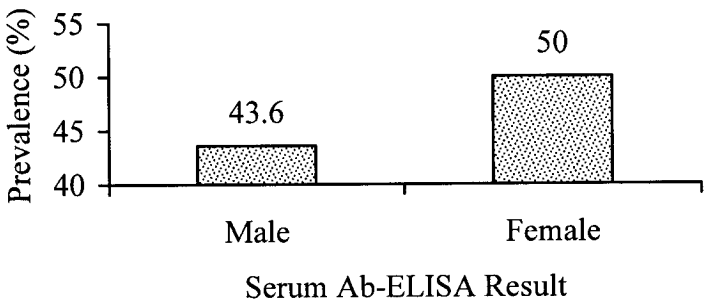

Figure 4.18 The prevalences of *T. solium* cysticercosis on serum Ag-ELISA in the two sex groups (n = 101).

4.3.3 Serum Ag-ELISA

Out of the 101 serum samples collected from the two districts, 9 were found positive on serum Ag-ELISA giving a total prevalence of 8.9 %. There was no statistical difference in prevalence ($\chi^2 = 0.128$, $p = 0.72$) between Monze with 6 (10.7%) positives and Gwembe with 3 (6.7%).

Fifty six serum samples were examined from Monze district and on Ag-ELISA cysticercosis prevalence by health centre ranged between 0% for Chikuni Mission Hospital, Kaumba Health Post, Mwanza Rural Health Centre and St. Mary's Health Post and 66.7% for Chisekesi Rural Health Centre (Table 4.14). From Gwembe district 45 serum samples were examined and only Chaboboma Rural Health Centre

and Lukonde Rural Health Centre recorded presence of individuals with circulating cysticercal antigens (Table 4.15).

Table 4.14 The numbers and prevalences (%) of cysticercosis using serum Ag-ELISA for the eight health centres in Monze district (n = 56).

Health centre	No. examined	No. +ve (%)
Chisekesi Rural Health Centre	3	2(66.7)
Chikuni Mission Hospital	15	0(0)
Hamapande Rural Health Centre	9	1(11.1)
Kaumba Health Post	5	0(0)
Monze Mission Hospital	0	0(0)
Mwanza Rural Health Centre	2	0(0)
Nampeyo Rural Health Centre	18	3(16.7)
St. Mary's Health Post	4	0(0)
Total	56	6(10.7)

Table 4.15 The numbers and prevalences (%) of cysticercosis using serum Ag-ELISA for the seven health centres in Gwembe district (n = 48).

Health centre	No. examined	No. +ve (%)
Bbondo Rural Health Centre	7	0(0)
Chaboboma Rural Health Centre	6	1(16.7)
Chipepo Health Post	4	0(0)
Gwembe District Hospital	7	0(0)
Lukonde Rural Health Centre	9	2(22.2)
Luumbo Rural Health Centre	7	0(0)
Munyumbwe Rural Health Centre	5	0(0)
Total	48	3(6.7)

The cysticercosis prevalence by age on serum Ag-ELISA ranged between 0 for the 37-48 and above 60 years age group and 18.2% for the group of 25-36 years of age (Figure 4.19).

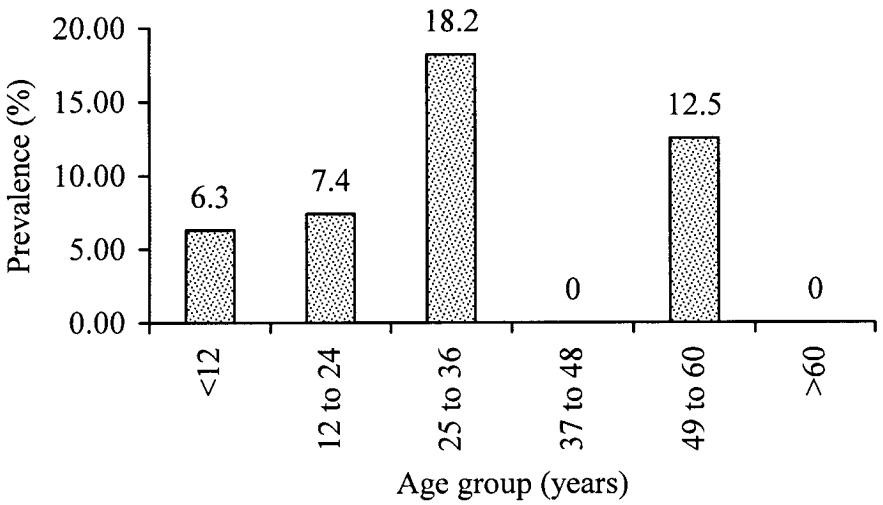

Figure 4.19 The prevalences of *T. solium* cysticercosis on serum Ag-ELISA in the six age groups (n = 101).

On serum Ag-ELISA, there was no significant difference in cysticercosis prevalence in the two sexes in the two districts (Monze: $\chi 2 = 0.000$; $p = 1.000$: Gwembe: $\chi 2 = 0.163$; $p = 0.685$) with males having a prevalence of 7.7% and females 9.7% (Figure 4.20).

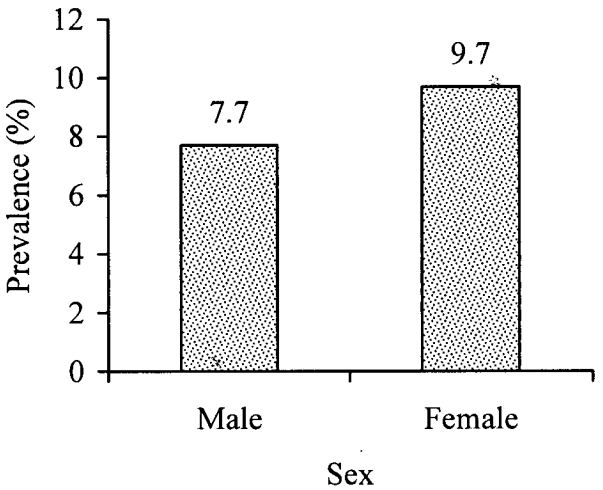

Figure 4.20 Prevalence of *T. solium* cysticercosis by sex on serum Ag-ELISA (n = 101).

4.3.4 Comparison of the three diagnostic tests (urine Ag-ELISA, serum Ab and Ag-ELISA)

Comparison of the tests could only be done on the results based on the 101 individuals from whom both urine and serum was collected.

Hence using the *Kappa* test, the three tests were compared two by two in order to find out the agreement between the tests in the detection of human cysticercosis. Only slight agreement was observed between the urine Ag-ELISA and the serum Ab-ELISA tests. The agreement here was statistically significant ($p < 0.05$) (Table 4.16) but the tests only agreed in less than 20% of the cases and hence a very slight agreement.

Of the 101 individuals from whom serum was collected, a total of 50 had cysticercal antigens in their urine, 9 had circulating antigens in serum while 48 were positive for antibodies (Figure 4.21). Out of the 50 urine Ag-ELISA positives, only 7 were also positive on serum Ag-ELISA and 17 were positive on serum Ab-ELISA. The study also showed that only 3 of the 9 serum Ab-ELISA positive individuals were also positive on Ag-ELISA. The findings also showed that out of the 101 study subjects from whom both urine and serum was collected, 20 (19.8%) were negative on all three diagnostic tests while only one individual was found to be positive on all three tests. On the other hand, 33 urine Ag-ELISA positive individuals had no antibodies against cysticerci.

