An epidemiological study of human tapeworm infections in the Eastern Province of Zambia

By

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Declaration

I declare that the thesis hereby submitted to the University of Pretoria for the degree Philosophiae Doctor has not previously been submitted by me for a degree at this or any other University. That it is my own work in design and in execution, and that all material contained herein has been fully acknowledged.

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Kabemba E. Mwape

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Protocol approval

This study was approved as PROTOCOL V063/09 by the Research Committee and the Animal Use and Care Committee of the University of Pretoria, under the Belgian Cooperation in the framework of the Institutional collaboration with the Institute of Tropical Medicine in Antwerp, Belgium.

Ethical considerations

Ethical clearance of the studies was obtained from the University of Zambia Biomedical Research Ethics Committee (IRB0001131, 012-04-09) and further approval was obtained from the Ministry of Health of Zambia and also from the local District health authorities of Petauke and Katete districts where the studies were conducted. Meetings were held with the community leaders (headmen) and their subjects where the purpose of the studies was explained and their permission requested to conduct the studies in their area. Informed consent was also sought from the individual subjects to participate in the study. Subjects were not forced to participate and participation was requested of individuals of all ages after written informed consent. For individuals below the age of 16, permission was sought from their parents or guardians by way of written informed consent. All participants found positive for taeniosis and other helminths were provided with treatment, namely niclosamide and mebendazole respectively. Those positive for cysticercosis were referred to the District hospital for follow-up and the recommended standard of care provided to them, if required. According to the guidelines of the Zambian Ministry of Health, the recommended management for cysticercosis consists of praziquantel with or without steroids (case dependent, prednisolone is mostly used).

For the part of the study conducted in Ecuador, ethical clearance was obtained from the Institutional Ethics Committee of the Central University of Ecuador, with further approval from the provincial and community authorities and the local health centers.

All activities were carried out with the highest ethical standards as guided by the appropriate guidelines and enforced by the Review Boards.

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List of abbreviations

Ab	Antibody
Ag	Antigen
Ab-ELISA	Enzyme-linked immunosorbent assay for the detection of antibodies directed against *T. solium* metacestode antigens
AI	Agreement index
AIDS	Acquired immunodeficiency syndrome
B160/B60 and HP10 Ag-ELISA	Enzyme-linked immunosorbent assay for the detection of circulating antigens of the metacestode of *T. solium*
BC	Before Christ
CART	Classification and regression trees
CI	Confidence interval/Credibility interval
CLTS	Community led total sanitation
CNS	Central nervous system
Copro-Ag ELISA	Enzyme-linked immunosorbent assay for the detection of *T. solium* antigens in faeces
Copro-PCR	Polymerase Chain Reaction assay for the detection of *T. solium* DNA in faeces
CSF	Cerebrospinal fluid
CT	Computer assisted tomography
DALY(s)	Disability Adjusted Life Year(s)
DIC	Deviance information criterion
DNA	Deoxyribonucleic acid
EITB	Enzyme linked immunoelectro-transfer blot
ELISA	Enzyme-linked immunosorbent assay
EPS	Electrolyte-polyethyleneglycol salt
ES	Excretory/secretory
ESP	Excretory/secretory proteins
GIS	Geographic information system
Gp	Glycoprotein

H_2SO_4	Sulphuric acid
HH(s)	Household(s)
HIV	Human immunodeficiency virus
M	Molar
MAFF	Ministry of Agriculture, Food and Fisheries
MoAb	Monoclonal antibody
MoH	Ministry of Health
MRI	Magnetic resonance imaging
N	Normal (H_2SO_2 4 N)
NBCS	New born calf serum
NCBI	National Centre for Biotechnology Information
NCC	Neurocysticercosis
OD	Optical density
OPD	Orthophenylenediamine
OR	Odds ratio
PBS	Phosphate buffered saline
PBS-Tween 20	Phosphate buffered saline in 0.05% Tween 20
PCR	Polymerase chain reaction
pD	Number of parameters effectively estimated by the model
PVPP	Polyvinyl-polypyrolidone
QS	Questionnaire
rDNA	Ribosomal deoxyribonucleic acid
RFLP	Restriction fragment length polymorphism
RHC	Rural health centre
Rnnh	Risk adjusted nearest neighbour hierarchical spatial clustering
Se	Sensitivity
Sero-Ag ELISA	Enzyme-linked immunosorbent assay for the detection of circulating antigens of the metacestode of *T. solium* in serum
Sp	Specificity
STH(s)	Soil transmitted helminth(s)

TCA	Trichloroacetic acid
th	Conditional probability
UNDP	United Nations Development Programme
US and USA	United States of America
US$	United States of America Dollars
WHO	World Health Organization

Thesis summary

Neurocysticercosis is an important neglected parasitic zoonoses caused by the metacestode stage of *Taenia solium*. The infection is endemic in most developing countries of Africa, Asia and Latin America. Most studies on the parasite in endemic regions of sub-Saharan Africa have been carried out in pigs with little or no data available on human taeniosis/cysticercosis. The aim of this thesis was to provide an understanding of the epidemiology of human taeniosis/cysticercosis in the Eastern province of Zambia where porcine cysticercosis is endemic, and to assess the performance of the existing diagnostic tests under field conditions.

Community-based cross sectional and longitudinal studies (Chapters 3 and 4 respectively) were carried out in two rural communities of Eastern Zambia to obtain the prevalence of taeniosis/cysticercosis and incidence of cysticercosis respectively as well as to study related risk factors. Given the constraints associated with blood collection in epidemiological studies, the use of urine, which is better accepted by communities, as a diagnostic specimen for human cysticercosis in field conditions, was evaluated (Chapter 6). Furthermore, three techniques, coproscopy, copro-Ag ELISA and copro-PCR, for the detection of taeniosis in stool samples were compared (Chapter 7).

Prevalences of 6.3% (45/718) and 5.8% (41/708) were determined for taeniosis based on copro-Ag ELISA and cysticercosis using Ag-ELISA assays, respectively in the study area in Petauke district with an increased frequency of cysticercosis positive individuals from the age of 30 onwards. In a neighbouring district, Katete, higher prevalences of 12.0% (27/227) and 14.5% (155/1069) were determined for taeniosis and cysticercosis using the same tests, respectively, but no significant relationship with age could be identified. A higher prevalence rate of cysticercosis based on sero-Ab analysis (EITB) ranging between 33.5 (54/161) and 38.5% (62/161) was recorded in the Katete study. The overall incidence rate for cysticercosis in Katete was determined to be 6.2% after 12 months. The longitudinal study revealed that even though many people were exposed to the infection as indicated by the antibody and antigen presence, few actually established active infections. This highlighted the importance of a multitude of factors, and not just exposure, that play a role in the establishment of infection and/or symptomatology.

Classification and regression analyses (CART) revealed that older individuals and those that came from households with many inhabitants were associated with higher prevalence rates of infection, listing these disease determinants as being very important for infection, and as such potential focus points of control programmes. Moreover, the CART highlighted differences in important disease determinants in neighbouring endemic areas, indicating the complexity of identifying optimal control measures.

Evaluation of the urine Ag-ELISA revealed a lower specificity as compared to the serum Ag-ELISA which was more pronounced testing the Zambian samples (78.6%) than the Ecuadorian samples (88.4%). Therefore, aspects of specificity have to be addressed for urine to be used as a sample in field conditions. Comparison of three different diagnostic tests for taeniosis showed coproscopy to be the least sensitive (52.5%) and the copro-PCR the most specific (99.6%).

Given the findings of this study, control of the tapeworm remains a challenge. It is proposed that, as there is a lack of knowledge of the parasite at all levels; health education should always be included in control programmes. It would be the best option in the long term, and would also determine the success of other more short term control measures as the more people get to know about the importance of the parasite the more they will accept control measures such as improved sanitation, vaccination and/or treatment of pigs and treatment of adult tapeworm carriers.

Chapters 3, 4 and 5 of this thesis demonstrated that human *T. solium* taeniosis/cysticercosis is endemic in the study area. It also indicated the need for further studies on transmission dynamics and burden of disease on the local people, as well as further improvement of diagnostic techniques. Finally, given the complexity of the disease determinants, as highlighted in chapter 5, the control of *T. solium* in our study area depends on multi-sectoral collaboration including the medical, veterinary and other relevant sectors as well as effective engagement of the affected communities.

CHAPTER 1

GENERAL INTRODUCTION

Taenia solium, *Taenia saginata* and *Taenia saginata asiatica* are important tapeworms causing taeniosis in man who is the definitive host for these cestodes, while cattle (*T. saginata*) and pigs (*T. solium* and *T. s. asiatica*) are the intermediate hosts, in which the metacestode larval stages (cysticerci) develop resulting in cysticercosis. Unlike *T. saginata* and *T. s. asiatica*, the metacestodes of *T. solium* can also infect man, who then acts as an accidental host and acquires human cysticercosis. Man acquires taeniosis following ingestion of undercooked meat contaminated with cysticerci. These develop into adult intestinal tapeworms which when mature release proglottids laden with eggs or just eggs in the faeces. The excreted eggs are immediately infective to the intermediate host (Murrell, 2005).

The lodging of the metacestodes of *T. solium* in the brain results in neurocysticercosis (NCC), one of the most important neurological parasitoses in man and the main preventable cause of acquired epilepsy in endemic areas (Carabin *et al.*, 2011). It is for this reason, that *T. solium* has a potentially higher public health impact than *T. saginata*, which mainly has economic implications (Gajadhar *et al.*, 2006). Adult tapeworm infections are largely asymptomatic, though some people may experience abdominal discomfort, nausea, diarrhea and loss of appetite and in the case of *T. saginata*, itchiness of the anal area due to the actively migrating proglottids (Muller, 1975).

While *T. saginata* has a more cosmopolitan distribution, *T. solium* is mostly reported in developing countries of Africa, Asia and Latin America, and *T. s. asiatica*, also known as the Asian *Taenia,* is restricted to East Asian countries and has not been reported elsewhere in the world including Africa (Eom *et al.*, 2009). *T. solium* endemicity in developing countries is reported to be associated with poverty, free ranging pigs and poor sanitary conditions especially lack of latrines (Phiri *et al*., 2003; Murrell, 2005; Sikasunge *et al.*, 2007). Many reports have documented *T. solium* infection in pigs in Africa with prevalence rates as high as 64% (Dorny *et al.*, 2004b). The cysticercosis/taeniosis disease complex remains a neglected tropical disease

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with very little information on the disease status in humans and, like many parasitic zoonoses, its true burden still needs to be determined (Carabin *et al.*, 2005; Praet *et al.*, 2009). The current global burden of *T. solium* cysticercosis in terms of Disability Adjusted Life Years (DALYs) has been estimated at 2-5 x 10^6, an estimate comparable to other neglected parasitic zoonoses but less than the "big three" global infectious diseases malaria, HIV and tuberculosis (Torgerson & Macpherson, 2011). The estimated burden for cysticercosis could be an underestimate as the calculations are done based on the few available data. Although epidemiological studies have contributed to the growing body of evidence on endemicity in some countries such as Mexico (Flisser and Gyorkos, 2007), this has not been the case in many endemic countries such as those in sub-Saharan Africa. A lot of information is still urgently needed in terms of disease prevalence in humans, transmission dynamics in endemic populations and NCC related clinical symptoms (Praet *et al.*, 2009). However, epidemiological and hospital based studies to provide such data require affordable, reliable and easy to apply diagnostic techniques which are currently insufficiently used in endemic countries.

As in many parts of the developing world, there is very little information on human *T. solium* taeniosis and cysticercosis disease status and burden in Zambia which is a developing country in sub-Saharan Africa with the Eastern, Southern and Western provinces reported to be endemic for *T. solium*, based on occurrence of porcine cysticercosis, with prevalence ranging from 17 to 30% based on sero-Ag analysis (Phiri *et al.*, 2002; Sikasunge *et al.*, 2008b). The country has seen an increase in the number of pigs and the consumption of pork in the past two decades (Phiri *et al.*, 2003). However, pigs are mostly raised under free-range management systems in resource poor rural communities. The free-ranging pig population in Eastern province accounts for more than 50% of this increase and all factors favouring transmission of the parasites are commonly present in this part of Zambia. As in most developing countries, sanitary conditions are poor with rampant lack of latrine use thereby allowing the free-range pigs access to human excreta. Moreover, the meat of pigs slaughtered in backyards and slaughter slabs is hardly ever inspected (Phiri *et al.*, 2003). Prevalence of porcine cysticercosis has been quite well documented in Zambia; however, little or no data is available on the infection status in humans or the factors at play in the maintenance of the parasite in the communities. It is assumed that presence of cysticercosis in pigs entails the presence of human tapeworm carriers (Flisser, 2002). Studies are

therefore needed to provide data on the epidemiology of the parasite in humans in areas reported to be endemic for porcine cysticercosis.

The aim of this research was, therefore, to study the epidemiology of human *T. solium* infections by determining prevalence and incidence rates of the infection and studying the risk factors associated with the infection in rural communities. The establishment of the disease prevalence would reveal how common the infection is while incidence would help in understanding the transmission dynamics. This thesis also explores a number of gaps in disease diagnosis. The use of urine as a diagnostic sample for human cysticercosis was evaluated, as it is generally easier to provide by communities in endemic areas, unlike blood samples. Lastly, this study compared the effectiveness of three tests namely coproscopic examination, coproantigen enzyme linked immunosorbent assay and polymerase chain reaction for the diagnosis of taeniosis. The results of this work will enhance the understanding of the epidemiology of *T. solium* in humans thereby aiding in decision-making regarding control measures in Zambia.

CHAPTER 2

LITERATURE REVIEW

2.1   Introduction

Three tapeworms of medical importance include *Taenia solium* (Linnaeus, 1758), *Taenia saginata* (Goeze, 1782) and *Taenia saginata asiatica* (Eom & Rim, 1993). The larval stages are known as metacestodes or cysticerci (Pawlowski & Schultz, 1972; Eom & Rim, 1993; Fan & Chung, 1998). Tapeworms are the causative agents of taeniosis in humans (Yamasaki *et al.*, 2004). Unlike *T. saginata* and *T. s. asiatica*, *T. solium* has a greater importance in public health because the lesser specificity for its intermediate host can give rise to human cysticercosis. This occurs when humans act as an accidental host harbouring cysticerci, which often lodge in the brain leading to NCC (Murrell, 2005) which is reported to be a major cause of epilepsy and is associated with considerable morbidity and even mortality and accounting for about 30% of all reported cases of acquired epilepsy in endemic areas (Ndimubanzi *et al.*, 2010). Not only does *T. solium* contribute to high morbidity and mortality, it also leads to livestock production losses implying considerable monetary costs to poor rural communities (Carabin *et al.*, 2005, 2006).

The current review provides updated information on the history, morphology, life cycle, epidemiology, diagnosis, treatment and control of the *T. solium* taeniosis/cysticercosis zoonotic disease complex. Special attention is paid to the current situation in sub-Saharan Africa and in particular Zambia.

2.2   History

Taenia species affecting man today could have diverged from *Taenia hyaenae*, a species closely resembling *T. solium* with hyaenids, canids and felids as definitive hosts and bovides as intermediate hosts (Hoberg *et al.*, 2001). The study of the mitochondrial and genomic deoxyribonucleic acid (DNA) of *T. solium* has revealed two different mitochondrial genotypes related to the biogeographic origin of the parasite, namely, the African/American and the Asian genotypes (Nakao *et al.*, 2002; Ito *et al.*, 2003; Martinez-Hernandez, F. *et al.*, 2009; Michelet *et al.*, 2010a). Using the available molecular data and Bayesian analysis, *T. solium* is reported to

have diverged into those two genotypes about 360, 000 years ago (Michelet & Dauga, 2012). The taxonomy of the *Taenia*, according to the GenBank database of the National Centre for Biotechnology Information (NCBI), is shown in Figure 2.1.
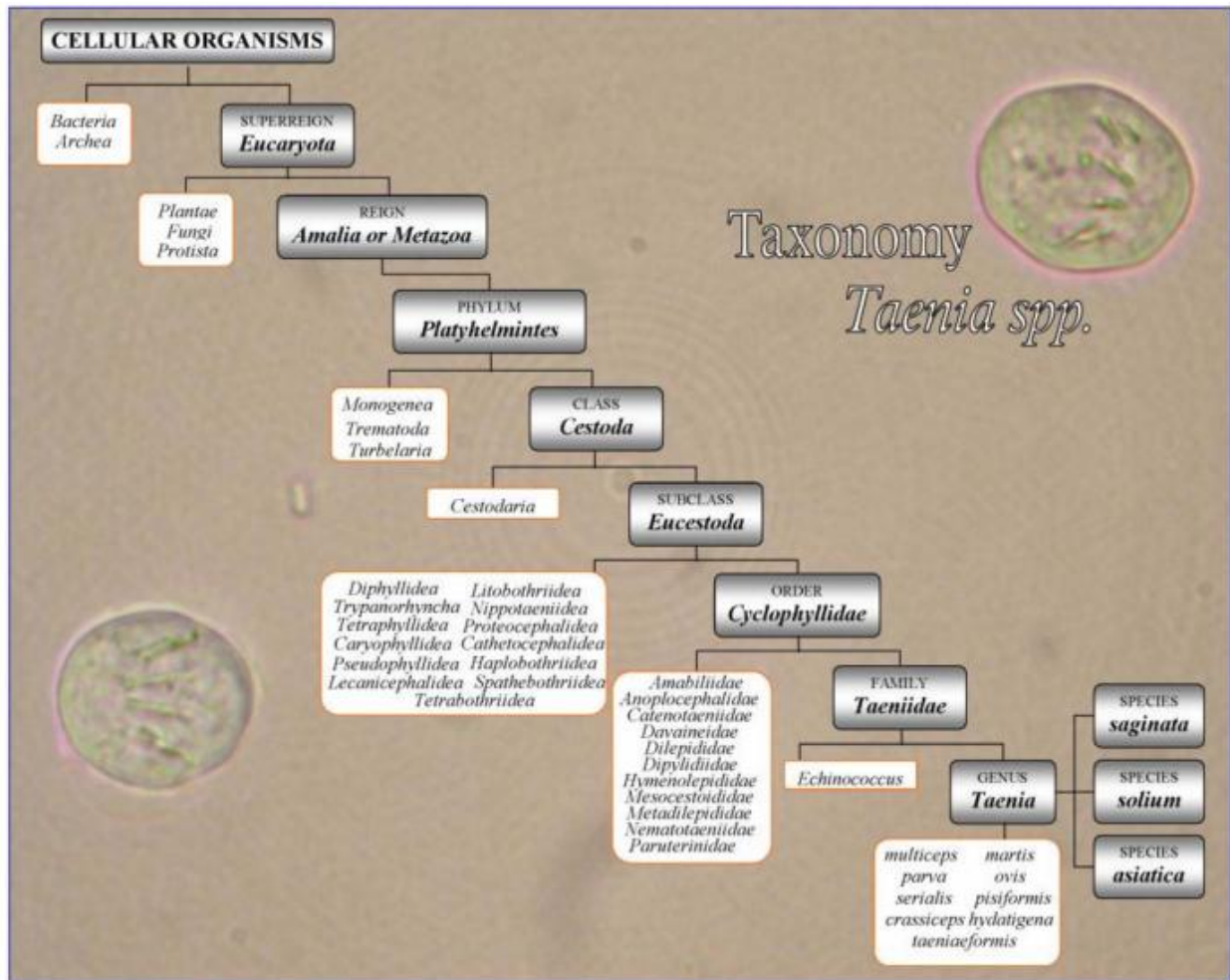
Figure 2.1: The taxonomy of *Taenia* species. (Source; www.ncbi.nlm.nih.gov)

The encysted *T. solium* larvae in pigs (cysticerci) are reported to have been well known to the ancient Greeks with Hippocrates (460 to 377 BC) suggesting that Greek physicians knew that humans harboured such cysts in the brain (Cox, 2002; Wadia & Singh, 2002). The scientific study of the taeniid tapeworms of humans can be traced back to the late 17th century. 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 other tapeworms such the broad tapeworm (*Diphyllobothrium spp.*) and the taeniid tapeworms that were identified, the distinction between *T. solium* and *T. saginata* was only made in the middle of the 19th century by Kuchenmeister, based on the morphology of the scolex (Cox, 2002).

T. s. asiatica is a species that closely resembles *T. saginata* and was only recently described as a new species in 1993 (Eom & Rim, 1993). However, controversies remain as to whether *T. s. asiatica* should be considered a new separate species with most authors considering it only as a subspecies of *T. saginata* and name it *T. saginata asiatica* as opposed to *T. asiatica* (Galan-Puchades & Mas-Coma, 1996; Loos-Frank, 2000).

Before the relationship between the adult tapeworm and their metacestode larval stage was understood, the larval stages were described with their own names, as if they were separate species or genera, a situation that still exists today (Acha & Szyfres, 2003). The first indication that intermediate hosts were involved in the life cycles of taeniid tapeworms emerged in 1784 from studies using the pork tapeworm. 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).

2.3   Morphology of *Taenia* spp.

2.3.1   Adult tapeworm

The adult tapeworm of the family *Taeniide* (*T. solium*, *T. saginata* and *T. s. asiatica*) are elongated flat, opaque white or yellowish, segmented parasites measuring between 1-12 meters (Bowman, 1999; Murrell, 2005). The body consists of a head or scolex, bearing attachment organs, a short unsegmented neck and a very long chain (strobila) of segments (Urquhart *et al.*, 1988). The body lacks a cavity or alimentary canal and each segment is called a proglottid and is hermaphrodite i.e. contains both male and female reproductive organs (Soulsby, 1982). The scolex has four suckers and a rostellum that may be armed with hooks (*T. solium,* Figure 2.2, A1), or unarmed and sunken (*T. saginata,* Figure 2.2, A2) or with rudimentary hooklets (*T. s. asiatica*). The hooks on the *T. solium* rostellum are arranged as a crown with two rows of 22 to

23 ranging in size from 110 to 180 μm. The scolex is followed by the neck, measuring about 5 to 10mm, from which the long chain of proglottids proliferates. The proglottids gradually increase in development and size so that the posterior end of the tapeworm has the gravid, broadest, longest and oldest proglottids (summarized by Murrell, 2005).

The proglottids making up the strobila or body of the tapeworm are in different stages of development and can be grouped as immature, mature and gravid segments. Immature segments are wider transversely than longitudinally while the mature segments are square with sexual organs completely formed. *T. solium* has a three-lobed ovarium while that for *T. saginata* is bilobed (as shown in Figure 2.2, B1 and B2). Gravid segments have a rectangular shape filled with a branched uterus filled with eggs, which resembles sacs full of eggs. The uterus develops 7 to 35 lateral branches depending on species, a feature that helps differentiation between *T. solium* (7-20 branches, Figure 2.2, C1) and *T. saginata* (14-35 branches, Figure 2.2, C2) (Schmidt, 1986; Harrison & Bogitsh, 1991). A gravid proglottid of *T. solium* may contain up to 50,000 eggs per proglottid and *T. saginata* up to 100,000 eggs (Soulsby, 1982).

T. solium   *T. saginata*

A1

1 mm

(without staining) 40X

The armed scolex

A2

0.5 mm

(without staining) 40X

The unarmed scolex

B1

Accessory lobe

4 mm

Three lobed ovarium

B3

5 mm

Bilobed ovarium

C1

6 mm

7-20 uterine branches

C2

30 mm

13-35 uterine branches
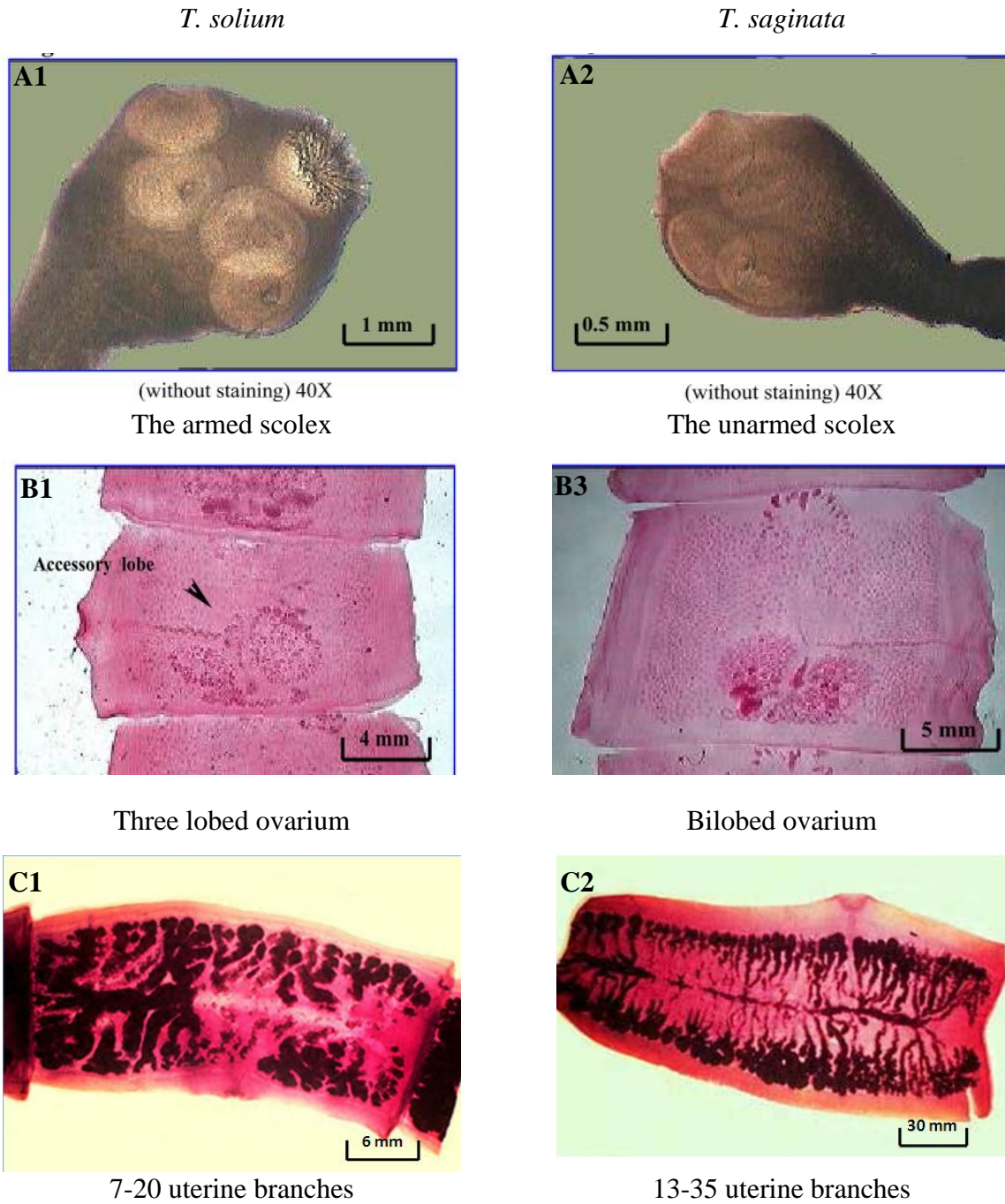
Figure 2.2: Morphological differences between *Taenia solium* and *Taenia saginata* as seen in the scolex (A), ovarium (B) and uterine branches in gravid proglottids (C). (Sources; www.dpd.cdc.gov; www.stanford.edu)

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2.3.2 Taeniid egg

The eggs or oncospheres (Figure 2.3) are rounded or subspherical ranging in diameter from 29 to 77 μm for *T. solium* and 39 to 50 μm for *T. saginata*, with a thick radially striated brown shell called the embryophore (Verster, 1969; Muller, 1975). The oncospheres of all taeniids are morphologically indistinguishable under light microscopy (Murrell, 2005). When released from the definitive host, many eggs are infective to a suitable intermediate host while others may be at different stages of maturation. The embryophore protects the oncosphere in the external environment enabling the eggs to withstand a wide range of environmental conditions and disinfectants and can remain viable for weeks or months. Inside the embryophore there is an embryonated oncosphere also called hexacanth embryo because it bears three pairs of hooks (Figure 2.3, B) (Bowman, 1999).
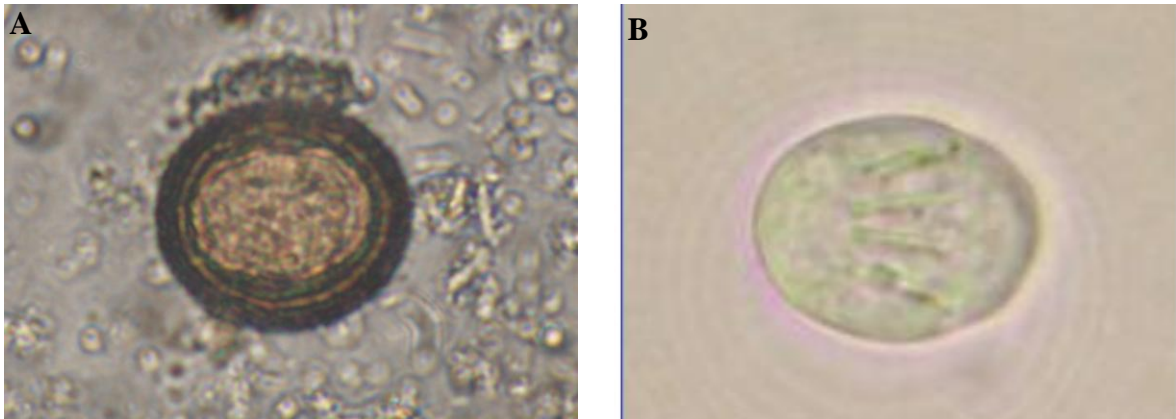
Figure 2.3: The rounded or sub-spherical taeniid egg with a thick radially striated embryophore, A (Source; Mwape K.E., 2009) and the oncosphere or hexacanth embryo with three pairs of hooks, B (Source; www.dpd.cdc.gov)

2.3.3 Cysticercus

The larval stage or metacestode is called cysticercus and is an ovoid vesicle measuring approximately 5-15 mm in diameter (Figure 2.4). The vesicle has a transparent membrane containing an invaginated scolex in a colourless fluid usually less than 0.5ml (de Queiroz & Alkire, 1998). In the muscles viable cysticerci appear as translucent, thin-walled cysts about 0.5

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to 1 cm in diameter with an eccentric white nodule corresponding to the invaginated scolex (Pal *et al.*, 2000). The size of cysticerci varies with the stage of development. By 20 days post infection, the cyst has 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 & Collins, 1992). Soulsby (1982) stated that the cysticerci in the intermediate host are infective to man after about nine to ten weeks. Although the longevity of cysticerci is not known, the young age at which pigs, unlike cattle, are slaughtered, the majority of cysticerci are viable.
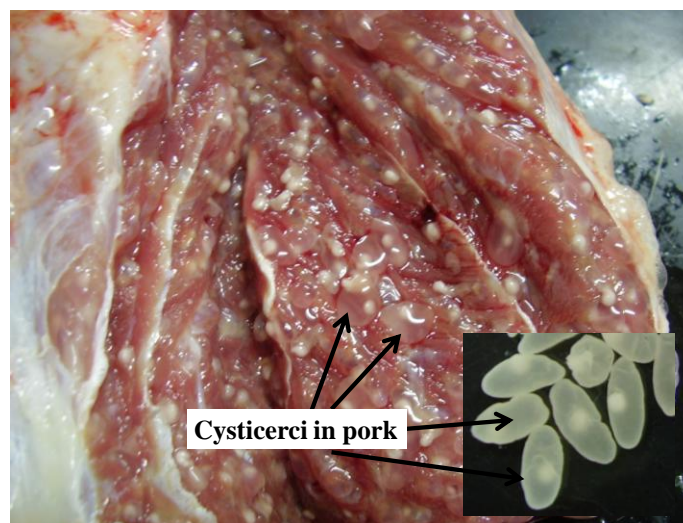
Cysticerci in pork

Figure 2.4: *T. solium* cysticerci in muscle tissue with a transparent membrane containing an invaginated scolex (Source; Mwape K.E., 2010)

2.3.4   Life cycle of *T. solium*

The life cycle of taeniid tapeworms involves two hosts and a free-living stage (Figure 2.5). The adult worm lives in the small intestines of man, who is the definitive host (Murrell, 2005). The tapeworm produces millions of eggs, which mature within the proglottids and passed to the environment (Toledo *et al.*, 2001). The eggs can either be intermittently expelled from the proglottids into the intestine or entire gravid proglottids may be passed in the faeces (White, 1997).
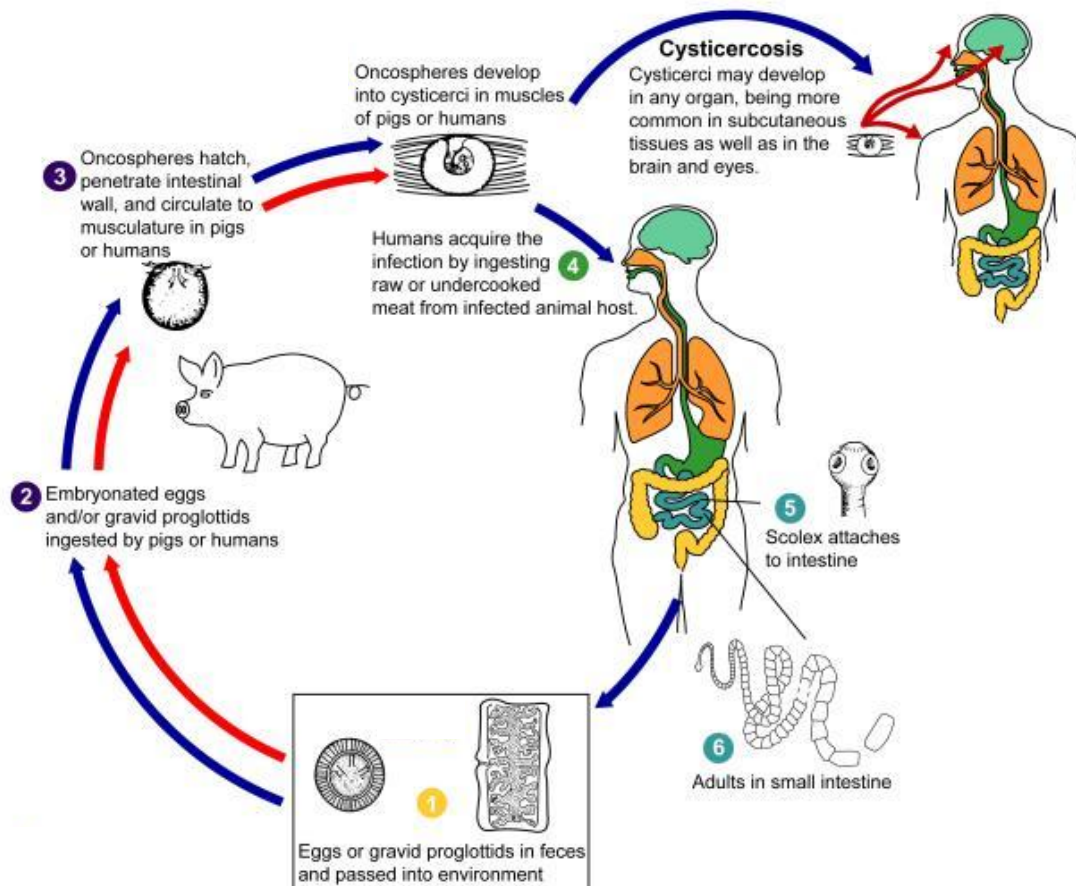
Figure 2.5: The life cycle of *Taenia solium* (Source; www.cdc.gov)

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 be released at the same time. The passage of these proglottids in faeces is intermittent (Allan *et al.*, 2003).

When ingested by pigs, 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 other parts of the body, such as striated and cardiac muscles, subcutaneous tissue and the brain where it encysts, forming cysticerci (Murrell, 2005). Excretory proteases produced by oncospheres facilitate their penetration into the intestinal mucosa and vessels in the submucosa (Flisser, 1994). The cysticerci develop primarily in the masseter, heart,

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tongue and shoulders, but may be disseminated throughout the body (Boa *et al.*, 2002; Phiri *et al.*, 2006; Soulsby, 1982). It is however, not known whether the oncospheres actively migrate to specific tissue or merely passively lodge in the tissues with high blood flow such as the muscles or the brain (White, 1997). In pigs, cysts can be found in the brain even in cases of low intensities of infection (Phiri *et al.*, 2006).

Human cysticercosis may occur if eggs are conveyed to the mouth by unclean fingers after defecation or other contamination with *T. solium* eggs (Murrell, 2005). The presence of a tapeworm carrier and non-sanitary conditions are the most likely vehicles of human infection (Schantz *et al.*, 1992). Flisser (1994) suggested that retrograde movement of intestinal movements 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, establishes in the muscle, subcutaneous tissue, eye or the brain. The highest number of cysts establishes in the subcutaneous tissue, then the brain, but may also be found in muscle, diaphragmor in the heart and eye (Gracey & Collins, 1992).

When inadequately cooked meat containing viable cysticerci is consumed by man, the metacestode evaginates, attaches to the small intestinal wall and grows into an adult tapeworm over a period of about three months (Peters & Pasvol, 2002). The adult worm develops forming gravid proglottids leading to the release of eggs containing oncospheres infective to the intermediate host and hence continuing the life cycle.

Man is the natural definitive host of the *T. solium* adult tapeworm, although, Allan *et al.* (1991) reported that the former may also be established in Lar gibbon (*Hylobates lar*), Chacma baboon (*Papio urnus*) and Golden hamster (*Mesocricetus auratus*). The larval stage of *T. solium* is found most commonly in pigs and man, though it can also occur in the wild boar, rabbits, sheep, and dogs (Sciutto *et al.*, 2000; Ito *et al.*, 2002; Wang *et al.*, 1999; Buback *et al.*, 1996; Theis *et al.*, 1996).

2.4 The importance of taeniosis and cysticercosis

2.4.1 Human

The presence of the adult tapeworm in the intestines of the definitive hosts (taeniosis) is generally asymptomatic. The most noticeable sign is the presence of proglottids either in faeces or actively migrating out of the host. *T. solium* releases a few gravid proglottids with the stools daily or two or three times a week, while proglottids of *T. saginata* migrate actively through the anus, single or in chains of up to 7 and are expelled daily (Flisser *et al.*, 2005). Although largely asymptomatic, carriers of *T. saginata* report peri-anal pruritus produced by the migrating proglottids (Muller, 1975) and the discomforting sensation of worms moving between the legs (Thornton, 1979). Other symptoms may include non-specific abdominal discomfort, hunger pains, nausea, flatulence; diarrhoea, increased appetite and weight loss (www.cdc.gov).

Zoli *et al*. (2003b) noted that in Africa the importance of cysticercosis on human health is rather difficult to estimate because of the highly variable clinical picture of the disease. The clinical presentation depends on the affected organ, with NCC and ophthalmic cysticercosis being associated with substantial morbidity. Outside the central nervous system (CNS), cysticercosis causes no major symptoms (Garcia *et al.*, 2003b). However, even when in the CNS, the clinical picture ranges from asymptomatic to severe headache, epilepsy and even death depending on the location, number of cysticerci and the inflammatory reaction (Del Brutto, 2012. Human NCC, if symptomatic, is characterized by varying neurological symptoms and is the frequent cause of neurological disorders in many developing countries. However, in spite of the localization of the cysticerci in the brain, many cases are asymptomatic (Alexander *et al.*, 2010; Carabin *et al.*, 2011). It is 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 (Del Brutto, 2005; Sotelo *et al*., 1990). Epilepsy is the most common and usually only clinical manifestation of NCC among symptomatic people (Carpio & Hauser, 2002) and it is estimated that in endemic countries, about 30% of acquired epilepsy cases are due to NCC (Ndimubanzi *et al.*, 2010). Other symptoms include nausea, vomiting, headache, ataxia, confusion, hydrocephalus, vasculitis and stroke (Del Brutto, 2012; Carabin *et al*., 2011).

Also, the cost of several visits to the physician, the costs for serology and/or computer tomography (CT) scan, transport and drugs have to be taken into account. Zoli *et al*. (2003) stated that although in many African countries, patients are not hospitalized during the treatment of NCC; losses due to the disease are thought to be quite insidiously significant. Cysticercosis can have as such an enormous socio-economic relevance, which besides treatment costs, also includes loss in man hours and direct economic 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 and Brazil were estimated at US$ 89 million and US$ 85 million respectively (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. High levels of very profound stigma have been reported in rural poor communities in Zambia (Birbeck *et al.*, 2007; Atadzhanov *et al.*, 2010).

The global burden of cysticercosis is presently unknown. In terms of DALYs the global burden of epilepsy is estimated to be 7.8 million with 6.5 million of these occurring in sub-Saharan Africa (Torgerson & Macpherson, 2011). A few local studies have shown estimates of the burden of cysticercosis in endemic areas. In West Cameroon, epilepsy due to NCC results in approximately 45,800 DALYs with a possible economic burden of over € 16 million (Praet *et al.*, 2009). A study in Mexico estimated that over 25000 DALYs were lost due to the clinical manifestations of NCC (Bhatarrai *et al*, 2012). The annual monetary burden of cysticercosis has been estimated at between US$ 18.6 million and US$ 34.5 million in the Eastern Cape of South Africa (Carabin *et al.*, 2006). Those studies also showed that more data are needed to more accurately estimate the true global burden of cysticercosis.

2.4.2 Animals

Clinical symptoms due to localization of metacestodes in the muscles of animals are rarely manifested. However, symptoms such as anorexia, fever, nausea, diarrhoea, staggering gait and

even death have been observed in massive infections in experimentally infected pigs (Pawlowski & Schultz, 1972). Neurological symptoms are not well documented in pigs but epileptiform convulsions have been rarely observed (Pawlowski & Schultz, 1972).

Porcine cysticercosis is an economically important parasitic disease because it affects a large number of pigs, making their meat unfit for human consumption, which greatly reduces its market value, thereby incurring sizable economic losses (Phiri *et al.*, 2003; Widdowson *et al.*, 2000). According to legislation of many African countries meat of infected pigs should be destroyed, but due to lack of well-organized meat inspection and very common illegal slaughtering, almost all infected carcasses are consumed or sold at a reduced price, thereby, causing a loss to either the farmer or the intermediary agent (Phiri *et al*., 2003; Zoli *et al.,* 2003a).