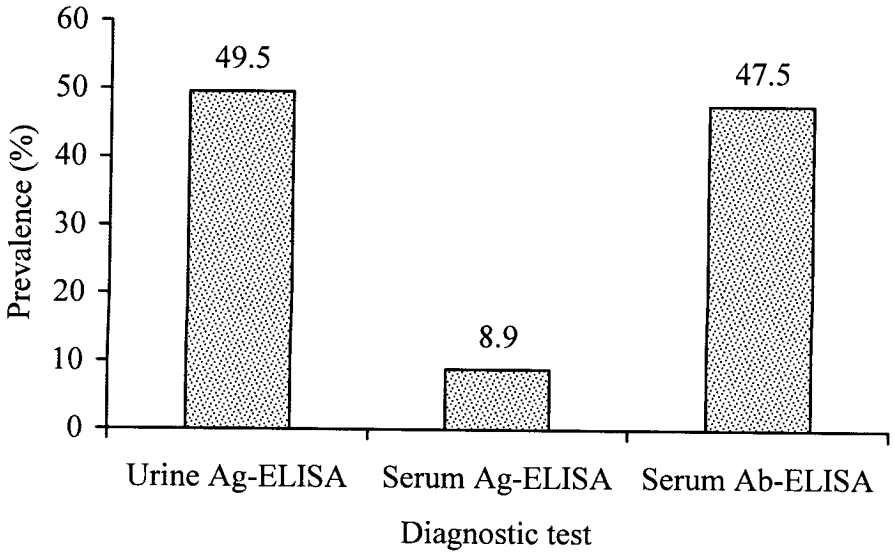

Figure 4.21 The prevalences of *T. solium* cysticercosis on urine Ag-ELISA, serum Ag and Ab-ELISA (n = 101).

Table 4.16 Measure of agreement (Kappa) among the three different ELISA tests; urine Ag-ELISA, serum Ag-ELISA and serum Ab-ELISA using the SPSS programme for the detection of human cysticercosis (n = 101).

		Serum Ag-ELISA		Serum Ab-ELISA	
		No.+ve	No.-ve	No.+ve	No.-ve
Urine	No.+ve	7	43	17	33
Ag-ELISA	No.-ve	2	49	31	20
	Kappa value (p)	0.1 (0.153)		-0.27 (0.013)	
Serum	No.+ve			3	6
Ag-ELISA	No.-ve			45	47
	Kappa value (p)			-0.05 (0.587)	

4.4 Investigation of risk factors associated with Taeniosis/cysticercosis

4.4.1 Sample description (Socio-demographics)

A total of 15 health centres were visited between November 2004 and March, 2005. Participants were recruited as they visited the health centres for unrelated problems and were also asked to bring forward any other member of the household willing to participate. Recruitment was done randomly and individual informed consent obtained. A total of 701 individuals from 387 households (189 in Monze District and 175 in Gwembe District) were recruited and information obtained. The number of individuals sampled per household ranged from 1 to 13 depending on the number of people in a household and the willingness of the individuals to participate in the study. The mean number of individuals in each household was 8 (Std. Deviation = 4.068) with a mode of 6. The highest number of people in a household recorded was 30 and the lowest 1. Of the 701 participants, 418 (59.6%) were females while 283 (40.4%) were males. The age of the participants ranged from 1 to 91 years (Range = 90) with the mean age being 23.61 years with a standard deviation of 16.647. The age and sex distribution of the respondents is shown in Figure 4.22 and Table 4.17 respectively. Overall, there were more females interviewed than males and most of the people interviewed were from the 12 to 24 years age group.

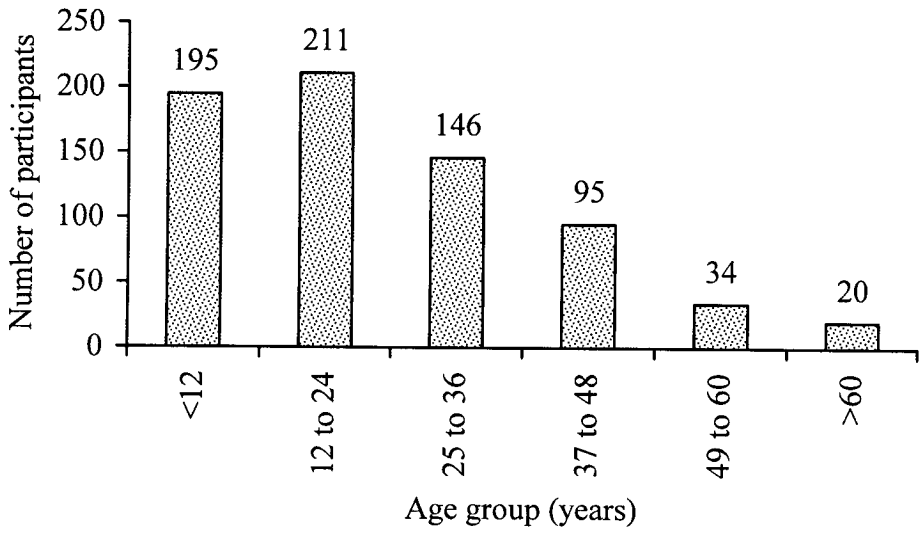

Figure 4.22 The age distribution of all the participants allotted into six age groups.

Table 4.17 The sex distribution of all the participants.

Sex	No. of participants (%)
Female	418(59.6)
Male	283(40.4)
Total	701(100)

The highest education level attained by the people in each household is shown in Table 4.18. Most of the respondents (52.2%) from the 387 households interviewed had reached secondary level of education.

Table 4.18 The education level attained by the respondents in each household in both Gwembe and Monze districts.

District	No.	Primary No. (%)	Secondary No. (%)	Tertiary No. (%)
Gwembe	168	84(50.0)	73(43.5)	11(6.50
Monze	219	79(36.1)	129(58.9)	11(5.0)
Total	387	163(42.1)	202(52.2)	22(5.7)

Most households (44.4%) in the two districts depend on crop sales as their main source of income while 26.6% on livestock and crop sales, 20.0% are in employment and earn a salary, 5.9 are involved in some form of business, 2.1% depend on fish sales and the remaining 1.0% combines fish and crop sales (Table 4.19).

Table 4.19 Number (%) of respondent according to household's main source of income in Monze and Gwembe Districts.

District	n	Crop sales	Livestock/ Crop sales	Salary	Business	Fish sales	Fish/ Crop
Gwembe	168	64(38.1)	40(23.8)	49(29.2)	4(2.4)	8(4.7)	3(1.8)
Monze	219	108(49.3	63(28.8)	28(12.8)	19(8.7)	0(0)	1(0.4)
Total	387	172(44.4	103(26.6)	77(20.0)	23(5.9)	8(2.1)	4(1.0)

n = number of respondents

The people of the Southern province of Zambia, including the two districts of Monze and Gwembe are traditionally cattle herders but the advent of cattle diseases

has caused them to start keeping other animal species. Pig keeping has offered a more profitable alternative and in this study information obtained from 387 households revealed that 33.9% of these kept pigs. These pigs are mostly kept on free range and left to scavenge and hence exposed to human faeces. Positivity on coproscopic examination and coming from a household that kept pigs did not yield any statistical significance ($\chi^2 = 0.000$, $p = 1.000$).

Of all the households that kept pigs, a greater number (91.6 %) of them admitted having at least one member that consumed pork. However, 47.5% of those that did not keep pigs also consumed pork.

4.4.2 Participant's medical complaints related to taeniosis/cysticercosis

Information in relation to the participant's medical complaints with regard to taeniosis/cysticercosis was obtained from all participating individuals and the responses are as shown in Table 4.20. Only 18.7% ($n = 701$) of the participants were found to have ever taken any anti-helmintic drug.

Table 4.20 Respondent's medical complaints related to taeniosis/cysticercosis in Gwembe (n = 311) and Monze (n = 390) district.

Complaint	No. in Gwembe (%)	No. in Monze (%)	Total No. (%)
Persistent headaches	97(31.2)	130(33.3)	227(32.4)
Seizures	30(6.4)	8(2.1)	38(5.4)
Chronic diarrhoea	57(18.2)	79(20.2)	136(1.9)
Abdominal pain	137(44.1)	152(39.0)	289(41.2)
Nausea	27(8.7)	87(22.3)	114(16.3)
Proglottids in stool	24(7.7)	42(10.8)	66(9.4)
Skin nodules	5(1.6)	22(5.6)	27(3.9)
Anti-helmintic drugs	79(25.4)	52(13.3)	131(18.7)

There was no statistical association between coproscopic examination positivity and chronic diarrhoea (3.8% versus, 2.9%; $\chi^2 = 0.053$, p = 0.818), nausea (2.7% versus, 3.2%; $\chi^2 = 0.000$, p = 1.000) and proglottids in faeces (3.2% versus 3.1%; $\chi 2 = 0.000$, p = 1.000). This was also observed when positivity on urine antigen ELISA was compared to persistent headaches (16.1% versus 12.1%; $\chi 2 = 1.584$, p = 0.208), seizures (19.2%; $\chi 2 = 0.358$, p = 0.550) and skin nodules (8.3%; $\chi 2 = 0.191$, p = 0.662). However, a statistical association was observed between being coproscopically positive and abdominal pain (5.0% versus 1.8%; $\chi^2 = 4.856$, p = 0.028). The effect of an individual having ever taken any anti-helmintic drug was also analysed and no association was found between taking an anti-helmintic drug and positivity on coproscopic examination (4.1% versus 2.9%; $\chi 2 = 0.173$, p =

0.677) and also positivity on Ag-ELISA (14.3% versus 13.2%; $\chi 2 = 0.023$, p = 0.880).

4.4.3 Transmission risk factors

Most of the households when asked whether they or any member of the household consumed pork, 60.0% (n = 378) of them in both Gwembe and Monze districts had at least one member of the household consuming pork, 30% (n = 387) of the households have slaughtered at least one pig at home and of these only 3.5 (n = 115) had their meat inspected (Table 4.21). Taeniosis prevalence based on coproscopic examination for participants coming from households where at least one member consumed pork was 3.5% and 2.4% for those without anyone consuming pork. Analysis of prevalence of taeniosis on coproscopic examination in households where pork was consumed did not yield a statistical significance ($\chi^2 = 0.342$, p = 0.559).

Table 4.21 The number and percentage of households in Gwembe (n = 168) and Monze (n = 219) districts with respect to pork consumption, home slaughter and status of pork inspection.

	Pork consumption	Home slaughter	Lack of pork inspection
District	No. (%)	No. (%)	No. (%)
Gwembe	102(60.7)	45(26.8)	44(97.8)
Monze	130(59.4)	70(32.0)	67(95.7)
Total	232(60.0)	115(29.7)	111(96.5)

It was also found that boreholes were a main source of drinking water to 83.9% of the households in Gwembe and 45.7% in Monze districts (Table 4.22). Other

households used rivers as their source of drinking water in Gwembe (8.9%) and Monze (11.4%) districts. In Gwembe district only 1.8% of the households used wells as their main source of drinking water and only 5.4% used tapped water. In Monze district, 31.5% of the households obtained drinking water from wells while, 11.4% used tapped water.

Table 4.22 The respondents in number and percentage grouped according to their main sources of drinking water for households in Gwembe (n = 168) and Monze (n = 219) districts.

Source of water	River	Borehole	Well	Tap
District	No. (%)	No. (%)	No. (%)	No. (%)
Gwembe	15(8.9)	141(83.9)	3(1.8)	9(5.4)
Monze	25(11.4)	100(45.7)	69(31.5)	25(11.4)
Total	40(10.3)	241(62.3)	72(18.6)	34(8.8)

Of the 387 households visited in Southern province, 53.4% used toilets while 46.6% did not (Figure 4.24). Out of the 219 households interviewed in Monze, 60.7% households used toilets with only 42.3 (n = 168) of the households in Gwembe that used toilets (Table 4.23).

Cysticercosis prevalence based on urine Ag-ELISA for participants that came from households without latrines was 15.4% and 11.6% for those that used latrines. Analysis of prevalence of human cysticercosis on urine Ag-ELISA for participants from households with or without a latrine yielded no statistical significance (χ^2 = 1.632, p = 0.201).

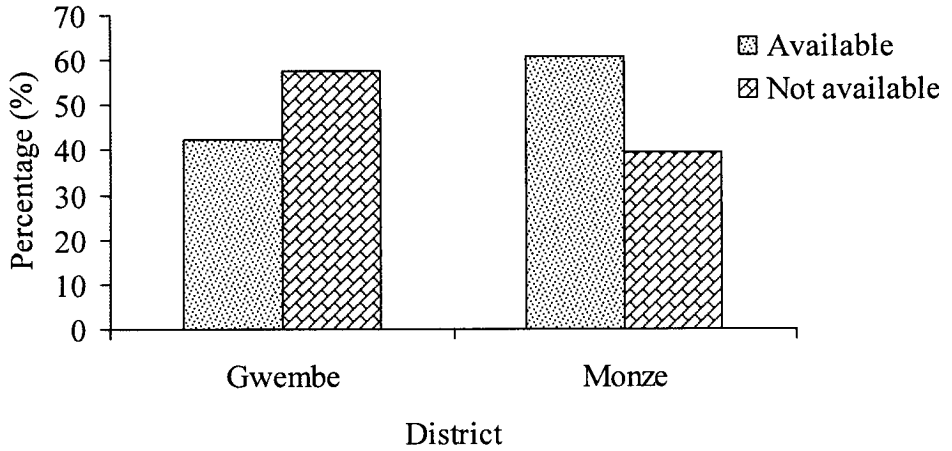

Fig. 4.23 Summary presentation of latrine availability (%) and their usage based on the households interviewed in Gwembe (n = 168) and Monze (n = 219) districts.

Table 4.23 The number and percentage of latrine availability based on the households interviewed in Gwembe (n = 168) and Monze (n = 219) districts.

District	No. Present (%)	No Absent (%)
Gwembe	71 (42.3)	97 (57.7)
Monze	133 (60.7)	86 (39.3)
Total	204 (52.7)	183 (47.3)

4.4.4 Knowledge of the parasite and its transmission

Individuals above the age of 12 years were further interviewed on how much they knew about taeniosis and porcine cysticercosis. Out of the 506 respondents, 172 (34.0%) admitted having heard of tapeworm infections in humans while 319 (63.0%) had never heard about the infection with 15 (3.0%) not responding at all. When asked if they knew how one gets to know that he/she has tapeworm infection,

70.8% showed total ignorance and did not respond. Those who acknowledged knowing that the presence of proglottids in faeces was an indication that one had the tapeworm infection were more in Monze district (8.3%) than Gwembe district (Table 4.24). The knowledge that tapeworm infection in humans was due to eating infected pork was only known to 16.7% and 9.2% of the respondents in Monze and Gwembe districts, respectively. Some (72.5%) were ignorant about how humans acquired the tapeworm (Table 4.25).