The monetary losses due to porcine cysticercosis have been estimated at almost € 480,000 in Cameroon (Praet *et al.,* 2009) and over € 2,000,000 in South Africa (Carabin *et al.*, 2006). As the parameters used in the estimations were based on tongue examination, which has a very low sensitivity, and questionnaire survey, the losses are probably higher than those estimated.

2.5   Diagnosis

2.5.1   Diagnosis of human taeniosis

Diagnosis of taeniosis is mainly based on the search for parasitic material in faeces. Coprological diagnosis is based on demonstration of *Taenia* proglottids or eggs or the demonstration of specific coproantigens by an enzyme-linked immunosorbent assay (ELISA) (Allan *et al*., 1990). The possibility of detecting *T. solium* specific antibodies in serum has also been demonstrated (Wilkins *et al.*, 1999).

2.5.1.1 Coproscopic examination

Coproscopic examination allows detection of *Taenia* eggs or proglottids in stool samples. Direct smears or concentration methods such as Kato-Katz and the formol-ether concentration technique (Ritchie, 1948) are widely used for the detection of *Taenia* eggs in faeces. These techniques have low sensitivities of around 38 to 69% (Allan *et al.*, 1996b; Sarti, 1997). This is

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primarily due to the intermittent nature of egg release leading to underestimation of the prevalence of taeniosis (Garcia *et al.*, 2003a). 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 and recommended the collection of samples over three days. Further, if destrobilation has led to a massive discharge of eggs, they may be absent from the faeces for up to several weeks thereafter, until more proglottids become mature and gravid (WHO, 1983).

Most importantly, *T. saginata* and *T. solium* eggs are identical under light microscopy (Murrell, 2005), leading to problems of diagnostic specificity. This is particularly relevant given the risks associated with *T. solium* infection (Allan *et al.*, 2003).

2.5.1.2 Identification and differentiation using morphological examination

Identification of human adult intestinal taeniids to species level relies on the recovery of gravid proglottids or scolices. This recovery has, however, proven difficult due to the disintegration of the proximal end of the worm when modern cestocidal drugs are used (WHO, 1983). Jeri *et al*., (2004) improved the treatment method to obtain a recognizable tapeworm by using pre-niclosamide and post-niclosamide electrolyte-polyethyleneglycol (PEG) salt purges to improve bowel cleaning and collection of the tapeworm scolex, making differentiation between *T. saginata* and *T. solium* easier. Nevertheless, since PEG has to be dissolved in two liters of water, it is very difficult to be accepted, especially in community studies.

Three morphological characteristics to distinguish *T. solium* from *T. saginata* were proposed by Verster (1969) in a taxonomic review of the genus *Taenia*. These characteristics are: presence of an armed rostellum on the scolex; three lobed ovary and absence of a vaginal sphincter. Additionally, the number of uterine branches in gravid proglottids is an indicative but not absolute difference between the two *Taenia* species. (Mayta *et al.*, 2000). Fixation and staining of proglottids with Semichon's acetocarmine allows for identification of these differences, as does injection of liquid black ink through the genital pore.

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2.5.1.3 Parasite coproantigen assays

Parasite coproantigens 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).

Several assays detecting *Taenia* coproantigens have been developed in different formats but all in the form of antigen capture ELISA using polyclonal antibodies obtained from hyper-immunized rabbits with either adult worm somatic or excretory-secretory (ES) products (Deplazes *et al.*, 1991; Maass *et al.*, 1991; Allan *et al.*, 1992; Machnicka *et al.*, 1996). These assays are reported to be genus specific and are independent of reproductive material (e.g eggs). Furthermore, the coproantigens are not detectable after treatment and the antigens are stable in faecal samples (Allan *et al.*, 2003).

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 some studies they are reported to have a specificity and sensitivity of 100% and 98%, respectively (Allan *et al.*, 1990; Allan *et al.*, 1992). Other studies in Guatemala and Peru have however, recorded lower sensitivities (Allan *et al.*, 1996a; Garcia-Noval *et al.*, 1996; Cabrera *et al.*, 2005). The tests are genus specific; as such *T. saginata* and *T. solium* infections cannot be differentiated. No cross reactions with faeces from other infections including *Hymenolepis* spp., *Ascaris*, *Trichuris*, hookworm and parasitic protozoa have been identified (Allan *et al.*, 2003). To achieve species specificity, Guezala *et al*. (2009) combined both polyclonal antibodies against *T. solium* adult whole worm extract and *T. solium* adult excretory/secretory proteins (ESP) in a hybrid sandwich ELISA format. This assay was reported to give a specificity of 100% and sensitivity of 95% for detection of *T. solium* carriers (Guezala *et al.*, 2009).

In epidemiological settings the coproantigen ELISA detects around 2.5 times more cases of taeniosis than basic microscopy (Allan *et al.*, 1996b; Garcia *et al.*, 2003a). It has also been reported to be a very useful test for the early evaluation of efficacy of antiparasitic treatment in human *T. solium* taeniosis (Bustos *et al.*, 2012).

2.5.1.4 Serological diagnostic assays

Wilkins *et al.* (1999) described *T. solium* specific antigens to detect antibodies against adult *T. solium* in serum by Western blot with sensitivity and specificity rates of 95% and 100% respectively. Even though no cross reactions were found in serum from individuals with *T. saginata* and other cestodes, including *T. solium* cysticercosis, one sample from a non intestinal *T. solium* carrier with positive NCC tested positive (Allan *et al.*, 2003). The serological diagnosis of taeniosis has obvious advantages over the faecal based methods e.g. species specificity, avoidance of potential biohazard associated with collection and handling of faecal samples and also the possibility of combining the test with other immunological assays in the diagnosis of cysticercosis. However, antibodies from treated infections remain for a long time and cause false positives (Allan *et al.*, 2003; Ito & Craig, 2003).

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 not yet commercially available for diagnosis but only for research purposes.

2.5.1.5 Molecular methods

Differentiation of human *Taenia* spp. by molecular assays is normally done on proglottids expelled from carriers after treatment (Eom *et al.*, 2002; Gonzalez *et al.*, 2002; Rodriguez-Hidalgo *et al.*, 2002). In recent years, polymerase chain reaction (PCR) tests for species-specific confirmation of *Taenia* spp. have been developed based on the detection of parasite DNA in faecal samples (copro-DNA) (Yamasaki *et al.*, 2004), or on cysticerci (Yamasaki *et al.*, 2002; Yamasaki *et al.*, 2004) or eggs present in the faeces (Yamasaki *et al*., 2004). Several methods and loci have been used for differentiating *Taenia* spp. Gonzalez *et al*. (2002) designated primers have been 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 the non-specific amplification found when using field samples. They, however, also used D*de*I as the restriction enzyme.

The major problem with PCR for DNA detection in stool samples has been that of sensitivity owing to the presence of PCR inhibitors in stool (Nunes *et al.*, 2003; Nunes *et al.*, 2006). Mayta *et al*., (2008) reported a nested-PCR assay targeting the Tso31 gene that was developed for the specific diagnosis of taeniosis due to *T. solium*. The specificity and sensitivity of the assay on archived samples were 97% (31/32) and 100% (123/123), respectively. Under field conditions, the assay had 100% sensitivity and specificity using microscopy and/or enzyme-linked immunosorbent assay coproantigen testing as the gold standard.

The high sensitivity of species-specific detection of *Taenia* spp. is a major advantage of the copro-PCR test for the diagnosis of taeniosis (Nunes *et al.*, 2003; Yamasaki *et al.*, 2004). However, current DNA extraction methods are too expensive to be used as a routine test and developing countries lack well equipped laboratories needed for molecular tests (Murrell, 2005), which makes their use under field conditions questionable.

2.5.2   Human and porcine cysticercosis diagnostic methods

The examination of the tongue of live pigs for presence of cysticerci and meat inspection of pig's carcasses have been the main methods for identifying infected pigs. When carried out properly by both palpation and visual examination of the entire tongue, tongue examination has a specificity of 100% (Gonzalez *et al.*, 1990; Dorny *et al.*, 2004b). Sensitivity however, depends on the degree of infection in the animal reaching 70% for heavily infected animals, lower than 50% for moderate infections and much lower for the lightly infected ones (Gonzalez *et al.*, 1990; Phiri *et al.*, 2002; Pouedet *et al.*, 2002). Using a Bayesian analysis, the sensitivity of tongue examination in Zambia was estimated at 21% (Dorny *et al.*, 2004b). Meat inspection on the other hand depends on visualization of different predilection sites in the carcass such as heart, tongue, masseter and shoulder muscles (Gracey & Collins, 1992). Although considered quite specific

(100%), meat inspection is not sensitive (22%) especially for pig carcasses with low cyst burdens (Gonzalez *et al.*, 1990; Sciutto *et al.*, 1998; Boa *et al.*, 2002; Dorny *et al.*, 2004b).

The asymptomatic nature of human taeniosis (Alexander *et al.*, 2010) and heterogenous nature of NCC symptoms makes a clinical diagnosis of the infection difficult (Fleury *et al.*, 2004; Fleury *et al.*, 2010; Carabin *et al.*, 2011). Diagnosis can sometimes be made parasitologically by the demonstration of a scolex with hooks in biopsy or autopsy material especially in Asia where subcutaneous cysticercosis are more frequent (Murrell, 2005).

Often, the diagnosis of NCC requires the use of sophisticated and expensive neuroimaging techniques such as CT and magnetic resonance imaging (MRI). However, even with these techniques, diagnosis of NCC is commonly uncertain. Diagnostic criteria have therefore been proposed based on clinical, radiological, immunological and epidemiological data of patients. The criteria are divided into absolute, major, minor and epidemiological, based on the diagnostic strength of each. Absolute criteria permits unequivocal diagnosis even if considered alone; major criteria strongly suggests the diagnosis but cannot be used alone to confirm or exclude the disease; minor criteria are frequent clinical and radiological manifestations of the diseases but are relatively non-specific and therefore unable to significantly distinguish the diagnostic possibilities: epidemiological criteria include potential exposure that favours a diagnosis of cysticercosis and living in endemic settings (Del Brutto, 2012). The CT and MRI technologies are however, not commonly available in developing countries due to their cost, leaving many patients to go undiagnosed.

2.5.2.1 Immunodiagnostic techniques

As there are no clinical features specific for cysticercosis, asymptomatic brain lesions are not uncommon and imaging methods unavailable for epidemiological studies; the definition of cases is often based solely on immunodiagnostic methods (Flisser, 2002). The development of immunodiagnostic tools has greatly contributed to our knowledge on the importance of taeniosis/cysticercosis by enabling sero-epidemiological surveys and community–based studies (Dorny *et al*., 2003). These tools also offer the possibility of surveillance of the infection in both humans and pigs during and after control programmes (Garcia *et al.*, 2000; Sarti *et al.*, 2000). 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 (Castillo *et al.*, 2009).

2.5.2.1.1 *Cysticercosis antibody detection assays*

Various techniques to detect antibodies to *T. solium* infections in man and pigs have been described such as the complement fixation test, immunoelectrophoresis, hemaglutination, radioimmunoassay, ELISA, dipstick ELISA, latex agglutination and immunoblot (Flisser *et al*., 1975; Miller *et al.*, 1984; Tsang *et al.*, 1989; Garcia & Sotelo, 1991; Ferreira *et al.*, 1997; Ito *et al.*, 1998; Rocha *et al.*, 2002). 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) have been evaluated. However, the use of crude antigens has resulted in moderate sensitivities and relatively poor specificities (Schantz & Sarti-Gutierrez, 1989; Fleury *et al.*, 2001). Research on the antigenic properties of cyst fluid and surface proteins as well as improvements in protein purification techniques have led to better tests with high sensitivities and specificities (Gottstein *et al.*, 1986; Parkhouse & Harrison, 1989; Tsang *et al.*, 1989; Ito *et al.*, 1998).

The most widely used antibody detecting test for the diagnosis of cysticercosis in human and pig serum samples is the enzyme linked immunoelectrotransfer blot (EITB) technique or Western blot. This is an immunoblot of seven cysticercus glycoproteins, purified by lentil lectin 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 it does not bound well to the plates compared to the crude extracts generally used in the ELISA (Dorny *et al.*, 2003). The EITB has been successfully used in field studies for antibody detection of *T. solium* cysticercosis in humans and pigs in endemic areas of Peru, Guatemala and Mexico (Allan *et al.*, 1997; Gonzalez *et al.*, 1990; Sarti *et al.*, 1997). The major disadvantage of the test, however, is the reliance on parasite material, the complicated nature of antigen preparation, the cost, instability of the reagents involved during production and the sophisticated equipment needed (Rodriguez-Canul *et al.*, 1997, 1998).

Purified glycoproteins from cyst fluid have been applied in the form of an immunoblot and ELISA on 53 parasitologically or pathologically confirmed NCC serum samples (from Korea, China and USA), resulting in a highly specific and sensitive test for differential serodiagnosis of NCC (Ito *et al.*, 1998). More recently, the use of recombinant antigens and synthetic proteins has been reported (Sako *et al.*, 2000; Sato *et al.*, 2003; Hell *et al.*, 2009). Even though the use of recombinant antigens has resulted in more specific tests able to differentiate *T. solium* infection in pigs from infections with other taeniids (Sato *et al.*, 2003), they have lower sensitivity (Dorny *et al.*, 2003).

Barcelos *et al*. (2007) reported the possibility of distinguishing between active and inactive cysticercosis by detecting immunodominant IgG in total saline extracts of *T. solium* cysticerci only in CSF and not in serum. Further, the use of different immunodiagnostics tests (EITB, Ab-ELISA and Ag-ELISA) on CSF has been shown to give indications of the location of the cysticerci and when combined with PCR can diagnose cases missed by the immunodiagnostic tests applied on CSF (Michelet *et al.*, 2010b). Though serological testing of NCC using CSF may be more appropriate, the collection of CSF is more cumbersome than blood and may only be possible in clinical settings (Deckers & Dorny, 2010). The use of saliva has also been reported in the diagnosis of NCC by EITB with good results, though serum remains a better sample (Feldman *et al*., 1990; Flisser *et al*., 1990).

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. 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) or surgical treatment (Ito *et al.*, 1999). The occurrence of cross-reactions with other diseases such as hydatidosis and ascaridosis has been observed with *T. solium* antigens (Pinto *et al*. 2000).

2.5.2.1.2   *Cysticercosis antigen detection assays*

Antigen detection has provided a suitable alternative for the drawbacks associated with antibody detection (Brandt *et al.*, 1992; Van Kerckhoven *et al.*, 1998; Dorny *et al.*, 2003). Two MoAb-based tests namely the B160/B60 Ag-ELISA (Dorny *et al.*, 2000) and the HP10 Ag-ELISA

(Harrison *et al.*, 1989) have been validated and continue to be used routinely for the detection of parasite antigens.

Sensitivities and specificities of the tests in pigs have been estimated at 76% and 84% respectively for the B160/B60 Ag-ELISA and 55% and 83% respectively for the HP10 Ag-ELISA (Krecek *et al.*, 2008). The B160/B60 Ag-ELISA has been used in sero-epidemiological studies for *T. saginata* bovine cysticercosis (Dorny *et al.*, 2002) and *T. solium* porcine cysticercosis (Phiri *et al.*, 2002; Sikasunge *et al.*, 2008b) in Zambia with an estimated sensitivity and specificity of 87% and 95% respectively (Dorny *et al.*, 2004b). Although some authors have been able to detect cysticercosis in pigs with one cyst using the serological tests (Nguekam *et al.*, 2003a; Dorny *et al.*, 2004b), they are less sensitive when applied to pigs with very low cyst burden (Sciutto *et al.*, 1998). In addition, the genus specificity does not allow the differentiation between metacestode infections of *T. solium* and *T. hydatigena* in pigs (Dorny *et al.*, 2004b), thus making their use for the diagnosis of *T. solium* cysticercosis not accurate in areas where these two parasites are sympatric. The Bayesian analysis has also been used to estimate the sensitivity and specificity of the B160/B60 Ag-ELISA for diagnosis of human cysticercosis in Ecuador and determined to be 90% and 98% respectively (Praet *et al.*, 2010b). The test has also good performance for antigen detection in urine in clinical settings (Castillo *et al.*, 2009). Most importantly the circulating antigen detecting technique offers the advantage over antibody detecting tests of confirming 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; Dorny *et al.*, 2003; Alexander *et al.*, 2010). It has been demonstrated that there is a positive correlation between the number of viable cysts and cysticercus antigen concentration (Deckers *et al.*, 2008).

Erhart *et al.* (2002) found a very good agreement between the B160/B60 Ag-ELISA, 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).

Antigen and antibody detecting tests require the collection of serum or CSF. However, the collection of CSF or blood is an invasive procedure that requires technical expertise. 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 teardrops other than serum. Of these, urine is increasingly used as a specimen for the diagnosis of many parasitic infections (Parija, 1998). Antigen detection in urine is being employed in the diagnosis 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.*, 1987a), toxoplasmosis (Ayi *et al.*, 2005), cystic echinococcosis (Parija *et al.*, 1997; Ravinder *et al.*, 2000) and cysticercosis (Castillo *et al.*, 2009; Parija *et al.*, 2004). Though the use of urine for diagnosis of cysticercosis has shown good results, it has only been used in clinical settings and its applicability in field conditions is yet to be evaluated.

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

Since the life cycle of *T. solium* requires two hosts and a free living stage, the parasite population at any one time consists of three distinct sub-populations; the adult worms in the definitive host (man), larvae or cysticercus in the intermediate host (pig) and the egg in the environment. Therefore, assessing the epidemiology of this important zoonotic parasite requires that all the three sub-populations are taken into account as they are interdependent (Murrell, 2005).

The transmission dynamics of taeniosis and cysticercosis are poorly understood in endemic areas. The pig is the essential intermediate host of *T. solium* and risk factors have been mostly studied in relationship with infection in this host. Poverty, traditional pig husbandry (free-range) systems, poor sanitation (open-air defaecation) and hygiene (Garcia *et al.*, 1996; Gonzalez *et al.*, 2001) and the use of human stool as fertilizer in agricultural fields have been identified as factors associated with infection in pigs (Phiri *et al.*, 2003; Murrell, 2005; Praet *et al.*, 2010a). Less attention has been paid to identify and document the risk factors involving human infection (Garcia-Garcia *et al.*, 1999). Consumption of raw or undercooked pork, lack of meat inspection, poor household conditions, lack of tap water, lack of knowledge about the parasite and lack of latrines have been described as important risk factors associated with infection in man (Diaz *et al.*, 1992; Sarti *et al.*, 1992; Schantz *et al.*, 1992; Sanchez *et al.*, 1998).

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. These carriers intermittently shed proglottids and/or substantial numbers of infective eggs in their faeces, thereby exposing the majority of the victims to cysticercosis due to the unhygienic preparation and serving of food (Schantz *et al.*, 1992).

In non-endemic countries, the disease is most likely to be imported through immigrant human tapeworm carriers or people travelling to endemic areas where they may acquire taeniosis through consumption of infected pork or cysticercosis from the contaminated environment (Yanagida *et al.*, 2012). Migration of tapeworm carriers from rural to urban areas predisposes a higher transmission risk of cysticercosis when there are poor environmental and social conditions (Schantz *et al.*, 1992).

Sero-epidemiological studies utilizing antigen and antibody detecting tests can give more insights in the actual transmission dynamics of the parasite. In a study in an endemic region of Ecuador where people are continuously exposed to the parasite, active infection (determined by the presence of antigen) significantly increased in elderly people (Praet *et al.*, 2010c). Immunosenescence, the gradual deterioration of the immune system brought on by natural age advancement, was proposed as the possible explanation for the increased rate of infection in the elderly as has been observed in NCC (Fleury *et al.*, 2004) and other parasitic infections such as, hookworms and *Strongyloides stercoralis* (Albright & Albright, 1994; Jardim-Botelho *et al.*, 2008).

Even though high rates of seroconversion (antibody based) are recorded in endemic areas, studies have shown that about 40% of infected individuals serorevert when re-sampled after one year (Garcia *et al.*, 2001). However, this was based on antibody testing, which, as stated above, can detect resolved infections and therefore, seroconversion and seroreversion rates based on antigen detection would give a clearer picture of the transmission dynamics in individuals with viable infections.

2.6.1 Global distribution of *T. solium* taeniosis/cysticercosis and prevalences in sub-Sahara African countries

T. solium taeniosis/cysticercosis is endemic in most developing countries of Latin America, Sub-Sahara Africa and East Asia (Figure 2.6) (WHO, 2010). Poor regions are said to be the most affected and presence of the disease is considered as a biological marker of socio-economic underdevelopment (Garcia *et al.*, 2003b). WHO reported that more than 80% of the world's 50 million people affected by epilepsy live in developing countries many of which are endemic for the parasite (WHO, 2010).

It has been suggested that colonization could have led to the introduction of *T. solium* into Africa and Latin America about 500 years ago (Nakao *et al.*, 2010; Martinez-Hernandez *et al*., 2009). Therefore, the migration of humans and development of farming and animal husbandry have contributed greatly to the spread and diversification of the tapeworms (Michelet & Dauga, 2012 Martinez-Hernandez *et al*., 2009).

T. solium infections are increasingly being diagnosed in Africa (Murrell, 2005). Data in humans is very limited due to the lack of adequate surveillance, monitoring and reporting systems despite increasing recognition of the infection as a serious emerging threat to public health (Mafojane *et al.*, 2003). The information on the infection in pigs is in many instances more extensive than for human infections because of the growing interest among veterinarians and agriculturalists in porcine cysticercosis. Regions of hyperendemicity have been identified (Phiri *et al.*, 2003; Zoli *et al.*, 2003a) (Table 2.1). Though the presence of porcine cysticercosis can be used to estimate the presence of the infection in humans, available data suggest the latter is often underestimated (Flisser, 2002). Reported prevalences in pigs range from 20 – 40% in most regions of sub-Saharan Africa. There is growing concern for the infection in both pigs and humans due to the rapidly expanding numbers of pigs raised on free-range in the past two decades (Phiri *et al.*, 2003). The scanty data available for the infection in humans show prevalences of up to 21.6%, in a study performed in the Democratic Republic of Congo (Kanobana *et al.*, 2011).
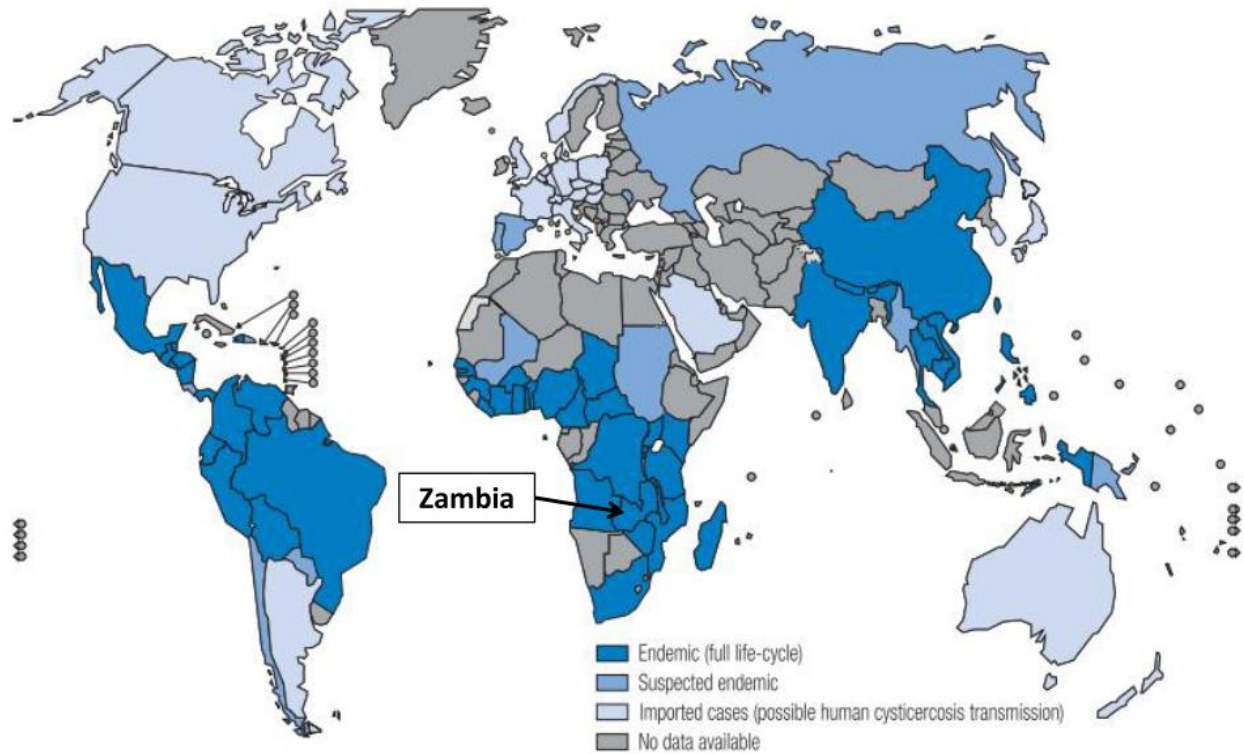
Figure 2.6: The global distribution of *Taenia solium* taeniosis/cysticercosis (WHO, 2010) with Zambia's location in sub-Saharan Africa

Prevalence data on taeniosis/cysticercosis in both humans and pigs on the African continent are presented in Table 2.1.

Table 2.1: Prevalence of porcine and human taeniosis and cysticercosis in African countries

Country	Year	% porcine cysticercosis prevalence	% Human taeniosis prevalence	% human cysticercosis prevalence	Reference
Angola	2003	0.0 – 6.8[a]	ND	ND	Zoli et al., 2003a
Benin	2003	ND	ND	1.3[b]	Zoli et al., 2003a
Burkina Faso	2009		ND	0.0 – 10.3[b]	Carabin et al., 2009
	2010	0.0 – 25.0[b]			Ganaba et al., 2011
Burundi	1997	2.0 – 39.0[b]	0 – 1.0[d*]	4.6[b]	Newell et al., 1997
Chad	2003	6.8[a], 25.7[c]	ND	ND	Zoli et al., 2003b)
Congo D.R.	2010	41.2[b]			Kanobana et al., 2011
	2011		0.33[d]	21.6[b]	Praet et al., 2010a
Gambia	2010	4.8 – 13.2[b]	ND		Secka et al., 2010b
	2010			1.7[b**]	Secka et al., 2010a
Ghana	2003	11.7[a]	ND	CR	Zoli et al., 2003b
Ivory Coast	2003	2.5[a]	ND	CR	Zoli et al., 2003b
Kenya	2003	10-14		ND	Phiri et al., 2003
	2009		2.5[d]		Asaava et al., 2009
	2010	4[b]			Kagira et al., 2010
	2010		4-10[d]		Wohlgemut et al., 2010
Madagascar	2003	7 – 21[b]	ND	ND	Andriantsimahavandy et al., 2003
Mozambique	2009	34.9[b]	ND		Pondja et al., 2009
	2011			15.0 – 21.0[b]	Afonso et al., 2011
Nigeria	1993		11.5[d]	ND	Dada et al., 1993
	2003	1.8 – 20.5[a]			Zoli et al., 2003b
Rwanda	2003	10 – 30[a]	ND	7[‡]	Zoli et al., 2003b
Senegal	2003	1.2			Zoli et al., 2003b
	2011		9.3[d†]	11.9[b]	Secka et al., 2011
South Africa	1990		ND	7.4[b]	Sacks & Berkowitz, 1990
	2008	64.6[b]			Krecek et al., 2008
Tanzania	2003	0 – 46.7[a,c]	ND	ND	Phiri et al., 2003
	2006	7.6[c]			Boa et al., 2006
Uganda	2009	8.6[b]	ND	ND	Waiswa et al., 2009
Zimbabwe	1992		ND	12.0[b**]	Mason et al., 1992
	2003	2.7 – 28.6[b]			Phiri et al., 2003

a. Meat inspection/autopsy; b. Serology; c. Tongue examination; d. Coproscopic examination; ‡ Autopsy; † Percentage of the cysticercosis positive individuals; *Study in school children; **Study involving epileptic patients; ND no data available; CR case report

2.6.2 Prevalence of *Taenia solium* infections in Zambia

Zambia is a landlocked country located in the southern part of Africa bordered by eight countries (Figure 2.6). Until recently the country was divided into nine provinces. As the rest of the eastern and southern African region the country has seen an increase in pig population with most of these reared under free-range management systems. According to the Food and Agricultural Organization website (www.fao.org), Zambia's pig population rose from 308,872 in the year 2000 to 500,000 in 2010. The Eastern and Southern provinces account for over 50% of this increase in pig numbers. With this increase in smallholder pig keeping came reports of cysts being observed in the pigs slaughtered in backyards without controlled meat inspection (Phiri *et al.*, 2003). Extensive studies have hence been carried out in pigs in six districts of three provinces of the country namely, Eastern, Southern and Western provinces (Figure 2.7). These studies have indicated very high prevalences of porcine cysticercosis (Table 2.2) (Phiri *et al.*, 2002; Sikasunge *et al.*, 2008b) and have highlighted the importance of free-range pigs and absence of latrines in maintaining the infection in pigs (Sikasunge *et al.*, 2007). Using the Bayesian approach, the porcine cysticercosis prevalence has been estimated as very high with 64.2% (Dorny *et al.*, 2004b).
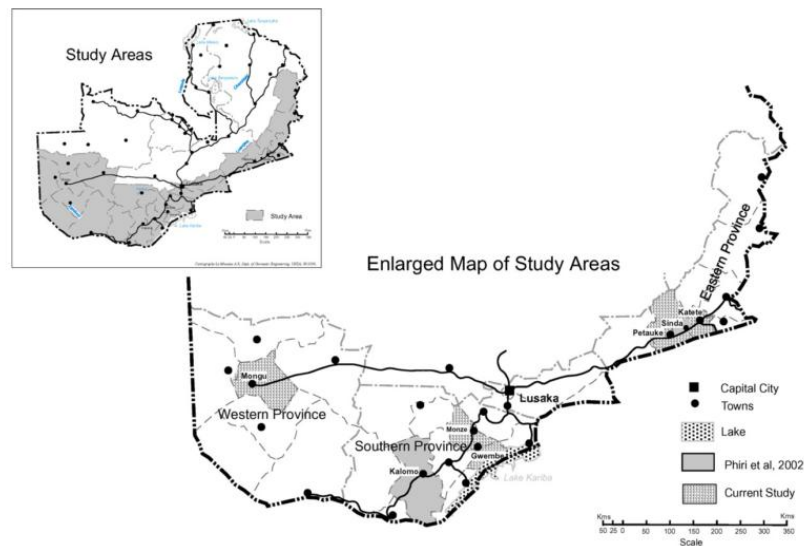
Figure 2.7: Map of Zambia showing districts were porcine cysticercosis prevalence has been determined (Source; Sikasunge *et al*. 2008b)

Table 2.2: Prevalence of porcine cysticercosis after tongue examination and antigen ELISA by district in Eastern, Southern and Western provinces of Zambia

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

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

The high porcine cysticercosis prevalences recorded in pigs are an indication of the presence of adult tapeworm carriers within the communities. Though data in pigs is available, information is still lacking on the disease status in humans in the country. The only information available is a University Master degree dissertation that indicated the prevalence of taeniosis and cysticercosis to be 3.1% and 13.4%, respectively, in two districts (Gwembe and Monze) of the Southern province (Mwape, 2006). The taeniosis prevalence was based on coproscopic examination and therefore could be an under estimation while that of cysticercosis was based on the presence of cysticercal antigens in urine using the B160/B60 Ag-ELISA, which had never been validated for use in field conditions.

2.7 Treatment of taeniosis in humans and cysticercosis in humans and pigs

2.7.1 Taeniosis

Two well-known synthetic and safe drugs with high efficacies against tapeworms in humans i.e. niclosamide and praziquantel are currently used for the treatment of taeniosis (Murrell, 2005). Niclosamide is given at a single dose of 2g in adults with an efficacy of 85% (WHO, 1983). Praziquantel, given at dosages from 5-10 mg/kg body weight, has an efficacy of 95% (WHO, 1995). However, a study in Mexico, reported that praziquantel for tapeworm treatment should not be given at doses lower than 10 mg/kg as this reduces the efficacy to about 50% (Sarti *et al*., 2000).

Albendazole, widely used for treatment of infection with soil transmitted helminths (STHs), and cheaper than niclosamide and praziquantel, has shown some efficacy against tapeworms but has to be given in larger doses over a few days (Chung *et al.*, 1991).

Nitazoxanide, a drug with a structure similar to niclosamide has recently been introduced as a broad spectrum antiparasitic agent. The drug is effective mainly against intestinal protozoan infections but has also some activity against STH and taeniosis (Murrell, 2005).

2.7.2 Human cysticercosis

The treatment of human neurocysticercosis (NCC) should undertaken in hospital conditions and depends on the number, localization (parenchymal or extraparenchymal), viability (viable, degenerated or calcified cysts), and the size of the cyst. Many neurologists advise against antihelmintic treatment of a single viable cyst, calcified cyst or asymptomatic NCC (Carpio *et al.*, 1998; Garcia *et al.*, 2002; Garcia *et al.*, 2005). The use of anti-parasitic treatment has been a debatable issue for some time (Del Brutto *et al.*, 2006) with many neurologists advising to only alleviate the symptoms in CT scan or MRI positive patients.

It is well proven that both albendazole and praziquantel are cestocidal and result in the resolution of *T. solium* cysts (Sotelo *et al.*, 1990; Gonzalez *et al.*, 1995; Gonzales *et al.*, 1996). Albendazole is currently the drug of choice as it has slightly better efficacy, better availability and is cheaper (Sotelo *et al.*, 1990; Garcia *et al.*, 2004). Although the clinical benefits of cestocidal treatment

continue to be controversial, increasing evidence points to clinical benefits for some forms of NCC (Del Brutto, 2012). Cestocidal drugs are reported to be responsible for some neurological symptoms provoked sporadically during treatment of asymptomatic cases of NCC due to the intense inflammatory reaction occurring around dying cysticerci (Flisser *et al.*, 1993). The management of cysticercosis and/or NCC should therefore include the use of symptomatic medication such as anti-inflammatory drugs (corticosteroids) and anti-convulsants (anti-epileptic drugs).

The contemporary role of surgery in the management of NCC has been restricted to placement of ventricle-peritoneal shunts in hydrocephalus and excision of well localized cysts, single giant cysts and excision of well-defined seizure foci (Murrell, 2005). Recently, excision of cysticerci by endoscopic microsurgery in a procedure known as minimal invasive flexible endoscopy surgery has been described (Proano, *et al*., 2009).

2.7.3 Porcine cysticercosis

Since there are largely no clinical symptoms in pigs, treatment is purely for public health and economical reasons. The use of albendazole or praziquantel has been mainly restricted to experimental studies. The need for repeated doses, secondary effects, high cost and low efficacy against cysticerci in the CNS has limited their use in large-scale programmes. Oxfendazole administered at a single oral dose of 30mg/kg body weight is reported to be 100% effective in treating porcine extra-neural cysticercosis (Gonzales *et al.*, 1996) however, has low efficacy against brain cysts (Gonzalez *et al.*, 1998; Sikasunge *et al.*, 2008a). Despite its good efficacy against cysticerci in pigs, oxfendazole has not yet been registered for use in pigs in many countries.

2.8   Prevention and control of taeniosis and cysticercosis

T. solium taeniosis/cysticercosis are reported to be potentially eradicable infections, however, since the mostly affected areas are developing countries, challenges such as lack of diagnostic facilities, of health infrastructures in rural areas, of effective taeniacides where they are needed, of cooperation between medical and veterinary services and lack of knowledge of the parasite, have hindered the implementation of control measures (Murrell, 2005). Several control options

that target the various potential intervention points in the life cycle of the tapeworm (Figure 2.8) have been described and are summarized in the following paragraphs, firstly with focus on the final host, and secondly on the intermediate host.
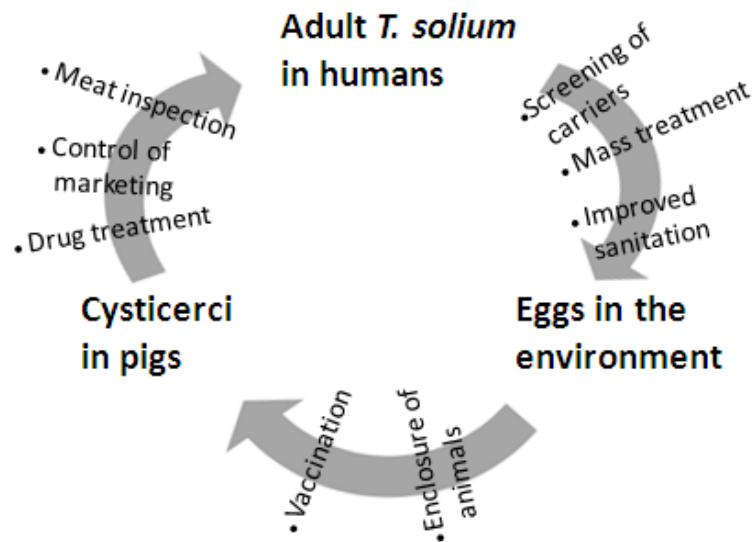
Figure 2.8: Potential intervention points for preventing transmission of *T. solium* (Adapted from Murrell, 2005)

The treatment or prevention of tapeworm infection in humans and the subsequent environmental contamination with taeniid eggs is of paramount importance in both prevention and control measures (Murrell, 2005). Identification and treatment of taenia carriers in communities is one of the strategies proposed for the control of cysticercosis (Flisser, 2006; Alexander *et al.*, 2011). This could be achieved by screening taeniosis by the less sensitive microscopy (Allan *et al.*, 1993; Allan *et al.*, 1996b) or the highly sensitive coproantigen ELISA (Allan *et al.*, 1996b; Rodriguez-Canul *et al.*, 1999; Sarti *et al.*, 1997, 2000), which however, is not readily available in endemic countries. The WHO (1983) recommended both the detection and treatment of tapeworm carriers or treatment of the whole population. In a cost implication analysis, Alexander *et al.* (2011) recommended that in endemic areas mass therapy or screening should be considered

and that incorporating mass therapy into existing mass drug distribution programmes was the most cost effective control strategy.

Meat inspection of pork before consumption prevents humans from acquiring taeniosis. However, the lack of safe slaughter houses, trained meat inspectors, low sensitivity of meat inspection for cysticercosis diagnosis, especially for lightly infected pigs, and the clandestine home slaughter of pigs, makes this strategy difficult in endemic areas (Murrell, 2005).

The use of closed clean sites for defaecating (latrines) and improved human hygiene are the most effective methods of preventing pigs from accessing human faeces and hence control the transmission of *T. solium* from pigs to humans (Sarti Gutierrez *et al.*, 1992). However, people in endemic areas need to be educated on the benefits of improved sanitation and personal hygiene. Health education may also play an important role in helping people to change eating habits (consumption of undercooked pork) and to improve the self-diagnosis of tapeworm infection. Though, health education has been reported not to change people's eating habits in 12 months, it results in a significant reduction in households consuming infected pork and incidence rates of porcine cysticercosis (Ngowi *et al.*, 2008). Despite the many advantages of free-range keeping of pigs in resource poor communities, the communities need to be educated on the public health implications of this form of pig management (Murrell, 2005).

The confinement of pigs by poor famers is impossible as not only is it costly to construct pens, it is also difficult for the farmers to provide a balanced diet to the pigs (Lekule & Kyvsgaard, 2003). Therefore, other options for controlling the infection in pigs should be employed. The potential of a vaccine for controlling porcine cysticercosis has been described in the past and some promising results were also reported (Lightowlers, 1999; Gonzalez *et al.*, 2005). 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 in developing countries and on the cost (Gonzalez *et al.*, 1999). One of the promising candidates is the TSOL18 vaccine. This vaccine has been reported to offer 100% protection against *T. solium* cysticercosis in pigs (Lightowlers, 2003; Flisser *et al.*, 2004; Assana *et al.*, 2010), however three vaccinations and a treatment are required. The use of two rounds of oxfendazole (30 mg/kg) has also been reported to reduce prevalence and incidence of infection in

pigs, especially when combined with human chemotherapy with praziquantel (Lightowlers *et al*., 2000; Garcia *et al.*, 2006; Lightowlers, 2010).

Though current efforts are centred on the control and potential eradication of taeniosis/cysticercosis, no control strategy has yet been proven effective and sustainable (Schantz *et al.*, 1993; Sarti *et al.*, 1997; Cruz *et al*., 1999). Pal *et al*. (1999) 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, and such changes were likely to be resisted because of the traditional and functional aspects of established pig-rearing practices (Sarti Gutierrez *et al.*, 1992). Therefore, an integrated approach has been proposed targeting multiple approaches recognizing 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 (Widdowson *et al.*, 2000; Lightowlers, 2003; Murrell, 2005; Flisser *et al*., 2006).

2.9   Justification for the study

As outlined in section 2.6.2 extensive studies have been conducted in pigs in Zambia and have reported high disease prevalences (Phiri *et al.*, 2002; Dorny *et al.*, 2004b; Sikasunge *et al.*, 2008b) as well as presence of risk factors important for the maintenance of the parasite in the intermediate host (Sikasunge *et al.*, 2007).

The Eastern province accounts for almost 50% of the total increase in the number of pigs recorded in the country in the past decade (Phiri *et al.*, 2003). These pigs are reared under the small-scale management system by free ranging (MAFF 1998; Phiri *et al*., 2002), making the risk of cysticercosis infection very high. Furthermore, cysticerci are commonly observed in pigs slaughtered in backyards. Studies are therefore required to study the infection in humans and it is for this reason that the area was chosen for the current study.

Gaps have been identified with regards to the epidemiology of human *T. solium* infections in Zambia due to the lack of studies that document the levels of infection in rural communities. Such epidemiological studies would rely on the currently available diagnostic tests of which the sensitivity is still low and their performance in field conditions requires assessment.