Table 4.24 Respondents who knew of how one knows that he/she has tapeworm infection from the interviewed individuals in Gwembe (n = 206) and Monze (n = 300) districts.

Sign	Gwembe No. (%)	Monze No. (%)	Total No. (%)
Proglottids in faeces	26(12.6)	42(14.0)	68(13.4)
Loss of weight	7(3.4)	14(4.7)	21(4.2)
Abdominal pain	16(7.8)	17(5.7)	33(6.5)
Diarrhoea	2(1.0)	14(4.7)	16(3.2)
Told at hospital	2(1.0)	2(0.7)	4(0.8)
Persistent hunger	4(1.9)	1(0.3)	5(1.0)
Skin nodules	1(0.5)	0(0.0)	1(0.2)
Ignorant	148(71.8)	210(70.0)	358(70.8)

Table 4.25 The number and percentage of participants who knew how humans acquire the tapeworm infection in the Gwembe (n= 206) and Monze (n = 300) districts.

Mode of infection	Gwembe No. (%)	Monze No. (%)	Total No. (%)
Eating infected pork	24(9.2)	50(16.7)	74(14.6)
Eating contaminated food	25(9.6)	31(10.3)	56(11.1)
Drinking contaminated water	1(0.5)	5(1.70	6(1.20
Eating soil	2(1.0)	1(0.3)	3(0.16)
Ignorant	154(74.6)	213(71)	367(72.5)

This study has revealed that 24.9% of the adults admitted to having observed cysts in pork (Table 4.26) while 73.3% had never observed cysts before and 1.8 % were ignorant. In Southern province, the cysts in pork were commonly known as *masese* meaning maize bran or *tukotokoto* meaning nodules.

Of the 126 individuals who had observed cysts in pork, only 4.9% acknowledged knowing how pigs acquire them and out of these 3.2% said pigs acquired the infection by eating human faeces. Interestingly, 14.8% of the respondents admitted eating pork infected with cysts and, 12.5% acknowledged selling pork with cysticercosis (Table 4.26).

Respondents in Gwembe district (27.7%) admitted seeing porcine cysticercosis relatively more frequently than their Monze colleagues (23.0%). A larger proportion of respondents in Gwembe (18.9%) also admitted to eating infected pork compared with 12.0% in Monze district (Table 4.26).

Table 4.26 The number (percentage) of respondents that had observed cysts in pork and those that ate or sold infected pork in Gwembe and Monze districts.

	Observation of cysts in pork	Ate pork with cysts	Sold pork with cysts
District	No. (%)	No. (%)	No. (%)
Gwembe	57 (27.7)	39 (18.9)	33 (16.0)
Monze	69 (23.0)	36 (12.0)	30 (10.0)
Total	126 (24.9)	75(14.8)	63 (12.5)

CHAPTER FIVE

5.0 DISCUSSION

The present study has confirmed the presence of taeniosis/cysticercosis among humans in the areas where the study was conducted. A complete discussion of the results obtained in this study have been divided in the following sections; taeniosis prevalence, cysticercosis prevalence, differentiation of *Taenia spp.*, comparison of the diagnostic tests and finally risk and transmission factors associated with the infection.

5.1 Human taeniosis prevalence

5.1.1 Prevalence on coproscopic examination

In this study a total of 21 (3.1%) samples were positive for taeniosis after coproscopic examination of 678 stool samples. These prevalence rates are quite high and thus confirm suggestions by Phiri *et al.*, (2003) and Sikasunge, (2005) that the high porcine cysticercosis prevalence recorded in their studies could indicate the presence of tapeworm carriers in the two districts. The prevalence of human taeniosis is much higher in this study than reported elsewhere in southern Africa. Goldsmid, (1968) in a study of intestinal helminths in the then Rhodesia, now Zimbabwe reported a taeniosis prevalence of 0.4% (n = 5,545). In Tanzania, Bennet *et al.* (1970) reported 2.0% (n = 295). Kyronseppa and Goldsmid (1978) reported a taeniosis prevalence of 0.5% in the northern part (Owambo and Kavango) of the then South West Africa, now Namibia. In the same country, but only in the Kavango area, Evans and Joubert (1989) detected a taeniosis prevalence of 0.9% (n = 4,174).

In a study to examine the prevalence of parasitic infection in HIV-positive and HIV-negative individuals in Ethiopia, Fontanet *et al.* (2000) found a taeniosis prevalence of 4.5% (n = 1239). This prevalence is not very different from what we found in our study and since it was done in immuno-compromised individuals, the result of this study is even higher as it was conducted in individuals without serious ailments. Only one faecal sample (0.4%) out of 269 was found positive for taeniosis in a study in Mozambique (Noormohamed, 2003). This taeniosis prevalence was lower than what was found in a similar age group in our study. In northern Nigeria, a study on the epidemiology of human taeniosis was carried out and it was found that 11.5% (n =1000) were infected (Dada, 1993). Two years later, in another state in Nigeria, human infection was assessed by coproscopically examining 1525 stool samples collected from one selected hospital and the overall taeniosis prevalence was 8.6% (Onah and Chiejina, 1995). This taeniosis prevalence was higher than what we found (3.1%) but like our study, the diagnostic technique used (coproscopic examination) has both low sensitivity and specificity and hence chances of false positives and/or negatives are very high.

In the Asian continent relatively high levels of taeniosis prevalences have been recorded; up to 6.0% in China, 0.4 – 23% in the Bali region of Indonesia, 10 – 50% from an area in Nepal populated by pig farmers and 0.5-6% in Vietnam with lower levels recorded in India (2.0%), Korea (0.06%) and 1.0% in the Philippines (Rajshekhar *et al.*, 2003). Wandra *et al.* (2006) reported that over a period of two years, from 2002-2004, the taeniosis prevalence in the Bali region of Indonesia ranged 1.1-27.5%. In most of these studies *T. saginata* infections were, however, found to be more common than *T. solium* infections.

The coproscopic examination showed an apparently higher taeniosis prevalence in the under 12 years of age group (4.2%) than in the other age groups though the differences were not statistically significant. These findings are in line with Rodriguez-Canul *et al.*, (1999), Sanchez *et al.* (1998, 1999) and Garcia *et al.* (2003) all of whom did not find any statistical association between age and positivity. Rodriguez-Canul *et al.*, (1999) found that the ages of their seven confirmed human taeniosis cases ranged from 8 – 60 years and they found no significant differences in age-specific prevalences. Sanchez *et al.* (1998) in their study of the prevalence of taeniosis and cysticercosis in an urban population in Honduras did not find significant differences between gender or age group and seropositivity using EITB. Similar results were obtained by Sanchez *et al.* (1999) who, in their population-based case control study found no statistically significant association between EITB-based seropositivity and age or gender. However, our results do not agree with those of Wandra *et al.* (2006) who, in a study in the Bali region of Indonesia, found that most carriers of the tapeworm were in the 30-44 years age group. They attributed this to the fact that adult males enjoyed eating raw beef. Adults in the study area, as in many areas of Zambia, consume roasted meat (pork or beef) as they socialize in alcohol drinking places and are, therefore, more at risk of contracting taeniosis than children especially that the meat is usually never thoroughly roasted.