Prevalence rates of human taeniosis/cysticercosis are unknown as well as incidence rates of human cysticercosis. Determination of these important disease parameters would not only improve the understanding of the transmission dynamics of *T. solium* in an endemic area but also contribute to the assessment of the disease burden. Though risk factors for the infection in pigs have been described in endemic areas of Zambia, there is still need to study the disease determinants in humans and establish which are important to be targeted in the formulation of appropriate control measures. Epidemiological studies of cysticercosis currently involve the collection of serum for detection of specific antibodies and/or antigens. The use of urine, as a diagnostic specimen in such studies, which would be better accepted by communities, requires evaluation. Further, a comparison of available tests for the diagnosis of taeniosis is required to determine the performances of these tests in field conditions in light of the absence of a gold standard test.

2.10 Aim and objectives

The aim of this thesis is to obtain a better understanding of the epidemiology of the human *T. solium* taeniosis/cysticercosis disease complex in Zambia and to explore the gaps that exist in the tools used for their diagnosis.

The aim of the study will be achieved by addressing the following:

1. Determining the prevalence of human taeniosis/cysticercosis in endemic rural communities and differentiating the *Taenia* spp. present in the area.

2. Determining the incidence of human cysticercosis in an endemic community.

3. Studying the determinants associated with human taeniosis/cysticercosis and explore their importance.

4. Evaluating the use of urine samples for the immunological diagnosis of cysticercosis under field conditions.

5. Comparing the performance of three tests, namely coproscopic examination, coproantigen ELISA and copro-PCR in the diagnosis of taeniosis in faecal samples.

CHAPTER 3

Taenia solium infections in a rural area of eastern Zambia
A community based study

Adapted from:

3.1   Introduction

Taenia solium taeniosis/cysticercosis is a neglected parasitic zoonosis, affecting mostly developing countries (Carpio *et al.*, 1998). Adult intestinal tapeworm infection (taeniosis) in man, who is the sole natural definitive host (Allan & Craig, 2006), is acquired by eating undercooked infected pork. Pigs are the intermediate host and are infected by ingestion of infective eggs (or proglottids), which develop into cysticerci (porcine cysticercosis). Humans can also acquire cysticercosis with cysts often lodging in the central nervous system causing neurocysticercosis (NCC) which is a major cause of acquired epilepsy in endemic regions associated with considerable morbidity (Serpa *et al.*, 2006).

In the last decade, many studies have been carried out in sub-Saharan Africa to determine the presence of *T. solium*. Until now, most studies have been carried out on porcine cysticercosis reporting endemicity in countries like Tanzania, Zambia, Mozambique and Kenya (Ngowi *et al.*, 2004; Boa *et al.*, 2006; Sikasunge *et al.*, 2008b; Pondja *et al.,* 2009; Kagira *et al.* 2010). Prevalence of human cysticercosis, which has been less studied, ranges from 7.4% in South Africa to 20.5% in Mozambique (both based on specific antibody detection) and 21.6% in the Democratic Republic of Congo (based on circulating antigen detection) (Sacks & Berkowitz,

1990; Vilhena *et al.*, 1999; Afonso *et al.*, 2011; Kanobana *et al.*, 2011). In Kenya, reports indicate taeniosis prevalences ranging from 4 to 10% (Wohlgemut *et al.*, 2010). These data emphasize the need for more studies in humans to gather information on the epidemiology of the parasite and to estimate the burden on affected communities.

In Zambia, pig keeping and pork consumption have increased significantly during the past two decades with Eastern and Southern provinces accounting for a greater part of this increase (Phiri *et al.*, 2003). Pigs are mostly kept by smallholder producers under free-range management. Several studies carried out in Zambia have indicated high prevalences of porcine cysticercosis. A study at a slaughter slab in Lusaka receiving village pigs, indicated a prevalence of up to 64.2% (Dorny *et al.*, 2004b) while field studies in Southern and Eastern provinces estimated sero-prevalences between 10.8 - 20.8% and 9.3 - 23.3% respectively (Phiri *et al*., 2002; Sikasunge *et al.*, 2008a). These studies clearly show that *T. solium* is present in rural areas of Zambia. However, except for preliminary unpublished data, no information was available on human cysticercosis and taeniosis.

The main objective of this study was to determine the prevalence of human taeniosis and cysticercosis in a rural community of Petauke district in the Eastern province of Zambia.

3.2   Materials and methods

3.2.1   Study area and population

The study was conducted in a rural community (Kakwiya) in Petauke district of the Eastern province of Zambia (Figure 3.1). Health care is provided by a Rural Health Centre (RHC) whose catchment population is 11,344 (Clinic headcount records). The people in this community practice subsistence farming, growing mostly crops like maize and groundnuts primarily for home consumption and cotton and bananas grown for household income generation. They also keep animals, mostly pigs with a few keeping cattle, goats and chickens. A preliminary visit to the area indicated that, as reported by Sikasunge *et al*. (2007), there was a high number of free roaming pigs and reports of cysts observed in pigs slaughtered in backyards.
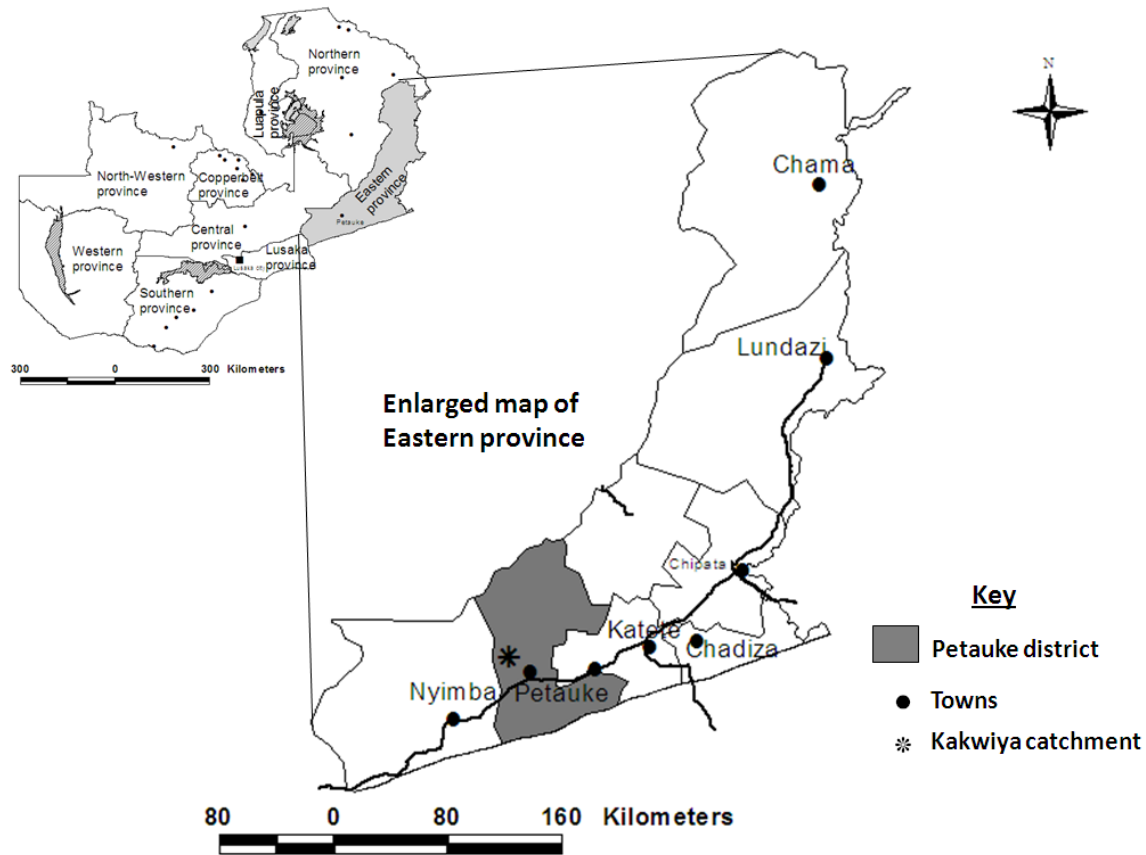
Figure 3.1: Map of Zambia indicating the study area (Kakwiya) in Petauke district of the Eastern province

3.2.2   Study design

A community-based cross sectional survey was conducted in the dry season between July and August 2009. The Kakwiya community was selected because it is a pig keeping community without any active ongoing sanitation programmes and cysticerci were observed in slaughtered pigs. The willingness of the community to participate in the study and the RHC to collaborate was also taken into account together with the availability of an adequate working space in the clinic and staff for the collection of samples. Only villages (n = 20) within a radius of 7km from Kakwiya RHC were selected. The selected villages were visited and all persons invited to participate in the study. Each participant was, after written informed consent, given two plastic sample bottles and requested to submit a stool and a urine sample at the RHC. Upon submission of these samples, a blood sample was also taken by qualified health personnel. A questionnaire,

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adapted from the questionnaire developed by the Cysticercosis Working Group in Eastern and Southern Africa (www.cwgesa.dk) to target risk factors associated with human infection, was formulated, translated into the local language, back translated and pre-tested in a neighboring district before administration in the study communities. A HH head was the targeted respondent and where not available, any adult member (above 18 years of age) of the HH was interviewed. The questionnaire targeted household characteristics (such as number of inhabitants in a household, main household income, highest level of education attained, main source of drinking water), presence of household level risk factors (such as keeping pigs and how they are kept, backyard pig slaughter, inspection of slaughtered pigs, consumption of pork by at least one member of the household, presence of a pit latrine, consumption or resell of pork with visible cysticerci) and knowledge of the parasite (such as observation of tapeworm in human faeces, how people acquired a tapeworm, observation of cysts in pork meat, what the cysts were and how pigs acquired them) was administered to each participating household. At the same time geographic coordinates of each participating household were obtained using a Global Positioning System (GPS) receiver (eTrex Legend® Cx, Garmin).

3.2.3   Sample collection and storage

Stool samples were divided into two aliquots; one placed in 10% formalin and the other in 70% ethanol and both aliquotes stored at 4°C until use. Urine samples were aliquoted in 1.8 ml vials and stored at -20°C. Analyses and results for the urine specimens are discussed in Chapter 7. About 5 ml of blood were collected into sterile plain blood collecting tubes and allowed to clot. To obtain the maximum amount of serum, the blood tubes were allowed to stand at 4°C overnight and then centrifuged at 3000g for 15 minutes. The supernatant (serum) was aliquoted into 1.8 ml vials and stored at -20°C until use. All the samples were transported to Lusaka where they were stored at -20°C until analysis.

3.2.4   Coproscopic examination

Presence of helminth ova in stool was examined microscopically using the formalin-ether concentration technique (Ritchie, 1948). Presence of a taeniid egg on a slide was recorded as being positive for taeniosis. The presence of other helminth eggs was also registered during the examination.

3.2.5   Copro-antigen enzyme-linked-immunosorbent assay (Copro-Ag ELISA)

An in-house copro-antigen detection ELISA (copro-Ag ELISA), as described by Allan *et al.* (1990) with slight modifications, was performed on stool samples. Briefly, the stool samples stored in 10% formalin were processed by mixing an equal amount of Phosphate Buffered Saline (PBS) and stool sample. This was allowed to soak for one hour with intermediate shaking and centrifuged at 2000g for 30 minutes. The supernatant was then used in the ELISA. The assay involved coating 96 well polystyrene plates (Nunc® Maxisorp) with the capturing hyper immune rabbit anti-*Taenia* IgG polyclonal antibody (from rabbits hyper immunized with adult worm somatic products) diluted at 2.5 µg/ml in carbonate-bicarbonate buffer (0.06 M, pH 9.6). After coating, the plates were incubated for 1 hour at 37°C, washed once with PBS in 0.05% Tween 20 (PBS-T20) and all wells blocked with PBS-T20+ 2% BCS. After incubating at 37°C for 1 hour and without washing, 100 µl of the stool supernatant was added and plates were incubated for 1 hour at 37°C followed by washing five times with PBS-T20. A biotinylated hyper immune rabbit IgG polyclonal antibody diluted at 2.5 µg/ml in blocking buffer was used as the detector antibody. One hundred microlitre was added and the plates were incubated for 1 hour at 37°C followed by washing 5 times. One hundred microlitres of Streptavidin-horseradish peroxidase (Jackson Immunoresearch Lab, Inc.) diluted at 1/10,000 in blocking buffer was added as conjugate. After 1 hour incubation at 37°C and washing 5 times, 100 µl of ortho phenylenediamine (OPD) substrate, prepared by dissolving one tablet in 6 ml of distilled water and adding 2.5 µl of hydrogen peroxide, was added. The plates were incubated in the dark for 15 minutes at room temperature before stopping the reaction by adding 50 µl of sulphuric acid (4N) to each well. The plates were read using an automated spectrophotometer at 490nm. To determine the test result, the optical density (OD) of each stool sample was compared with the mean of a series of 8 reference *Taenia* negative stool samples plus 3 standard deviations (cutoff).

3.2.6   Serum antigen enzyme-linked-immunosorbent assay (sero-Ag ELISA)

The presence of circulating cysticercus antigens in serum was measured by the monoclonal antibody based B158/B60 Ag-ELISA (sero-Ag ELISA) (Brandt *et al.*, 1992; Dorny *et al.*, 2000). Sera from two known highly positive pigs (obtained from a local pig market and confirmed by dissection) were used as positive controls. The OD of each serum sample was compared with a

sample of negative serum samples (N = 8) at a probability level of $P = 0.001$ to determine the result in the test (Dorny *et al.*, 2004a).

3.2.7 Differentiation of *Taenia* species.

Differentiation of the *Taenia* species. was done using molecular methods. Taeniosis positive individuals were treated with niclosamide (2g single dose) followed by a purgative (Magnesium sulphate, 30g). The collected tapeworm segments were stored in 70% ethanol until use. DNA was extracted from the parasitic material using the Boom extraction method slightly modified as described by Rodriguez-Hidalgo *et al*. (2002) and PCR used to amplify the mitochondrial 12s rDNA gene fragment. Restriction fragment length polymorphism (RFLP) was then used to differentiate the *Taenia* species. (Rodriguez-Hidalgo *et al.*, 2002).

3.2.8 Statistical analysis

All collected data was entered into an Excel (Microsoft Office Excel 2007®) spreadsheet and analyses were conducted in Stata 10 (http://www.stata.com). Chi square test was used to check for differences between disease positivity and gender. Uni- and multivariate logistic regressions were used to investigate the relationship between taeniosis and cysticercosis positivity and individual gender and age. The age variable was first used as a continuous variable and then categorized into categories of 10 years each, in order to identify changes in positivity frequencies as a function of the age of individuals. A change point analysis was used to simplify the observed relations into antigen patterns as a function of age (Speybroeck *et al.*, 2006; Praet *et al.*, 2010c). The level of significance was set at $P < 0.05$ for all statistical analyses.

The geo-reference data collected was used for spatial analysis using ArcView GIS 3.2 (http://www.esri.com). Analysis of the possibility of geographical clustering of households with latrines or those that kept pigs and also cases of taeniosis and cysticercosis was done by means of the risk-adjusted nearest neighbour hierarchical spatial clustering (Rnnh) using Crimestat® III (Levine, 2010). Given the limited number of individuals infected with taeniosis and cysticercosis, the minimum number of cases per cluster was set at 3 while the minimum number of households with a latrine or that kept pigs was set at 20. Monte Carlo simulations were run in this software to determine the significance of the clusters. Significance level of a cluster in the

simulation was set at 95% and a cluster was determined significant if the density of the points was higher than that obtained at the 95th percentile after 1000 simulations.

3.3 Results

3.3.1 Study population

A total of 720 individuals from 20 villages belonging to 255 households participated in the study. Of these, 428 (59.4%) were female and 292 (40.6%) were male and the age ranged from 1 to 96 years with a median age of 12 years. The age distribution, with a majority of the younger age group, was typical of rural areas in developing countries (Garcia *et al.*, 2003a). The number of people living in a household ranged from 1 to 13 with a median of 7. Stool samples were obtained from 718 people and 708 gave a blood sample. At least one participant from each participating household gave a sample depending on the willingness of the household members. The number of individuals sampled from each household ranged from 1 to 11. Some household characteristics recorded from the questionnaire administered to the 255 households included; 32.6% kept pigs with 99.6% of these rearing on free-range, 47.8% of the households did not have latrines (Figure 3.2) and 94.5% of the households had at least one individual who consumed pork. Three clusters each of households with latrines (14.13881S, 31.19501E, density = 748.97; 14.14338S, 31.20369E, density = 151.95 and 14.09718S, 31.17940E, density = 117.15; 95th percentile density = 0.02) and those that kept pigs (14.13891S, 31.19493N, density = 299.89; 14.14390S, 31.20374E, density = 134.33 and 14.09773S, 31.17961E, density = 86.79; 95th percentile density = 0.01) were identified in the study area (Figure 3.2). About 44% of the households reported to have slaughtered a pig in their backyards. None of them had the meat inspected before either home consumption or resell to members of the community. Pork was reported to be consumed in a variety of ways including boiling, frying and roasting. The data obtained in the questionnaire on risk factors is described in more detail in Chapter 5.
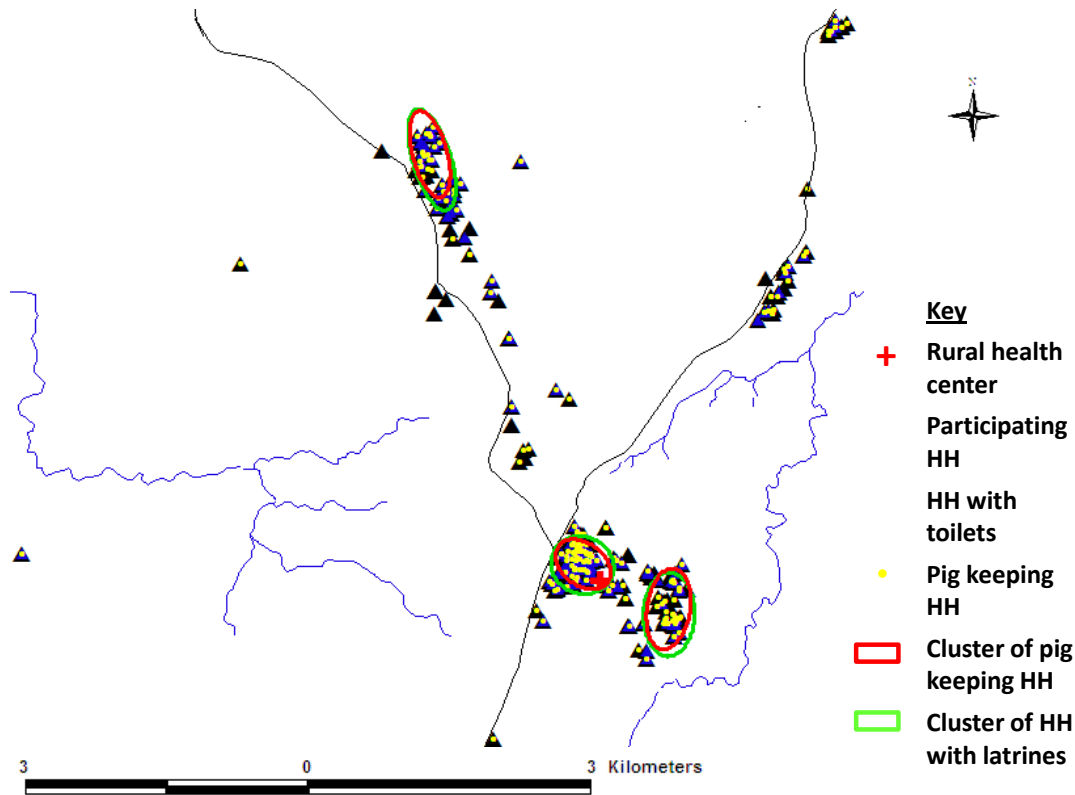
Figure 3.2: Spatial pattern of the participating households. Map of the study community showing participating households (HH), HH with pit latrines and pig keeping HH

3.3.2 Prevalence of taeniosis

The results for both the coproscopic examination and copro-Ag ELISA, are shown in Table 3.1. Two (0.3%) individuals were positive for taeniosis on coproscopic examination while copro-Ag ELISA detected 45 (6.3%) positives. The two coproscopic positives were also positive on copro-Ag ELISA. Figure 3.3 shows the copro-Ag ELISA results in function of 10 age groups of 10 years each. The highest prevalence was determined in the 80-89 years age group, though this was not significantly different from the other age groups. A univariate logistic regression analysis did not indicate any relationship between copro-Ag ELISA positivity and sex ($p = 0.548$) or age ($p = 0.311$). This finding was the same for the multivariate analysis with p values of 0.139 and 0.645 for sex and age respectively. One cluster (14.13868S, 31.19509E, density 116.55; 95th percentile density = 49.33) of taeniosis cases was identified in the study community (Figure 3.4). At household level, the number of positives per household ranged from 0 to 3. All taeniosis positive

individuals were treated with 2g niclosamide *per os* and given a purgative (Magnesium sulphate) two hours later. One tapeworm was collected and confirmed to be *T. solium* by PCR-RFLP.