After comparing the overall prevalence results on microscopy by gender, there was no evidence to suggest a significant difference in taeniosis prevalence (between that of females (3.0%) and males (3.2%). This was also observed when taeniosis prevalence of females was compared to males within districts. These findings agree with Evans and Joubert (1989) and Rodriguez-Canul *et al.* (1999) who found an

even distribution of *Taenia* infections among the sexes but disagree with Wandra *et al.* (2006) who found a higher prevalence in males than in females.

The prevalence for taeniosis on coproscopic examination obtained in this study in the two districts could have been an underestimate of the true prevalence because the diagnostic technique used has both low sensitivity as well as specificity (Allan *et al.*, 2003). The intermittent nature of egg excretion leads to underestimation of the prevalence of taeniosis (Allan *et al.*, 1996a). Because of this it is advisable to collect three stool samples at two day intervals. The yield of microscopy in confirmed taeniosis infections has been found to range from 36 to 56% while detection of taeniosis copro-antigen is more sensitive than microscopy even though also less sensitive than the immunoblot techniques (Allan *et al.*, 1990; Allan *et al.*, 1993). Sanchez *et al.* (1999) reported that about 30% of taeniosis cases detected using three samples would not have been detected if only one sample had been collected from each subject. The fact that only one sample was collected from each individual in this study means that our prevalence estimate is lower by at least 30%.

T. solium taeniosis tends to have a low prevalence, typically of $\leq$ 1%, even in endemic communities (Allan *et al.*, 1996a), and a community with a prevalence of $\geq$ 1% is considered hyper-endemic (Cruz *et al.*, 1999). The estimated prevalence of taeniosis amongst the present subjects, revealed by examination of faecal samples after formal-ether concentration, was 3.1%. Hence it can be concluded that the two districts of Gwembe and Monze are hyper-endemic areas for the pork tapeworm and the high number of tapeworm carriers are a risk not only to themselves and other humans but also to pigs.

5.1.2 Prevalence on PCR

On PCR, out of the sub-sample of 200 faecal samples, 21(10.5%) were positive for taeniosis. Like coproscopic results, the prevalence rates on PCR were not significantly different in the two districts of Gwembe and Monze. The findings on PCR confirmed 16 of the coproscopically positive samples that were included in the sub-sample and detected *Taenia* DNA in 5 coproscopically negative samples. This, therefore, indicates that PCR is a more sensitive test than microscopy. Even though PCR has thus far only been used for the differentiation of *Taenia* spp. after collection of parasitic material (Allan *et al.*, 2003; Mayta *et al.*, 2000), it can be used as a diagnostic tool but due to the cost involved it is rarely used. Our taeniosis results on PCR are much higher than what has been reported elsewhere using coproscopic examination.

In Papua, Indonesia, a study was carried out to detect human worm carriers using the more sensitive copro-Ag ELISA technique (Margono *et al.*, 2003). Although only 58 faecal samples were examined microscopically for presence of eggs, *Taenia* eggs were only found in one person. In contrast, five (8.6%) of the 58 stool samples were found to be positive using the copro-antigen test. This finding does not differ markedly from our result on PCR where we obtained a taeniosis prevalence of 10.5%. Margono *et al.* (2003) results, like our PCR results, confirm that the use of more sensitive tests gives a more representative result. The use of these tests would therefore give higher prevalences than what is usually obtained. The underestimation of the real taeniosis prevalence by microscopy was also confirmed by Garcia *et al.* (2003) who found eight cases (n = 1,317) with *Taenia* eggs on microscopy but on copro-Ag ELISA two times more carriers were detected. In an epidemiological study by Rodriguez-Canul *et al.* (1999), 10 (n = 475)

coproscopically negative individuals were found positive on copro-Ag ELISA and thus further confirming the sensitivity of the copro-Ag ELISA.

The PCR results showed that prevalence was higher in the 49-60 years age group than in the other age groups, though not statistically significant. This was unlike what was found on microscopy where prevalence was higher in the less than 12 years age group. The results, therefore, show that more adults were found positive on PCR than on coproscopic examination. This concurs with the assumption that adults, especially males, are more at risk of contracting taeniosis than children. De Giorgio *et al.* (2005) in their study of the seroprevalence of *T. solium* taeniosis in the USA found a higher seroprevalence in adults than in children. Howevér, unlike our study, Garcia *et al.* (2003) reported that community studies done in Guatemala and Peru showed that tapeworm infections are more common in younger than older individuals, especially in the female populations.

Even though there was no significant difference in taeniosis prevalence on PCR between males and females, the former had a higher prevalence than the later. The reason for this finding could be because females were more co-operative in the study and hence more of them were recruited to participate in the study than males.

The low sensitivity of the coproscopic examination test was also proved by the PCR which detected 5 more positives that were missed by the former. Therefore if all of the 678 faecal samples were examined using PCR, more positives would have been detected resulting in an even higher taeniosis prevalence rate.

5.2 Human cysticercosis prevalence

5.2.1 Prevalence on urine Ag-ELISA

The detection of specific antigen in urine is the most recent approach in the diagnosis of parasitic infections (Parija, 1998) and is based on the fact that soluble antigens released by living parasites are found in the blood during active infection; and it is hence possible that the same antigens are also excreted in urine.

The urine Ag-ELISA assay detected a cumulative human cysticercosis prevalence of 13.4% thus 84 individuals out of 627 had cysticercal antigens in their urine. There was no significant difference in cysticercosis prevalence by urine AG-ELISA between the two districts. The high cysticercosis prevalence recorded on urine Ag-ELISA tallies well with the high taeniosis prevalence because a high cysticercosis prevalence is more likely to occur in an area where taeniosis is prevalent.

Our cysticercosis results by urine Ag-ELISA did not differ significantly from those reported in other parts of Africa as well as the world where serum was used. In a prevalence study among children (aged 0-15 years) in Mozambique, seroprevalence of cysticercosis by Ab-ELISA was found to be 20.8% (n = 269) (Noormohamed, 2003). The cysticercosis prevalence rate found in this study in Mozambique was much higher than what was found in our study. Another sero-prevalence study in the same country of human cysticercosis reported a cysticercosis prevalence of 12.1% (n = 489) on Ab-ELISA (Vilhena *et al.*, 1999) which was similar to our study. A sero-prevalence study using Ab-ELISA for screening and EITB for confirmation to determine the epidemiological situation of cysticercosis in the island of Madagascar found a marked variation in the prevalence of cysticercosis from 7 to 21 % between the different provinces: less than 10% in coastal regions increasing to 20% in the

central regions (Andriantsimahavandy *et al.*, 2003). These results did not differ significantly with our urine Ag-ELISA results of just above 13% and they showed that Madagascar, like Zambia, is an endemic country for cysticercosis.

An Ab-ELISA for cysticercosis antibodies was performed on serum from 230 random admissions to a hospital in South Africa and a seroprevalence of 7.39% was obtained (Sacks and Berkowtz, 1990). This study also showed that the infection is endemic in some communities of South Africa. Our cysticercosis results agree with those of Theis *et al.* (1994) who found a cysticercosis prevalence of 13% in the Bali region of Indonesia. In the same country but in another district a human cysticercosis prevalence of 50.6% was recorded by Subahar *et al.* (2001) and this is one of the highest recorded anywhere in the world.

Community-based studies in Latin American countries where *T. solium* is endemic, such as Mexico, show seroprevalence ranging from 4.9%-10.8% (Sarti *et al.*, 1992, 1994; Schantz *et al.*, 1994). In Ecuador reported prevalences vary between 8% in rural villages to 18% in patients referred to neurological clinics (Tsang and Wilson, 1995). Those in Guatemala (10% - 17%) reported by Garcia-Noval *et al.* (1996) just like those found in Honduras by Sanchez *et al.* (1999) are similar to our findings. It is possible that the relatively poor socio-economic conditions in the Central American region, as is the case in Gwembe and Monze districts of Zambia, make it a particularly important focus of *T. solium* infection (Sanchez *et al.*, 1999).

After comparing the overall prevalence by gender, there was no evidence to suggest a significant difference in cysticercosis prevalence by gender on urine Ag-ELISA. This finding agrees with that of Garcia *et al.* (2003) and Sanchez *et al.* (1998) who both found no statistical association between gender or age group and seropositivity.

Sanchez *et al.* (1999) also found no significant association between EITB-based seropositivity and gender (OR = 0.47; CI = 0.18 - 1.17). However, in our study significantly higher cysticercosis prevalence was observed in females (17.4%) as compared to males (7.9%) in Gwembe district. This finding agrees with Garcia *et al.*, (1998) who demonstrated a strong association between being female and EITB-based seropositivity (p = 0.002). A possible explanation for this high cysticercosis prevalence is that females are more exposed to *Taenia* eggs in that they are the ones that take care of children, and this includes washing them; hence if the child has the tapeworm then the mother is more at risk than any other member of the household.

The urine Ag-ELISA cysticercosis prevalence was observed to increase with increasing age and then declined in the > 60 years age group. This finding conforms to the observations by Garcia *et al.* (2003) observation that the age-prevalence curve of intestinal taeniosis peaks at an earlier age than do human cysticercosis antibodies.

From our results, 8 individuals who were positive for taeniosis after coproscopic examination were also positive for cysticercosis on urine Ag-ELISA. It is possible that these had contact with the cestode's eggs by direct exposure since the possibility of self infection has been mentioned as a mechanism for transmission (Martinez-Maya *et al.*, 2003). The results agree with the findings that intestinal *T. solium* carriers have been found to be significantly more likely to be metacestode antibody positive than other members of the general public (Craig *et al.*, 1960).

The presence of intestinal *T. solium* tapeworm carriers increases the likelihood of human cystic infections, thus accurate identification and treatment of such cases has been identified as the priority in the control o f the parasite (Gemmel *et al.*, 1981).

5.2.2 Prevalence on serum Ab and Ag-ELISA

Of the 101 serum samples analyzed, only 9 (8.9%) were positive for cysticercal antigens and 48 (47.5%) for antibodies. These findings agree with what was found in many researches in other parts of the region (Garcia *et. al.*, 2003 and Sanchez *et al.*, 1998). However they disagree with Garcia *et al.* (1998) who demonstrated a strong relationship between being female and EITB-based seropositivity, a situation observed in the urine Ag-ELISA test of the present study. The increased risk recorded by Garcia *et al.* (1998) was because more females handled raw, infected pork more frequently that did males. This also applies to our study, but also that females handle all the activities in a household and hence more exposed to infection with *Taenia* eggs.

Only seven serum samples from the 50 urine positive individuals were positive on serum Ag-ELISA and 17 of these individuals were positive on serum Ab-ELISA. Two samples were positive on serum Ag-ELISA yet negative on urine Ag-ELISA. These could have been new infections considering the time that elapsed between urine and serum collection. This could also be the reason as to why samples that were urine Ag-ELISA positive were found negative on serum Ag-ELISA. The individuals who turned out negative on serum Ag-ELISA but who were positive on urine Ag-ELISA could have had the infection at the time of urine sample collection but were able to get rid of it by the time the serum was collected. Nguekam *et al.* (2003) stated that the advantage with the Ag-ELISA is that it detects only viable cysticerci and is very sensitive since it could detect a pig infected with one live cyst. The same should apply to humans since it's the same ELISA used to detect cysticercal antigens in circulation. Thus, the individuals positive on urine Ag-ELISA

no longer harbored viable cysts in their bodies and hence the negative result obtained on serum Ag-ELISA. The proposition that the urine Ag-ELISA could have false positive reactions was ruled out because the assay has not yet been found to cross react with other parasitic infections [Dorny, (2005), personal communication].

However, more research needs to be conducted so as to validate the urine Ag-ELISA and hence make it a more reliable test for diagnosis of human cysticercosis.

5.3 Differentiation of *Taenia spp.*

The differentiation of *Taenia* species is important because of their very different clinical and epidemiological consequences. Proglottids obtained from stool samples, after treatment, could easily be identified by carmine staining (Mayta *et al.*, 2000). However, this requires the obtaining of intact gravid proglottids as squashed proglottids give equivocal or uncertain results.

From the three proglottids collected, two were identified as *T. solium* because 7-10 uterine branches were counted in the gravid proglottids. In one sample the uterine braches could not be counted as the proglottids were too decomposed. Mayta *et al.*, (2000) reported that parasitic material, either scolex or mature gravid proglottids, naturally expelled or following treatment, may in some cases be too decomposed to allow assessment of morphological characteristics. Thus, in this study the morphological examination results could not be relied upon for the differential diagnosis of the *Taenia* species. Furthermore, the small number of recovered parasitic material could not allow the determination of the proportions of the *Taenia* species in the two districts.

Since recovery of parasite material after treatment was unsuccessful, copro-PCR was used as the best alternative for the differential diagnosis of the tapeworm. PCR methods (Eom *et al.*, 2002; Yamasaki *et al.*, 2004) offer a better alternative for identification of *Taenia* samples because apart from being applied on samples that are partly digested they can also be applied on faecal samples. However, since we only had three samples of parasitic material, they could not be very helpful in the differential diagnosis. It is for this reason that a sub sample of 200 faecal samples was chosen and PCR-RFLP conducted.

The PCR results showed that 21 out of the 200 samples were positive for *Taenia* and when RFLP was done all of these turned out to be *T. solium*. This finding disagrees with many studies in many parts of the world where different proportions of *T. solium* and *T. saginata* have been found (Somers *et al.*, 2006; Nguyen Thi Lan Anh, 2004; Mayta *et al.*, 2000). However, the findings agree with Margono *et al.* (2003) and Prasad *et al.* (2002) who found all the recovered adult worms after treatment to be *T. solium*. Wandra *et al.* (2006) on the other hand found all recovered proglottids after treatment to be those of *T. saginata*. The PCR-RFLP technique confirmed the coproscopic result for 16 samples that were included in the sub sample. It was also able to detect *Taenia* in five samples that were negative coproscopically and thus confirming that the PCR technique is more sensitive in the detection of tapeworm infection. However, the cost of the test and the kind of equipment involved cannot allow the use of PCR as a diagnostic tool in the field especially in developing countries which are endemic for the *Taenia* infection (Allan *et al.*, 2003).