Table 3.1: Results of the coproscopic examination and copro-Ag ELISA of the stool samples from Kakwiya RHC together with their 95% confidence intervals

Diagnostic test	Number of positive individuals	Prevalence % (95% CI)
Coproscopic examination	2/718	0.3 (-0.1-0.7)
Copro-Ag ELISA	45/712*	6.3 (4.5-8.1)

* No sample was left in 6 containers after coproscopic examination for copro-Ag ELISA as very little material was provided by the participants.

Age group (Years)	0-9	10-19	20-29	30-39	40-49	50-59	60-69	70-79	80-89	90-99
Proportion	0.05	0.07	0.10	0.04	0.10	0.07	0.06	0.00	0.17	0.00
n	258	215	62	49	42	28	33	18	6	1
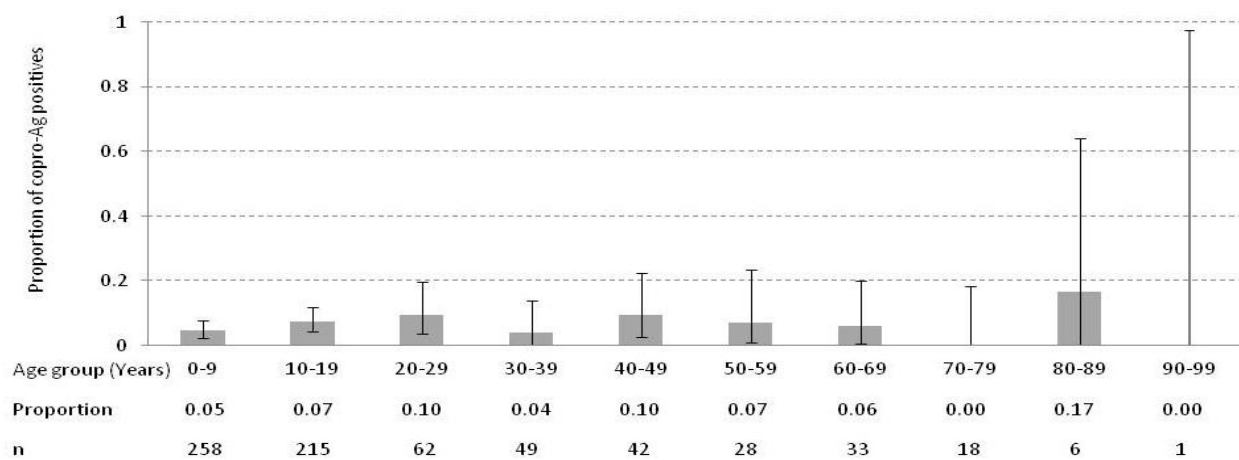
Figure 3.3: Taeniosis prevalence per age group. Results of the copro-Ag ELISA in a function of the 10 age groups of 10 years each. The upper and lower 95% exact binomial confidence intervals for the prevalence in each age group are represented through error bars. The proportion of positives and number of individuals sampled in each age group are also shown

3.3.3 Prevalence of cysticercosis

The results for the sero-Ag ELISA are shown in Figure 3.5 in function of 10 age groups of 10 years each. Circulating cysticercus antigens were detected in 41 (n = 708) participants giving an apparent prevalence of 5.8% (95% CI, 4.1 – 7.5). Uni- and multivariate logistic regression analysis revealed a very strong relationship between sero-Ag positivity and age ($P < 0.001$).

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Figure 3.6 indicates that the prevalence of cysticercosis is initially low and a change point analysis indicated a significant increase in positivity frequencies at 30 years of age. The logistic regression model indicated that the proportion of sero-Ag ELISA positive individuals remains at a constant level until the age of 30, and from this age onwards a significantly higher level is observed ($P < 0.001$).

A relationship was observed between copro-Ag positivity and sero-Ag positivity ($P = 0.03$) indicating that a copro-Ag positive individual was at an almost three-fold higher risk of being sero-Ag positive than the one who was not (OR = 2.9, $P = 0.029$).

There was no statistically significant difference in prevalence between males and females ($\chi^2 = 0.034$, $p = 0.854$). Two clusters (14.14048S, 31.19692E, density 31.31; 14.08460S, 31.22085E, density 195.17; 95th percentile density = 23.16) of cysticercosis cases were identified in the study community with the larger cluster spatially related to the taeniosis cluster (Figure 3.4).
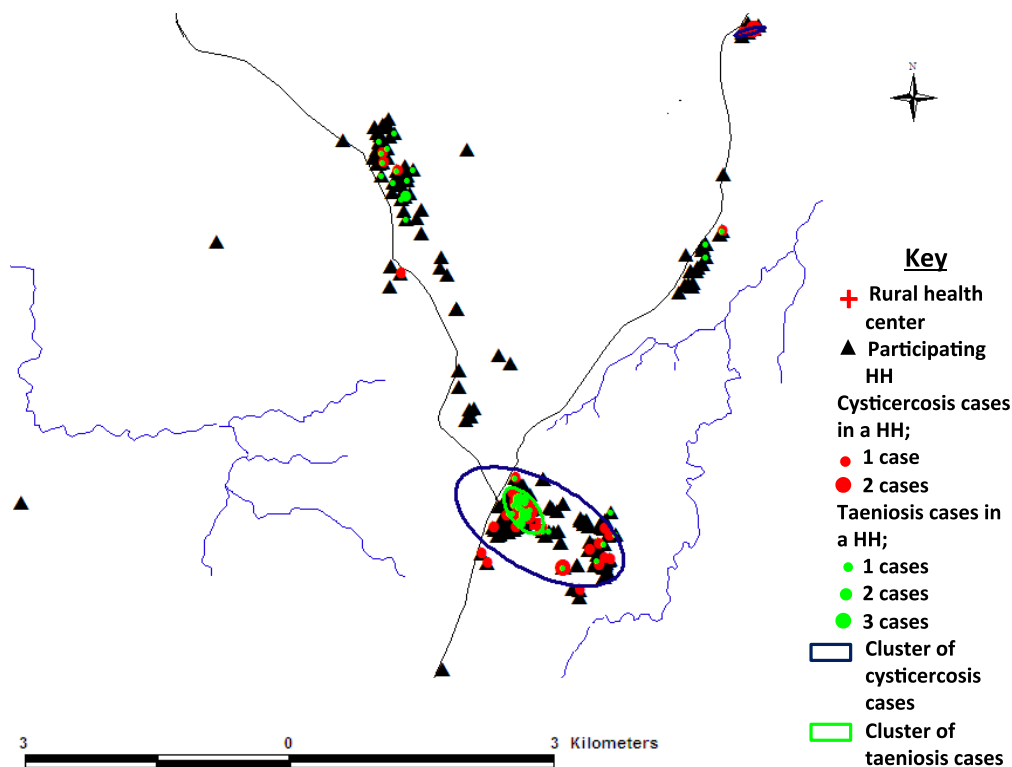
Figure 3.4: Spatial pattern of diagnosed taeniosis and cysticercosis cases. Map of the study community showing cysticercosis and taeniosis cases by sero-Ag ELISA and copro-Ag ELISA at household (HH) level; respectively

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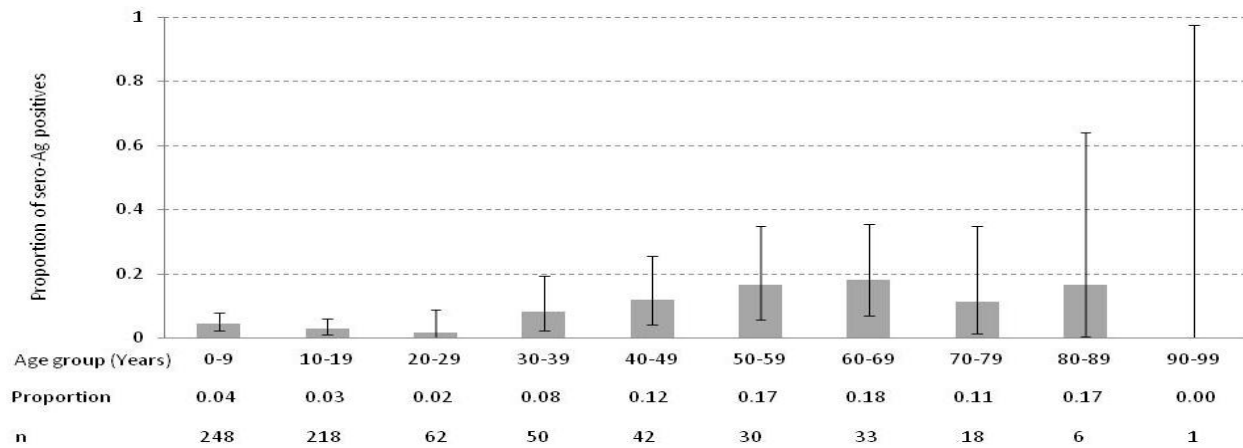
Figure 3.5: Cysticercosis prevalence per age group. Results of the sero-Ag ELISA in a function of the 10 age groups of 10 years each. The upper and lower 95% exact binomial confidence intervals for the proportion in each age group are represented through error bars. The proportion of positives and number of individuals sampled in each age group are also shown
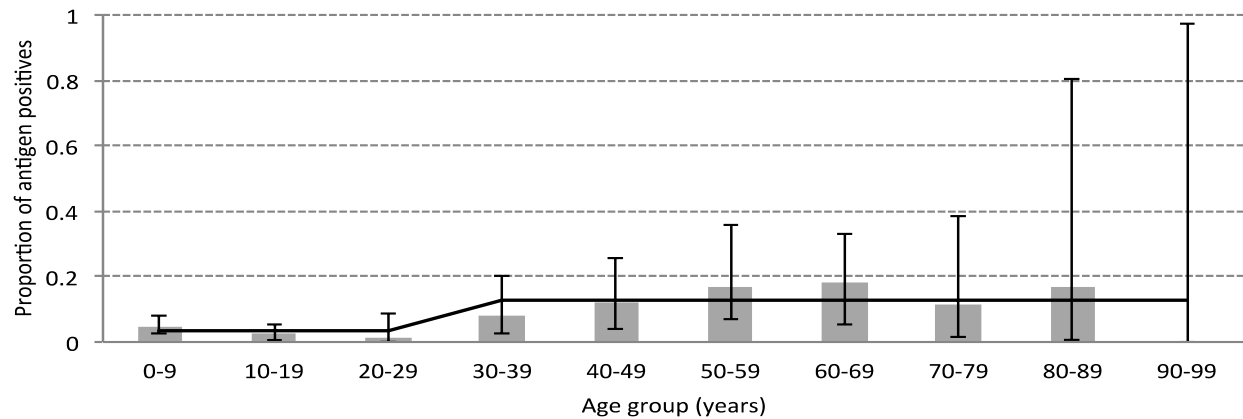
Figure 3.6: Change point analysis. Results of the sero-Ag ELISA (grey bars) in a function of 10 age groups of 10 years each, including the logistic regression predictions (black line). The upper and lower 95% exact binomial confidence intervals are shown with error bars

3.3.4 Prevalence of other parasites

Other intestinal parasites detected on coproscopic examination included hookworms, *Schistosoma mansoni*, *Trichuris trichiuria* and *Ascaris lumbricoides*. Table 3.2 shows the prevalence rates for these parasites with their respective 95% confidence intervals.

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Table 3.2: Prevalence rates of other parasites diagnosed on coproscopic examination of the stool samples and their 95% confidence intervals (n = 718)

Parasite	Number positive	Prevalence % (95% CI)
Hookworms	107	14.9 (12.3-17.5)
Trichuris trichiuria	69	9.6 (7.4-11.8)
Schistosoma mansoni	16	2.2 (1.1-3-3)
Ascaris lumbricoides	6	0.8 (0.2-1.5)

3.4   Discussion

The objective of this study was to determine the prevalence of taeniosis and cysticercosis in a rural community in the Eastern Province of Zambia, where risk factors for the transmission of *T. solium* are present.

3.4.1   Taeniosis

T. solium taeniosis tends to have a low prevalence, typically less than 1%, even in endemic communities (Allan *et al.*, 1996a), a higher prevalence is considered hyper-endemic (Cruz *et al.*, 1989). In this study a prevalence of 6.3%, based on copro-Ag ELISA, was determined, indicating a hyper-endemicity in the study community. As in a number of other studies, no significant association between age/sex and taeniosis positivity could be determined (Garcia *et al.*, 2003a; Rodriquez-Canul, 1999; Sanchez *et al.*, 1999).

Even though similar high taeniosis prevalences have been recorded in Kenya (4 -10%) (Wohlgemut *et al.*, 2010), the 6.3% prevalence determined in this study should be looked at critically. The sensitivity and specificity of the copro-Ag ELISA are estimated at 96 - 98% and 98 - 100%; respectively (Allan *et al.*, 2003; Allan & Craig, 2006). However, the possibility of false positive test results due to cross-reactions with other pathogens present in the community should be considered. The assay has been reported not to cross-react with other parasite species including *A. lumbricoides*, *T. trichiuria*, *Hymenolepis nana*, *H. diminuta* or parasitic protozoa (Allan *et al.*, 2003). Also in our laboratory, stool samples with known *H. nana*, *Schistosoma* species, *T. trichiuria* and *A. lumbricoides* infections were analyzed, and all results remained

under the cut off level (Unpublished data). As the assay is not species specific (Allan *et al.*, 1990), the possibility of the high taeniosis prevalence to be partially due to *T. saginata* infections cannot be ruled out. However, bovine cysticercosis in Zambia has so far only been reported in the Central and Southern provinces (Dorny *et al.*, 2002) and Western province (I.K. Phiri, personal communication) and no studies on *T. saginata* have been conducted in the Eastern province of Zambia.

Interviews with local people in the study area revealed that pig slaughter and pork consumption increases in the dry season as it is time for harvest and residents have then the means to buy either an entire pig or pieces of pork for home consumption. During this period, pig owners not only slaughter more pigs for the market but also for their own home consumption. Higher pork consumption could have led to new (still immature) taeniosis infections at the time of sampling, which will be detected by copro-Ag ELISA but not yet by coproscopy (Allan & Craig, 2006).

Only one tapeworm (from a participant positive on both copro-Ag and coproscopic examination) could be recovered after treatment of the 45 copro-Ag positive participants. The low recovery rate of tapeworms can be explained by: (1) stools were obtained only over one day and not over 3 days post treatment (Jeri *et al.*, 2004) due to logistical constraints, (2) usually after antiparasitic treatment, small and unrecognizable fragments are expelled by most patients (Jeri *et al.*, 2004) and these are easily missed, and (3) treatment of copro-Ag positive individuals was conducted over six months after collection of stool samples; natural expulsion may have occurred in this period, especially if it is during harvest when pumpkin seeds are eaten and they promote tapeworm release.

3.4.2 Cysticercosis

The sero-Ag ELISA detected an apparent cysticercosis prevalence of 5.8% indicating the presence of viable cysts and, as such, active infections in these individuals. The prevalence of cysticercosis recorded in our study is comparable with that recorded in other endemic areas, based on Ag-ELISA, such as in the Andean region of Ecuador and in north Vietnam (Rodriguez-Hidalgo *et al.*, 2003; Somers *et al.*, 2006), higher than in west Cameroon (0.4 to 4.0%) and southern Ecuador (2.3%) (Nguekam *et al.*, 2003b; Rodriguez-Hidalgo *et al.*, 2006) but lower than that reported in the Democratic Republic of Congo (21.6%) (Kanobana *et al.*, 2011). Other

studies that have recorded higher seroprevalences include those that used antibody detection techniques such as in Mozambique (12.1%), South Africa (7.4%) and Peru (13.9%) (Sacks & Berkowitz, 1990; Vilhena *et al.*, 1999; Garcia *et al.*, 2003a). However, antibody detection indicates exposure to the parasite and not necessarily established infection and hence is likely to detect more positives than the antigen detecting assay used in the current study (Garcia *et al.*, 2001).

Change point analysis of the association of antigen seropositivity and age revealed that the number of individuals in which circulating antigens were detected was significantly higher in people older than 30 years, indicating that viable cysts were more frequently present in individuals above this age. Studies have shown that a higher proportion of vesicular stage cysticercii is found in older (60 years and above) NCC patients (Fleury *et al.*, 2004; Cavellani *et al.*, 2007) and this has been attributed to immunosenescence since a weaker immunity in the elderly would facilitate the establishment and maintenance of viable cysticerci unlike in fully immunocompetent younger individuals (Albright & Albright, 1994). The significant increase in sero-antigen positive individuals in the elderly was also observed in Ecuador where the number of positive individuals was higher in people order than 60 years (Praet *et al.*, 2010c). However, in our study we see an increase already in young adults (from 30 years onwards) who are probably immunocompetent.

Establishment and development of infection is influenced by a range of complex factors; among which are parasite factors (e.g. parasite virulence/pathogenicity influenced by genetic differences, number, stage, location), host factors (e.g. age, gender, genetics influencing the immunological responses of the host when exposed to infection) and environmental factors (e.g. presence of risk factors, level of exposure, presence of other infections) (Fleury *et al.*, 2010). It is very difficult to pinpoint exactly those factors present in the study area/population/age groups; that can explain this early increase in establishment of viable infection.

The high taeniosis prevalence recorded in the studied community entails a possible very high exposure risk to infective eggs. In a study in India, higher infection rates (as indicated by sero Ag detection) were noted in areas with higher taeniosis prevalences (Jayaraman *et al.*, 2011). A study in an Orthodox Jewish Community in New York City also demonstrated that a high egg

burden due to a tapeworm carrier in a home leads to NCC cases (Shantz *et al*., 1992). Also in our study a significant positive relationship between copro-Ag positivity (presence of tapeworm) and sero-Ag positivity (cysticercosis) was established (logistic regression and cluster analysis). Level of exposure/infection with which the host is confronted can have an important effect on the immunological response of the host (Fleury *et al.*, 2004), and as such on the establishment of viable infection.

The general factors that lower immunity in groups of individuals in the population could be at play making the people in our study community more susceptible to infection. According to the United Nations Human Development Indices of 2008, about 64% of Zambia's population lived on less than $1 per day as compared to only 20% for Ecuador (UNDP, 2007). Poverty is an indication for poor nutritional status, which has an impact on the immune system (Field *et al.*, 2002). Also the presence of other diseases such as HIV-AIDS, malaria and tuberculosis, other helminthic infections and physical environmental conditions (Horne, 1997) can influence greatly the host's reaction to other infections. In 2008, Zambia's HIV prevalence stood at 14.3% with the age group between 20 and 40 years being the most affected (Kandala *et al.*, 2008). The country is also endemic for malaria (MoH, 2006) and helminthic infections are widely reported in rural areas, as reported in this study.

Genetic polymorphism of the parasite is another important determining factor for the establishment and development of infection (Martinez-Hernandez *et al*., 2009). Nakao *et al.* (2002) have described a cluster of isolates from Asia, and another cluster from isolates from Latin America and Africa. However, genetic differences within a cluster (within a continent/country/region) need to be evaluated as well. Several Zambian isolates are currently being examined, and preliminary results indicate a high genetic variability (Unpublished results, K. Kanobana), which might explain differences in development of infection between regions.

We have, in this study, shown that *T. solium* taeniosis and cysticercosis are present in the study community. Many issues remain unclear and obviously more work is required to understand the many factors that contribute to the transmission dynamics of the parasite and disease development in endemic rural areas. Also the economic impact and burden of disease in rural pig keeping communities of Zambia needs to be determined.

CHAPTER 4

The incidence of human cysticercosis in a rural community in Katete district of eastern Zambia

4.1 Introduction

Human neurocysticercosis (NCC), an infection caused by the metacestode larval stage of the pork tapeworm *Taenia solium*, is a serious but neglected zoonotic disease and constitutes a major public health problem in many developing countries of Latin America, Asia and Africa (Del Brutto *et al.*, 2001; Parija *et al.*, 2007). The disease is associated with poverty, absence of latrines and hence free access to human stool by scavenging pigs (Sarti *et al.*, 1997; Sikasunge *et al.*, 2007). Humans are the definitive hosts harboring the adult tapeworm (taeniosis) while pigs are the intermediate hosts harboring the metacestode larval stage (porcine cysticercosis). Carriers of the tapeworm shed eggs into the environment that are infective not only to pigs but also to humans who then also act as an accidental host (Murrell, 2005) leading to human cysticercosis. When the larval stages invade the nervous system they cause NCC, which is the most important parasitic disease affecting the nervous system. NCC can be at the origin of various neurological problems, among which is acquired epilepsy (Garcia & Del Brutto, 2005) and accounts for about 30% of all epilepsy cases in endemic areas (Ndimubanzi *et al.*, 2010). NCC not only causes neurological morbidity but also imposes economic hardships on the already impoverished communities. In terms of DALYs, the global burden of epilepsy is estimated at 7.8 million DALYs with 6.5 million of these occurring in *T. solium* endemic regions of the world (Torgerson & Macpherson, 2011).