Our PCR-RFLP results cannot be taken as a prevalence rate because when selecting the sub sample there was bias towards those individuals who were either

coproscopically positive or had a medical history suggestive of tapeworm infection. However, the results have shown that the most prevalent *Taenia* species in the two districts of Gwembe and Monze is *T. solium*. Even though *T. saginata* was not detected, its presence in the study area cannot be entirely ruled out because no studies in bovine cysticercosis have been conducted in the area. A sero-epidemiological study of bovine cysticercosis conducted by Dorny *et al.*, (2002) found a bovine cysticercosis prevalence of 6.1% with a significantly higher prevalence in feedlots (10.8%) than in traditional farming systems (6.2). A factor that was put across for this high prevalence in feedlots was the continuous man to animal contact of casual workers in feedlots. The study was conducted in the Central and Southern provinces of Zambia but the two districts of Gwembe and Monze were not included. Hence the true situation of bovine cysticercosis in many parts of the country is not yet known. Bovine cysticercosis in the study area has not been detected yet and it is assumed to be absent [Phiri, (2005), personal communication] until extensive epidemiological studies are conducted to disprove this assumption.

The presence of *T. solium* carriers, therefore, further confirms the high presence of porcine cysticercosis recorded by other researchers (Sikasunge, 2005; Phiri, *et al.*, 2002) in the same study areas.

The confirmation of the presence of only *T. solium* in Gwembe and Monze districts, therefore, entails that the people are at an even increased risk of conducting cysticercosis and ultimately neurocysticercosis. When the tapeworm carriers migrate to urban areas, they become a risk for transmitting the *Taenia* eggs to other people.

5.4 Comparison of the different tests

Despite coproscopic examination being the most common and cheap diagnostic method for the detection of taeniosis, it has both low sensitivity and specificity (Allan *et al.* (2003). Hence, to obtain true prevalence values, more sensitive tests need to be utilized. PCR is one of the most sensitive tests that can be used for both diagnosis as well as species differentiation. When two or more tests are used to detect infection, statistical comparison of the two tests needs to be carried out so as to determine how well the tests agree. Statistical comparison of the two tests (i.e. coproscopic examination and PCR) to check agreement between them yielded a strong agreement (kappa = 0.852). A kappa value of 0.852 indicates that the tests agreed in 85.2% of the samples analyzed. This therefore means that although microscopy can be used to detect tapeworm carriers cheaply, it does not give a very representative prevalence rate and hence more sensitive but yet expensive tests are required (Allan *et al.*, (2003). The fact that PCR was able to detect five more taeniosis cases confirms that it is a more sensitive test than microscopy and can hence be used to determine a true prevalence value.

The results from the three assays performed to detect human cysticercosis only showed slight agreement between the urine Ag-ELISA and serum Ab-ELISA. Despite the agreement being statistically significant, the tests only agreed in less than 20% of the cases and hence very slight agreement. A high incidence of cysticercal antibodies were detected in the 101 serum samples (47.5%). It is possible that many of these may have had contact with the cestode's eggs by direct exposure due to the presence of many carriers of the adult worm and hence had transient infections. The six Ag-ELISA positive individuals but negative on Ab-ELISA

implies that they could have had low cyst burdens because the Ab-ELISA, especially when using the crude cyst fluid as antigen, has a poor sensitivity (Sikasunge, 2005). In pigs, Sciutto *et al.* (1998b) found that neither the Ag-ELISA and Ab-ELISA nor the EITB are adequate for the diagnosis of porcine cysticercosis in lightly infected village pigs. This, with the time factor, may explain the variation in the detection of cysticercosis by the three tests used in this study. Another factor could be that the number of serum samples collected was very small as compared to the urine samples and that serum sampling was biased towards positivity on urine Ag-ELISA. The collection of both urine and serum at the same time and from the same number of participants would have given a better picture on how much the tests agreed with each other. Hence, for future research work all samples should be collected at the same time.

5.5 Risk factors associated with taeniosis/cysticercosis transmission

In this study, we found that the majority of households had an average number of inhabitants of 8 implying that these numbers of inhabitants per households were at risk of acquiring taeniosis and/or cysticercosis, especially, if there are infected pigs and human tapeworm carriers in the household. Sarti *et al.* (1992b) in their study reported that belonging to a household owning a pig with cysticercosis may increase the risk of acquiring taeniosis and more so if some members of the household consume pork. This may explain why the levels of cysticercosis are higher than those of taeniosis in most endemic countries because if one individual in a household is a tapeworm carrier then all the other members are at risk of contracting

cysticercosis even if they do not consume pork. This kind of scenario is made worse if the levels of hygiene in a household are very poor.

Our results did not find any association between taeniosis and chronic diarrhoea, abdominal pain or with proglottids in faeces. There was also no association between cysticercosis and persistent headaches, seizures and skin nodules. The medical complaints recorded could thus have been due to other causes and not to taeniosis or cysticercosis. These results agree with Sanchez *et al.* (1999) who also did not find a significant association between EITB-based seropositivity and confirmed intestinal infection with *T. solium.* The non association of passage of proglottids to taeniosis could be that the respondents did not know how the proglottids looked like, even after explaining to them hence what they perceived as proglottids might have been totally something else. There was also no difference in taeniosis prevalence between individuals who had at one time taken some anti-helmintic drugs and those that had not. This could mean that even though the health centres sometimes carried out mass treatments, the anti-helmintic drugs that they used might not have any effect on taeniid tapeworms. Or that not everyone in the villages got the anti-helmintic drug.

The high levels of cysticercosis recorded in this study could suggest widespread neurocysticercosis. This could confirm the findings by Birbeck, (2000) who reported that epilepsy and febrile seizures were responsible for a significant burden of disease in the Southern province. However, in our study very few people admitted experiencing seizures and this may mean that epilepsy is a stigmatized condition in these areas. According to Birbeck (2000), patients at a rural Mission Hospital in Chikankata in Southern province with epilepsy had significantly less education than their sex-matched siblings and that there was evidence that epilepsy is

underreported, under recognized and under treated 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).

Sikasunge (2005) reported that pig keeping in Southern province of Zambia is quite a recent activity and that porcine cysticercosis infection rate has escalated to very high levels within a short period of time. Limited use of toilets was found to be a major factor accounting for this escalation and also that some people in some parts of the province could have been keeping pigs for a longer period and thus acted as a source of infection. Sarti *et al.* (1997) showed that extensively raised pigs have a higher seroprevalence of cysticercosis than intensively raised pigs and hence the humans are at even greater risk when the infected pork is consumed without proper cooking. 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 infection and that the transmission dynamics of taeniosis/cysticercosis are poorly understood especially with regard to relationship of human and porcine infection under field conditions. Analysis of prevalence of taeniosis in households with or without pigs on coproscopic examination yielded no statistical significance ($p = 0.570$). However, Sanchez *et al.* (1998, 1999) in their prevalence and population based case-control study, respectively, in Honduras found an association between pig raising by a household and EITB-seropositivity. Garcia *et al.* (2003) reported that domestic pig raising, taeniosis and human cysticercosis

are intimately interrelated. One explanation could be that even though almost all (91.6%) of the households that keep pigs have at least one individual that consumes pork, people who do not keep pigs also consume pork and are at an equal risk of infection. A study carried out in the north of India by Prasad *et al.* (2002) in a pig farming community found a very high taeniosis prevalence (38.0%) and hence further indicating that pig raising is a major risk factor for taeniosis.

The results show that the majority of households (83.9%) use boreholes as their source of drinking water and statistical analysis did not show any association between urine ELISA positivity and source of drinking water. Since borehole water is a form of portable water, these results agree with Sanchez *et al.* (1998) and Vilhena *et al.* (1999) who reported that seropositivity was statistically associated with lack of portable water. Lack of sanitary toilet facilities has been reported to be statistically associated with seropositivity for cysticercosis (Vilhena *et al.*, 1999; Sanchez *et al.*, 1998). In our results, 53.4% of the households used toilets and analysis of the results did not show any association even though lack of toilets has been proved elsewhere as a major risk factor for cysticercosis. Since the presence and usage of latrines was not physically inspected because the questionnaire was administered to participants as they visited the clinics, it can be concluded that some respondents to the questionnaire were not truthful. Sikasunge (2005) physically inspected the toilet facilities in households that he visited and found that a higher percentage of households (63.1%) did not use toilets. Thus, had we physically inspected the households many would have been found not to have toilets. Poor hygiene and living conditions puts people at risk of developing cysticercosis (Sarti *et al.*, 1992b and Sanchez *et al.*, 1997).

Of the above 12 years of age respondents interviewed, majority admitted not having ever heard of tapeworm infections in humans and a greater percentage of these were ignorant about how one gets to know whether they have the infection or not. Very few respondents admitted that man got tapeworm infection through consumption of infected pork. 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 taeniosis positive or seropositive. It therefore follows that the majority of people in the study areas could be at risk only because they are not aware of the parasite.

The results also show that the majority of the people that keep pigs at their households have at one time slaughtered a pig at home 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. Though some people admitted eating infected pork, others could have eaten infected pork unknowingly if the meat had light infections of cysticerci. Some of the risk factors of taeniosis and cysticercosis highlighted by Sarti *et al.* (1994) were eating infected pork and close proximity to a carrier of the adult *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.

Though this study found a high prevalence of both taeniosis and cysticercosis, the individuals in the two districts of Gwembe and Monze are very ignorant about the infection both in man and in pigs. Thus, the life cycle of the parasite is maintained because people are not only ignorant about the disease but also the parasite.

5.6 Conclusions and recommendations

The present study has provided important, previously unknown data on the prevalence of taeniosis and *Taenia solium* cysticercosis in the two districts of Gwembe and Monze of the Southern province of Zambia. This study has not only shown the endemicity of the parasite in the two districts but also the presence of factors that ensure maintenance and perpetuation of the parasite. A taeniosis prevalence of 3.1% by coproscopic examination is one of the highest not only in the region but also in the world. It, therefore, means that there are many carriers of the adult tapeworm who disseminate the infection not only to pigs but also spread eggs to other humans. The carriers are also a risk to people living in the urban areas when they migrate from the rural areas.

Despite the urine Ag-ELISA test being a relatively new test, a cysticercosis prevalence of 13.4% is quite high and similar to other studies in the region. A high human cysticercosis concurs well with the high taeniosis prevalence and may indicate the occurrence of neurocysticercosis and hence epilepsy. This is further compounded by the fact that the most prevalent tapeworm found in this study is *T. solium* whose larval stage causes neurocysticercosis. We therefore recommend further research in this area so as to determine the levels of epilepsy and the role that neurocysticercosis may be playing in the epidemiology of this important neurological disease.

It has been found out in this study that many people are unaware of the tapeworm infection, how it comes about and are hence at an even higher risk of infection as ignorance also plays a major role in the transmission of the parasite. We hope that this study will increase awareness about tapeworm infection not only among the

medical personnel but the public. This will lead to greater efforts to identify and treat tapeworm carriers who transmit *Taenia* eggs to others. It also follows that neurocysticercosis should, therefore, be considered in the differential diagnosis of neurological disturbances, even populations where pig cysticercosis is not observed.

Since this study has proved the presence of *T. solium* infection in the two districts it is incumbent upon the public health authorities and policy makers to formulate and carry out control measures. This will help prevent the maintenance and transmission of the tapeworm which has proved to be a public health problem not only in the rural areas but equally in the urban areas. It is recommended that sensitization measures be instituted to inform the public about the taeniosis/cysticercosis complex and ways to avoid infection of man and their pigs. People should be informed about the importance of sanitation, the need to improve on pig husbandry practices, the importance of having their meat inspected and importantly, dangers of consuming improperly cooked infected pork. Appropriate advice on personal hygiene and proper environmental control measures are crucial if high standards of public health are to be achieved. Studies should also be carried out in other pig keeping areas of the country so that the true status of tapeworm infection is ascertained. Undoubtedly, similar high prevalence figures might be found in many pig rearing areas of the country where surveys are yet to be carried out.

In conclusion, it can be said that, as for many other diseases, prevention is the key to reducing disease occurrence. We also know that simple changes in social habits and hygiene can prevent infection and disease in humans, 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 presence and consequences of *T. solium* infection, then sustainable and long lasting control programmes can be instituted.

The baseline data gathered in this first study on the status of the taeniosis/cysticercosis disease in humans should give the impetus to conduct further studies in humans in these areas. The assessment of the burden of the disease in humans should then be followed by intervention programmes such as extension programmes, community (civic) education and other prevention and control initiatives such as monitoring and surveillance. This may inevitably entail the creation of 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: Human Taeniosis/Cysticercosis Research Questionnaire

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

B. RESPONDENT

B1. Name:.....

B2. Age: .....

B3. Sex: .....

B4. Position in the household: .....

B5. Highest education level in household:.....

B6.Occupation:.....

Appendix 1 continued

C. HOUSEHOLD DESCRIPTION

C1. Household identification number: .....

C2. Name of household head: .....

C3. Ethnic background: .....

C4. Main income: .....

C5. Number of members of the household: .....

C6. Is it a pig keeping household?.....

D. RESPONDENT MEDICAL HISTORY

D1. Persistent headaches: .....

D2. Seizures: .....

D3. Chronic diarrhoea: .....

D4. Abdominal pain: .....

D5. Nausea: .....

D6. Proglottids in faeces: .....

D7. Ever taken anti-helmintic drugs: .....

D8. Skin nodules: .....

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(s) 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 (4) others (specify)

Appendix 1 continued

F. AWARENESS OF TAENIASIS 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? (Y/N)

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 (mention). (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)

World Health Assembly, item 14.2, 1-5 (Modified)

Appendix 2: Mean optical densities for sera dilutions at different antigen dilutions.

a) 1/50 antigen dilution (24μg/ml)

	Sera dilution		
	1/50	**1/100**	**1/200**
B	0.015	0.014	0.016
N	0.033	0.026	0.023
P1	0.109	0.101	0.071
P2	0.135	0.092	0.063

b) 1/100 antigen dilution (12μg/ml)

	Sera dilution		
	1/50	**1/100**	**1/200**
B	0.015	0.014	0.015
N	0.034	0.028	0.026
P1	*0.112*	*0.095*	*0.067*
P2	*0.116*	*0.089*	*0.063*

c) 1/200 antigen dilution (6μg/ml)

	Sera dilution		
	1/50	**1/100**	**1/200**
B	0.015	0.014	0.013
N	0.036	0.026	0.021
P1	0.123	0.085	0.058
P2	0.144	0.083	0.054

d) 1/400 antigen dilution (3μg/ml)

	Sera dilution		
	1/50	**1/100**	**1/200**
B	0.016	0.013	0.014
N	0.033	0.025	0.021
P1	0.108	0.078	0.057
P2	0.099	0.073	0.051