The few prevalence studies carried out in Africa have indicated sero-prevalences of human cysticercosis ranging from 7 – 22% (Kanobana *et al.*, 2011; Secka *et al*., 2011; Carabin *et al.*, 2009; Sacks & Berkowitz, 1990; Vilhena *et al.*, 1999). Studies that report incidence of human cysticercosis are even more scanty and absent for sub-Saharan Africa. Studies in the United States of America (USA), which is a non-endemic area, reported estimated incidences of NCC of 0.5 per 100,000 person years (O'Neal *et al*., 2011) and 1.5 per 100,000 person years (Townes *et*

al., 2004), with increased incidences of 5.8 (O'Neal *et al*., 2011) and between 8 and 10 per 100,000 among Hispanic people (Townes *et al.*, 2004). To our knowledge only few reports have been published on incidence studies of human cysticercosis in endemic areas. Two longitudinal studies in villages in Peru indicated human cysticercosis incidence rates of 25% and 8% by specific antibody analysis (Garcia *et al.*, 2001). It was suggested that the dynamic nature of the infection resulted in many newly exposed people, who actually didn't develop an established infection, as such mounting only a transient antibody response. In a simulation model, described by Praet *et al.* (2010c), an annual incidence rate of 14% was described in a study in Ecuador.

Obviously, there is a need to gather more information on the transmission dynamics of this parasite. The present study aimed at determining the incidence of human cysticercosis in an area endemic for porcine cysticercosis and with risk factors associated with the maintenance of the pork tapeworm.

4.2   Materials and methods

4.2.1   Study area and population

The study was carried out in the Vulamkoko community in Katete district of the Eastern province of Zambia (Figure 4.1). The Vulamkoko Rural Health Center (RHC) provides health care in this community with a catchment population of 23,613 (clinic headcount records). The climate is tropical with two main seasons, the rainy season (November to April) and the dry season (May to October/November). The mean rainfall varies from 500 to 1200mm/year with temperatures above 20°C most of the year. The most common ethnic group in Katete are the Chewa people who practice subsistence agriculture as described for the community in Petauke district in section 3.2 of Chapter 3. Pigs are commonly bought and sold among villagers and also to intermediary traders that often go to the villages to purchase the pigs for the market in the nearby towns. People's homes in this area are of adobe, usually have no sanitary facilities with hand pumps being the source of water for most villages. Since sanitation is poor, pigs have access to human faeces in the nearby bushes that are used as latrines by the villagers. The selection criteria for the community were, as for the Petauke area, described in Section 3.2.1 of Chapter 3.
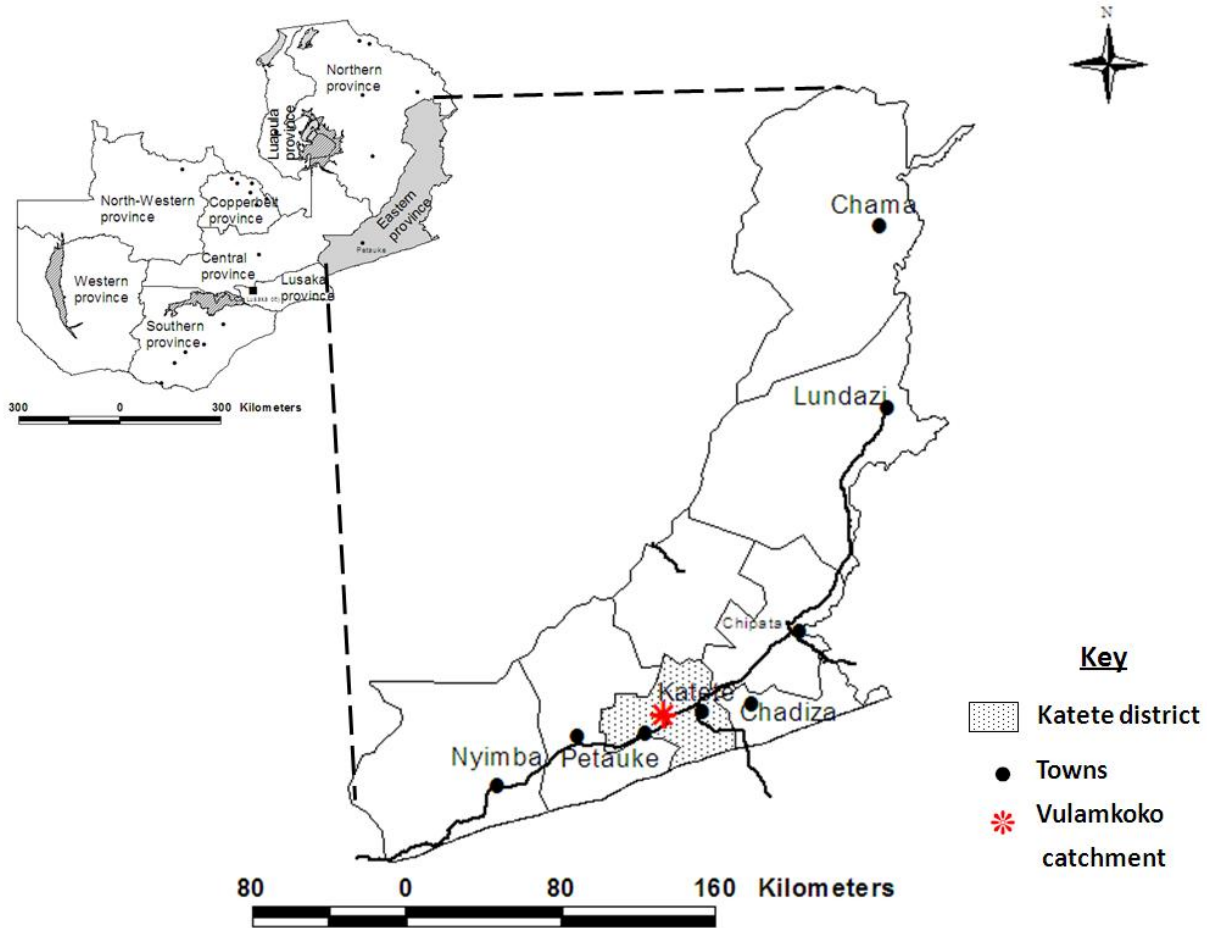
Figure 4.1: Map of Zambia showing the study area (Vulamkoko catchment) in Katete district of the Eastern province

4.2.2   Study design

A community-based longitudinal study was carried out between October 2009 and October 2010, with three main sampling rounds (R1, R2, R3) with six months intervals (Figure 4.2). Intermediate short trips were performed shortly after the main sampling rounds to include participants that were not present in the village at the time of the main sampling round. Participants who were not sampled in the first round of sampling and willing to participate were entered in the study only during the second round of sampling.

Meetings were held in the selected villages and individuals of all ages from all households invited to participate in the study. The sampling unit was an individual in a household and the

sampling was based on an individual's willingness to participate in the study. Each participant, after written informed consent, was registered and had a blood sample taken by qualified health personnel every six months during a 12-month period (a total of 3 samples). During the last sampling round, a stool sample was also requested from the participants.

A questionnaire was administered to each participating household and the same information as described in section 3.2.2 of Chapter 3 obtained. At the same time geographic co-ordinates of each participating household were obtained using a Global Positioning System (GPS) receiver (eTrex Legend® Cx, Garmin).

4.2.3 Sample collection and analyses

The collection, aliquoting, storage and analyses of serum and stool samples were as described in section 3.2.3 of chapter 3.

The serum samples were also tested for circulating specific antibodies against cysticercosis. However, due to budgetary restrictions, not all samples could be analyzed for specific antibodies. Therefore, from the individuals that gave samples for all the sampling rounds, a Stata® (Stata Corp., College Station, TX) generated random subset sample, taking into account the age and sex distribution, was tested for specific antibodies against cysticercosis using a commercial kit, Immunetics® (Immunetics Inc.). The assay was performed according to the manufacturer's instructions. In brief, the assay is an enzyme linked immunoelectrotransfer blot (EITB), which uses seven purified *T. solium* antigens (diagnostic bands being Gp-50, Gp-39 to -42, Gp-24, Gp-21, Gp-18, Gp-14 and Gp-13, where Gp stands for glycoprotein and the number is the molecular weight of each antigen expressed in kilodaltons). Reactions to any one or more of the bands are considered positive.

4.2.4 Statistical analysis

All collected data were entered into an excel (Microsoft Office Excel 2007®) spreadsheet and analyses were conducted in Stata 10 (Stata Corp., College Station, TX). Individuals were defined as at risk if they were seronegative at the beginning of a period. An incidence case was defined as an individual whose serology changed from negative to positive between two sampling periods. Incidence was determined for both antigen and antibody results for three periods namely

the first 6 months (Period 1, P1), the second 6 months (Period 2, P2) and over 12 months, that is between R1 and R3 (Period 3, P3). Only individuals who were sampled during all three sampling rounds were included in the incidence analysis. For every period, antigen and antibody seroconversion and seroreversion rates were estimated, analyses were also carried out for seroconversion and –reversion rates for the different age categories of 10 years intervals as well as for sex. Cysticercosis prevalence was calculated for each sampling round, the taeniosis prevalence for the final sampling round, and these were determined for all age categories and for sex. Logistic regression analysis was used to examine factors potentially associated with the infection/ exposure (including age and sex). Prevalence differences per sampling round, age category and by sex were estimated using the Chi square test and crude odd ratios to check for risk levels. The significant level was set at 0.05.

4.3 Results

4.3.1 Sampling

A total of 3167 serum samples (from 1206 individuals from 32 villages) and 226 stool samples were examined for cysticercal antigens (sero-Ag ELISA) and taeniosis (by coproscopy and copro-Ag ELISA), respectively.

A total of 1129 individuals were sampled at baseline, R1, and 77 joined the study at R2. Of the 1129 sampled at R1, 992 (87.8%) and 867 (76.8%) were re-sampled during R2 and R3, respectively. Fifty individuals from the R1 group were not sampled at R2 but re-appeared at R3 (Figure 4.2). Fifty-two (67.5%) of the 77 that entered the study at R2 gave a second sample during R3. A total of 867 (76.8%) gave samples during all sampling rounds. Reasons for lack of follow up at R2 consisted of refusal to continue participating (2.7%), away at time of sampling (3.8), reported sick and could not be sampled (1.2%), died of other causes, as assessed by the RHC, during the time interval between samplings (0.3%), relocated to other areas (1.2%) and those that could not be traced (2.8%). For those that gave a sample at R2, reasons for failure to give at R3 were as follows; refusal to continue participating (5.3%), away at time of sampling (6.6), reported sick and could not be sampled (1.1%), died during the time interval between samplings of other causes, as assessed by the RHC (0.7%), relocated to other areas (2.2%) and those that could not be traced (3.1%).
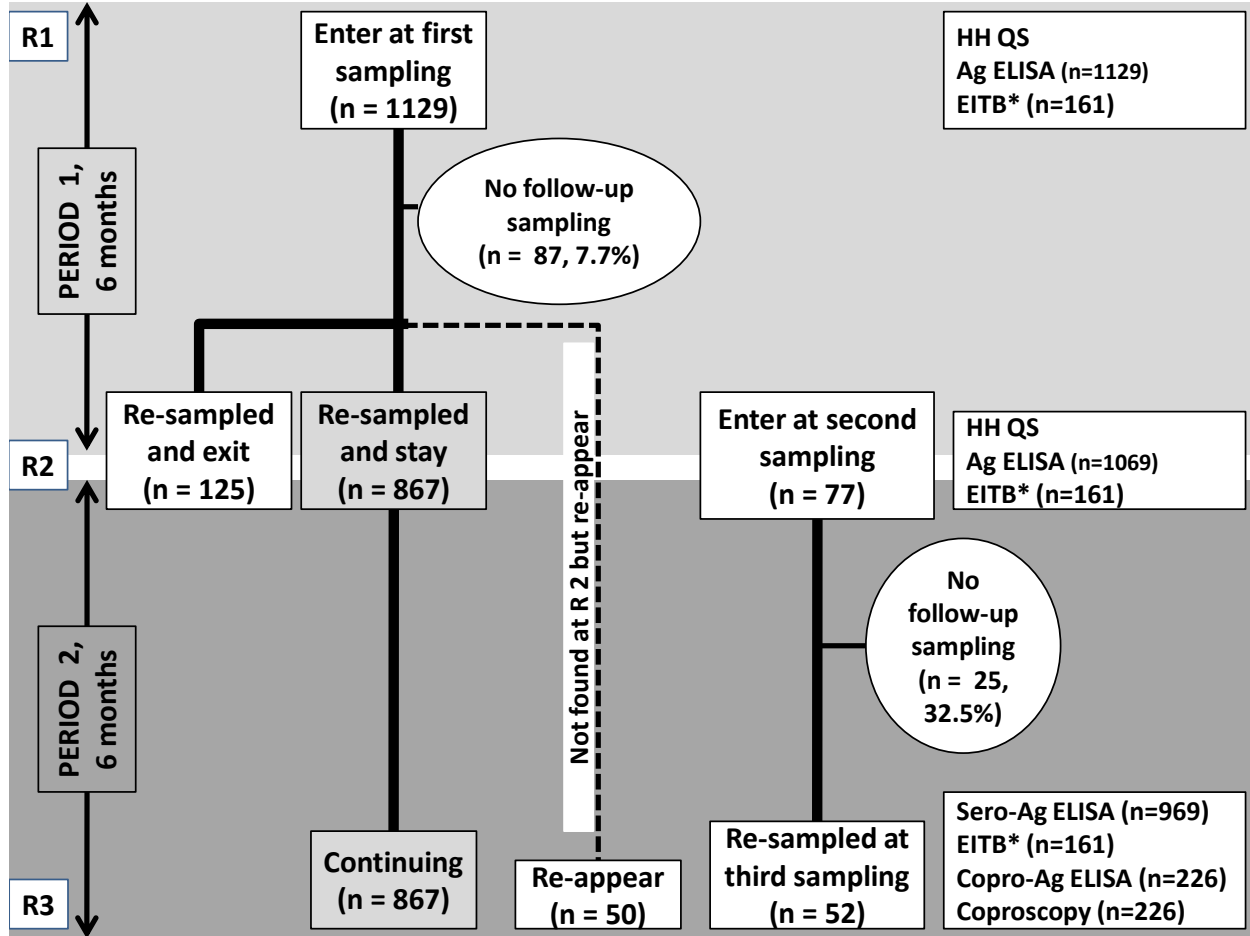
Figure 4.2: Cohort composition and changes during the three sampling rounds. HH QS stands for household questionnaire (Adapted from Garcia *et al*. 2003c)

From the 867 individuals sampled at R1, R2 and R3, 358 (41.3%) were male and 509 (58.7%) women; the age ranged from 2 to 87 years with a median age of 18 years. The number of people living in a household ranged from 1 to 15 with a median of 6. At least one participant from each household gave a sample. From the 867 individuals that gave samples for all the sampling rounds, a random sample of 161 was tested for specific antibodies against cysticercosis for each round (the same 161 participants were tested for each round).

4.3.2 Household characteristics

Household characteristics recorded from the questionnaire administered to the 516 households included; 69% kept pigs with 98% of these rearing on free-range, 46.6% of the households did not have latrines and 96.2% of the households had at least one individual who consumed pork. About 72.2% of the households reported to have slaughtered a pig in their backyards. Only 0.6% of them had the meat inspected before either home consumption or selling it to members of the community. Pork was reported to be consumed in a variety of ways including boiling, frying and roasting. The data obtained in the questionnaire on risk factors is described in more detail in Chapter 5.

4.3.3 Cysticercosis and taeniosis prevalences

Table 4.1 shows the overall and by sex cysticercosis prevalences per sampling for both the sero-Ag-ELISA and the EITB. Significantly higher sero-Ab prevalences (33.5-38.5%) were observed than sero-Ag prevalences (12.2-14.5%). Non-significant variations were observed in the prevalences per sampling round. Multivariate logistic regression analysis did not demonstrate any association between sero-Ag positivity and age or sex, and this for the 3 rounds. The interaction between sex and age was however, demonstrated to be significantly associated with sero-Ag positivity in all three sampling rounds ($P < 0.05$) (multivariate with interaction). Sero-Ab positivity was demonstrated to be significantly associated with only age and only in the second round of sampling. A change point analysis revealed a significant increase in antibody positives from the age of 40 years.

Taeniosis prevalence was determined to be 11.9% by copro-Ag ELISA and positivity was not related to either age or sex ($P > 0.05$) on both uni- and multivariate analyses. *Taenia* eggs were not detected by coproscopic examination in any of the stool samples. Other helminth ova detected included hookworms in 20 of the samples (8.8%), *Schistosoma* spp. in 7 (3.1%) and *Trichuris trichiuria* in 2 (0.9%).

Table 4.1: Seroprevalences of cysticercosis by Ag-ELISA and EITB for the three sampling rounds

Sampling round	Sex	Ag-ELISA			EITB		
		No. tested	% positive	95% CI	No. tested	% positive	95% CI
R1	All	1129	12.5	10.6-14.6	161	34.2	26.9-42.0
	M	464	13.6	10.6-17.0	67	34.3	23.2-46.9
	F	665	11.7	8.8-13.6	94	34.0	24.6-44.5
R2	All	1069	14.5	12.4-16.8	161	33.5	26.3-42.4
	M	440	16.8	13.4-20.6	67	34.3	23.2-46.9
	F	629	12.9	10.4-15.7	94	33.0	23.6-43.4
R3	All	969	12.2	10.2-14.4	161	38.5	31.0-46.5
	M	403	13.4	10.2-17.1	67	41.8	29.8-54.5
	F	566	11.3	8.8-14.2	94	36.2	26.5-46.7

R1, R2 and R3 stand for first, second and third round of sampling. Ag-ELISA: detection of circulating cysticercus antigen in serum; EITB: detection of specific antibodies in serum

4.3.4   Incidence

4.3.4.1 Antigen

For period 1 (6 months), 758 sero-Ag negative (naïve) individuals were at risk of infection; 52 (6.9%) seroconverted. For period 2 (6 months), 742 were at risk and 30 (4.1%) seroconverted while 48 (6.2%) of 758 individuals at risk of infection became sero-Ag positive for period 3 (12 months). Although males recorded higher incidence rates than females for all periods, this was not significant (Table 4.2). A significantly higher proportion of sero-Ag positive individuals became negative (seroreversion) at the end of each period: 36/109 (33.0%) for period 1, 47/125 (37.6%) for period 2 and 48/109 (44.0%) for period 3 (Figure 4.3).

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Figure 4.4 presents the seroconversion and reversion rates for the different age groups for period 3. Seroreversion was observed to be significantly higher than the seroconversion up to the age group of 30-39 years.

Table 4.2: Sero-incidence rates of human cysticercosis in function of sex for both circulating antigen and specific antibody analyses

Period	Sex	New cases	Individuals at risk*	Incidence (%)	95% CI
Ag-ELISA					
P1	All	52	758	6.9	5.2-8.9
	M	26	331	7.9	5.2-11.3
	F	26	447	5.8	3.8-8.4
P2	All	30	742	4.0	2.7-5.7
	M	14	297	4.7	2.6-7.8
	F	16	445	3.6	2.1-5.8
P3	All	48	758	6.2	4.6-8.2
	M	24	311	7.7	5.0-11.3
	F	23	443	5.2	3.3-7.7
EITB					
P1	All	18	106	17.0	10.4-25.5
	M	11	51	21.6	11.3-35.3
	F	7	55	12.7	5.3-24.5
P2	All	22	107	20.6	13.4-29.4
	M	11	42	26.2	13.9-43.8
	F	11	65	16.9	8.8-28.3
P3	All	25	106	23.6	15.9-32.8
	M	15	51	29.4	17.5-43.8
	F	10	55	18.2	9.1-30.9

P1 stands for period 1 (between Round 1 and Round 2, 6 months), P2 for period 2 (between Round 2 and 3, 6 months) and P3 for period 3 (between Round 1 and 3, 12 months). Ag-ELISA: detection of circulating cysticercus antigen in serum; EITB: detection of specific antibodies in serum.*Individuals at risk at the start of a period.
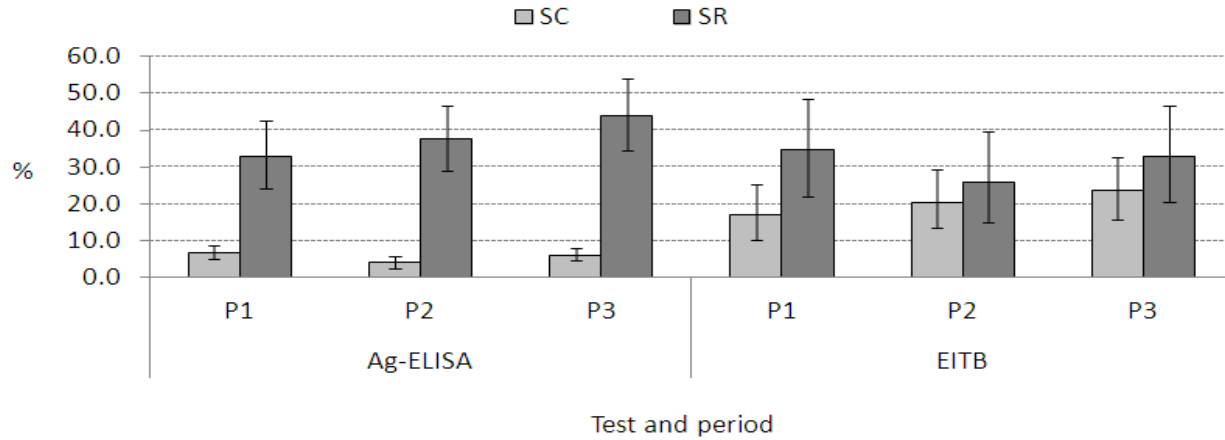
Figure 4.3: Rates of conversion and reversion on both serum antigens (by Ag-ELISA) and antibodies (by EITB) for the three sampling periods. SC and SR stand for seroconversion and seroreversion, respectively
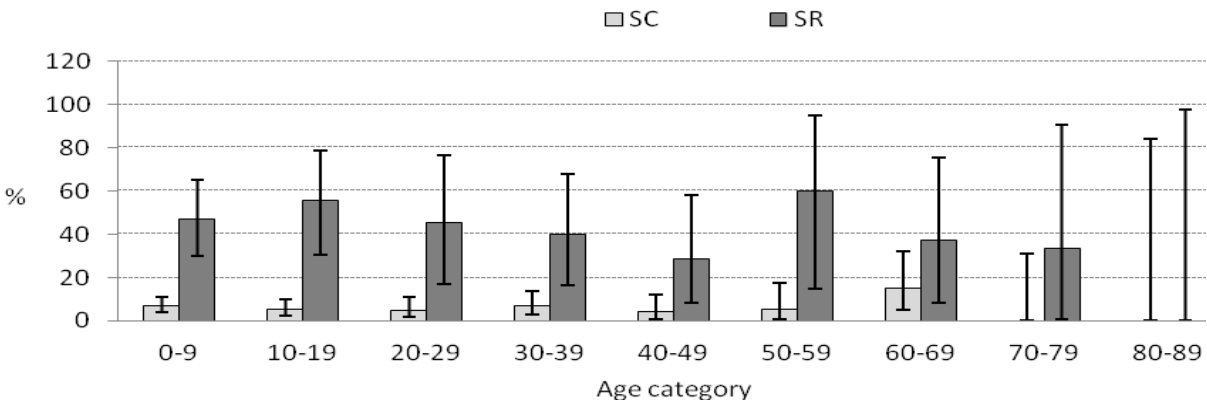
Figure 4.4: Rates of seroconversion and seroreversion on sero-antigen analysis in function of age categories for Period 3. SC and SR stand for seroconversion and seroreversion, respectively

4.3.4.2 Antibody

For Period 1, 106 sero-Ab negative (naïve) individuals were at risk of infection (on a group of 161 that were tested); 18 (17%) seroconverted. For Period 2, 107 were at risk and 22 (20.6%) seroconverted while 25 (23.6%) of 106 individuals at risk of infection became sero-Ab positive

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for Period 3 (Table 4.2). As for sero-Ag, the differences in incidence rates between males and females were not significant. Antibody seroreversion rates (34.5% for P1, 25.9% for P2 and 32.7% for P3) were higher than seroconversion rates, although not significantly (Figure 4.3). No significant differences were observed between seroconversion and seroreversion rates in the different age groups for Period 3 (Figure 4.5).
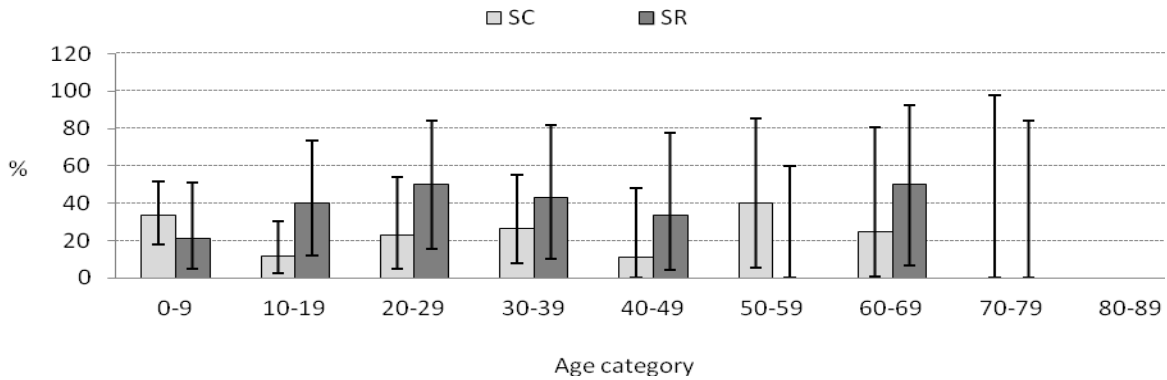
Figure 4.5: Rates of seroconversion and seroreversion on sero-antibody analysis in function of age categories for Period 3. SC and SR stand for seroconversion and seroreversion, respectively

Table 4.3 shows the infection dynamics, as measured by antibody and antigen presence, throughout the three sampling rounds. A high percentage (78.4%) of individuals remained sero-Ag negative throughout the study as compared to 44.7% for sero-Ab analysis. Almost 7% and about 19% of the individuals remained sero-Ag and sero-Ab positive throughout the study, respectively. Thirty one of 867 (3.6%) (sero-Ag) and 9 of 161 (5.6%) (sero-Ab) of the participants were negative at the start of the survey, became positive at R2 and turned negative again by R3. Vice versa, 0.5% (sero-Ag) and 3.7% (sero-Ab) of the participants tested were positive at R1, became negative at R2 and again positve at R3.

Table 4.3: Infection status changes (towards positivity) on sero-antigen and sero-antibody analysis throughout the study period

Infection status per sampling round			Test			
			Ag-ELISA		EITB	
R1	R2	R3	No.	%	No.	%
N	N	N	680	78.4	72	44.7
N	N	P	26	3.0	16	9.9
N	P	P	21	2.4	9	5.6
N	P	N	31	3.6	9	5.6
P	N	N	32	3.7	13	8.1
P	N	P	4	0.5	6	3.7
P	P	N	16	1.8	5	3.1
P	P	P	57	6.6	31	19.3

4.4 Discussion

The present study is the first in the world to estimate the incidence of human cysticercosis based on specific antibody as well as antigen detections; adding to the very short list of publications reporting the incidence of *T. solium* associated human infections (Villaran *et al.*, 2009). The determined high prevalence of human cysticercosis (12.2-14.5% sero-Ag prevalence and 33.8-38.5% sero-Ab prevalence) and taeniosis (11.9%) in this study confirm the endemicity of the parasite in this area. These results, as well as the high porcine cysticercosis prevalence previously identified (Sikasunge *et al.*, 2008) are strongly indicative for a high environmental contamination with *T. solium* eggs. The high sero-antibody positive results corroborate this finding, as presence of specific antibodies is indicative for exposure to infection (Garcia *et al.*, 2001; Dorny *et al.*, 2003). Less than half of the sampled population (44.7%) remained negative (sero-Ab) throughout the study period. About 32% (34/106) of the participants negative at the start of the study turned Ab positive at one point; another 3.7% of the participants positive at R1, but negative at R2, turned positive again at R3 (Table 4.3), indicating that more than one on three people (35.7%) have been (re)exposed and reacted to infection during the study period. The sero-Ag results present a different picture. A much higher percentage (78%) of people remaining

negative throughout the study was noticed; and 11.5% of the participants negative at the start of the study turned positive at one point (Table 4.3). As presence of antigen indicates establishment of infection rather than exposure, these results strongly indicate that about one on three people are exposed to infection, whereas the infection only establishes in about one on ten people. Findings from studies in Peru in pigs and human and in Ecuador in human (Praet *et al.*, 2010a) also suggest exposure without infection or mild infections that are aborted by the natural immunity of the individual, expressed by the presence of transient antibodies (Garcia *et al.*, 2003c). A study in Mexico reported that the rapid humeral response against *T. solium* in exposed individuals enables them to either avoid parasite establishment or spontaneously resolve the infestation (Meza-Lucas *et al*., 2003). The higher levels of sero-Ab prevalence and seroconversion in comparison with sero-Ag prevalence/ conversion, as well as the high seroreversion levels, identified in this study, contribute to this finding.

Another interesting outcome in this study is the rather short-term presence of antigen in 31 participants (negative at R1, positive at R2, and again negative at R3, table 3). Whether this is due to an only partial establishment of infection (immature cysticerci), or establishment and quick degeneration (self cure?) of the cysticerci is not clear. It was noted that individuals who became seronegative were those with samples that had low antigen titers (Data not shown). In humans, it is described that cysticerci in the brain usually stay viable during years, while probably cysticerci in the muscles tend to degenerate more quickly (Garcia & Del Brutto, 2005). However later, Garcia *et al.* (2010) challenged this theory in the case of single cysticercal granulomas, for which they hypothesize that instead of being caused by a late degenerative process, the granulomas are rather due to an early parasite death. In experimental infections in pigs often infections do not establish, or (partially) establish (with the corresponding increase in antigen levels) and abort shortly afterwards. Deckers *et al.*, (2008) demonstrated circulating cysticercus antigens as early as three weeks after experimental infection, which is before full maturation of the cysticerci. Many factors, among which the size of the (re)infection, the immune status of the host, age, sex play a determining role for the (non)establishment of infection (Fleury *et al.,* 2004). Results from this study suggest that the presence of antigen does not necessarily always signify presence of a viable, well established infection, however it could be indicative for short term partial establishment, and perhaps a 'transient' antigen presence should be considered. Since the tests used are not 100% sensitive and specific, the possibility of

force positives cannot be ruled out and could account for the observations made. The short-term presences of specific antibody and antigen levels can also explain the lack of difference in incidence rates whether calculated over 6 months or 1 year, as a number of people have seroconverted and reverted within a year. Also, in the light of this high occurrence of transient antibodies and antigens, serological results from field studies, in contrast to clinical studies, should critically be looked at. Therefore, individuals with positive test results should not be automatically considered as having cysticercosis, as is often done in reports from field studies.

Seroconversion and seroreversion rates were not significantly different for specific antibody levels, indicating an endemic stability. For antigen levels, significantly higher seroreversion rates were determined up to the age of 30 years, where after this difference could not be identified. In the Petauke study area (Chapter 3), significantly higher prevalences of cysticercosis (determined by antigen detection) were described in people of 30 years and older, which was suggested to be due to a lower host immune response. The higher seroreversion rates observed in younger people, but not in older people in this study, could indeed be indicative of an improved clearing of the infection in younger people in this area, which supports the earlier finding. However, these results should be considered carefully, as even though the seroconversion and reversion rates are different, the actual number of people seroconverting and seroreverting is almost the same. The latter can explain why no significant changes in prevalence have been observed in this study. The simulation models described in Praet *et al.* (2010c) suggest a continuous exposure of the population with seroreversion (antibody) rates depending on the number of exposures, which is related to age and the immunological status of the individual. Seroreversion rates of 60% after first exposure and 20% after second and subsequent exposures were obtained. In this study, no significant differences were identified in antibody seroreversion rates between the different age groups.

This is the first study to report cysticercosis incidence based on sero-Ag analysis (6.2%).The sero-Ab incidence rate (23.6%) is comparable to that reported in Peru by Garcia *et al*. (2001) and in Ecuador (Praet *et al.*, 2010c). A higher average porcine cysticercosis sero-Ab incidence rate of 53% has been reported in Peru (Garcia *et al.*, 2003c). Since pigs are highly coprophagic, it is expected that they would be exposed more frequently and to higher levels of infection as compared to humans and hence record a higher incidence rate especially for sero-Abs.

The cysticercosis prevalence recorded in this study is higher than what has been reported in a neighboring district (5.8%, Chapter 3) possibly indicating a higher risk of infection in the Katete area than in Petauke area. This could be attributed to the major differences that have been recorded between the two districts in terms of risk factors such as lack of toilets, pig keeping households and consumption of undercooked pork (Chapter 5).

It is unclear whether the recorded risk for cysticercosis infection in our study area can be extrapolated to risk for NCC and whether clinical symptoms are mainly due to re-infection over a period of time. As the number of cysts and site in the brain has a bearing on the establishment of clinical symptoms (Dorny *et al.*, 2009), one would assume that re-infections increase the risk of NCC in an endemic area. However, a long-term follow-up of asymptomatic sero-Ag positive individuals in India revealed that such individuals do not develop symptoms of NCC even after a period of four to five years (Alexander *et al.*, 2010).

In conclusion, this study has shown the dynamic nature of *T. solium* infections, many of the people at risk become (re)infected due to the high environmental contamination, with a high number turning seronegative within a year after infection. An important number of infections probably never fully establish, leading to transient antibody responses and possibly even 'transient' antigen presence. Control measures should therefore, highlight reduction of this contamination. Monitoring the latter can be done by environmental sampling for eggs and diagnosis of carriers; however, the two methods are not very successful (Diaz *et al.*, 1992). Besides their obvious usefulness in the understanding of the transmission dynamics of the parasite, incidence studies can be of great help in this evaluation.

CHAPTER 5

Study and ranking of determinants of *Taenia solium* infections by classification tree models

Adapted from:

Kabemba E. Mwape, Isaac K. Phiri, Nicolas Praet, Pierre Dorny, John B. Muma, Gideon Zulu, Niko Speybroeck & Sarah Gabriël (2012). Study and ranking of determinants of *Taenia solium* infections by classification tree models. *Tropical Medicine and International Health* (Submitted article).

5.1   Introduction

The disease determinants associated with *Taenia solium* infections have been widely reported. In most studies, data analyses that determine associations between the infection and a particular determinant have been utilized. Commonly used are the multinomial models such as the classical logistic regression analyses that utilize linear combinations as the primary method of expressing relationships between variables. These models fail to rank the factors according to their importance in light of multiple interactions among the various predictor variables (Thang *et al.*, 2008). This ranking is an important tool for the establishment of a decision guide for control measures by determining the priority foci of prevention/control methods. However, until now, these analyses have not been carried out for the determinants of *T. solium* infections.

The classification and regression tree (CART) is a useful statistical model that can deal with a large number of independent variables and allows exploring the relationship and the relative importance of the variables and also their possible interactions. The method was successfully applied in different parasitological contexts (Thang *et al.*, 2008; Yewhalaw *et al.*, 2009; Protopopoff *et al.*, 2009; Bhattarai *et al.*, 2010).

Two community based studies were conducted in two endemic districts of eastern Zambia to determine the prevalences of taeniosis and cysticercosis (results presented and discussed in Chapters 3 and 4) and to study the factors associated with human taeniosis and cysticercosis. The objective of this study was to evaluate relationships and interactions between predictor variables (risk factors) and disease outcome and to rank the factors in order of importance. For this, two different methods of factor analyses i.e. CART and logistic regression were used.

5.2   Materials and methods

5.2.1   Study area and population

The study was conducted in Katete and Petauke districts of the Eastern province of Zambia (Figure 5.1). The two districts are reported to be endemic for porcine cysticercosis (Sikasunge *et al.*, 2008b) and preliminary visits revealed the presence of free roaming pigs.
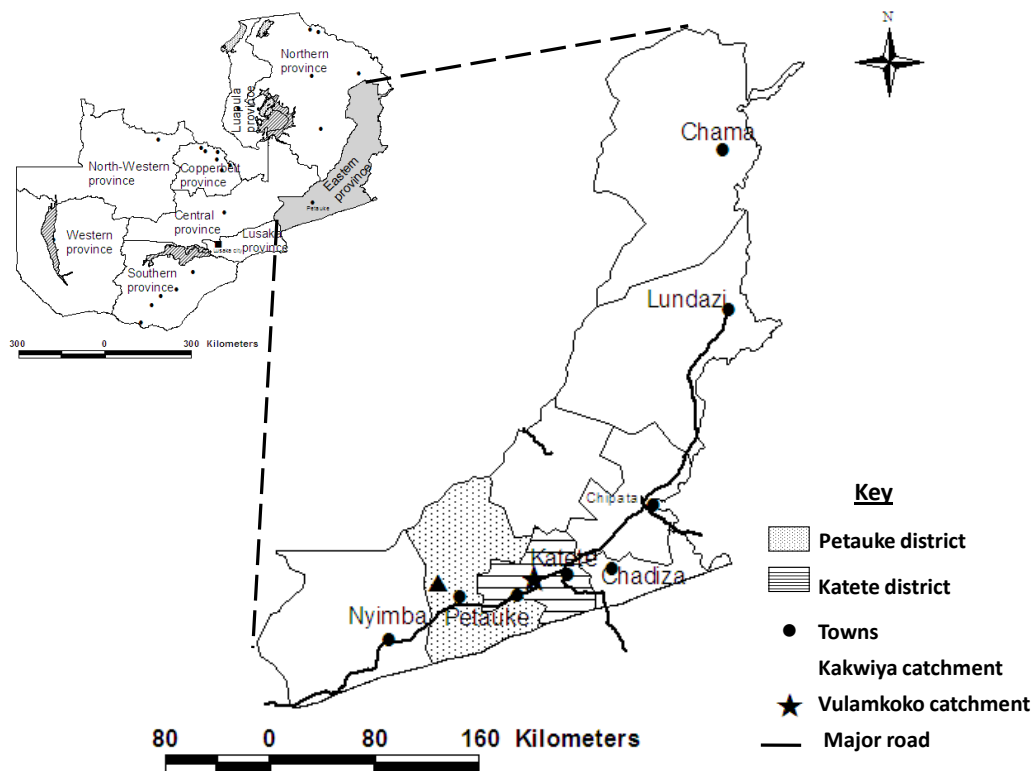
Figure 5.1: Map of Zambia showing the districts of Petauke and Katete and the two catchments of Kakwiya and Vulamkoko Rural Health Centres respectively

The climate is tropical and modified by altitude with two main seasons, the rainy season (November to April) and the dry season (May to October). The most common ethnic groups in Katete and Petauke districts are the Chewa and Nsenga people, respectively. Both groups practice subsistence agriculture raising animals like cattle, goats, pigs and chickens and grow crops like maize, groundnuts, bananas and cotton.

5.2.2 Study design

Two community based questionnaire cross sectional surveys, one in Petauke and another in Katete, were conducted between July and November 2009 as part of the studies described in Chapters 3 and 4. The administration of the questionnaire is extensively described in section 3.2.2 of Chapter 3. The questionnaire covered aspects of HH characteristics and knowledge of the pork tapeworm infection in humans and pigs as shown in Table 5.1. As people could not differentiate between roundworms and tapeworms, questions on their knowledge of the adult tapeworm in faeces were modified to ask about helminths in general and not specifically the tapeworm. For cysticercosis, questions included having seen or heard of anyone in the village suffering from epilepsy, chronic headaches, madness and skin nodules.

Table 5.1: Predictor variables introduced in the CART analysis for both taeniosis and cysticercosis

Variable classes	Predictor variables
Host characteristics	
	Age
	Gender
	Not taken an anthelmintic in one year
	Coproantigen positivity*
	Positive for other helminth infections
Household characteristics	
	Number of inhabitants
	Income
	Highest education level
	Pork consumption
	Boiling of pork before consumption
	Keeping pigs
	Free range Slaughtered a pig in the backyard
	Slaughtered pig inspected
	Presence of a latrine
Knowledge of helminth infection in humans	
	Heard of helminth (tapeworm) infections
	Knew how to acquire the infection
	Seen subcutaneous nodules
	Heard of someone with chronic headache
	Heard of someone with madness
Knowledge of infection in pigs	Observed cysts in pork
	Knew what cysts were
	Knew how pigs acquireinfection
	Ate infected pork
	Sold infected pork
	Threw away infected pork

*Predictor variable only introduced in the analysis for cysticercosis

5.2.3 Sample analyses

The methodologies of sample collection and laboratory tests are described in Chapters 3 and 4.

5.2.4 Statistical analysis

All collected data were double entered, checked and cleaned in an Excel (Microsoft Office Excel 2007®) spreadsheet. A CART (Salford Predictive Miner, Version 6.6 Salford Systems Inc., California, USA) analysis was conducted on the data set. Disease positivity (taeniosis by copro-Ag ELISA and cysticercosis by sero-Ag ELISA) was used as the dependent variable and a set of different variables as independent or predictor variables. The predictor variables introduced in the CART analysis are listed in Table 5.1 for both taeniosis and cysticercosis in the two districts. CART analysis is a non-linear and non-parametric model that is fitted by binary recursive partitioning of multidimensional covariate space (Speybroeck, 2012). The analysis successively splits the dataset into increasingly homogeneous subsets until it is stratified to meet a specified criterion (Speybroeck *et al.*, 2004; Thang *et al.*, 2008). The building of a classification tree begins with a root (parent) node, containing the entire set of observations, and then through a process of yes/no questions, generates descendant nodes. Beginning with the first node, CART finds the best possible variable to split the node into two child nodes. In order to find the best variable, the software checks all possible splitting variables (called splitters), as well as all possible values of the variable to be used to split the node. In choosing the best splitter, the program seeks to maximize the average "purity" of the two child nodes so that the child nodes will be as homogeneous as possible with respect to the outcome variable. In terms of "purity", CART aims at obtaining nodes with only positive or only negative observations and in this respect the determinants are especially appropriate in isolating positive or negative observations (Suman *et al.*, 2010). The Gini index was used as the splitting method. A 10-fold cross-validation was used as the method for testing the predictive capacity of the obtained trees. The one-standard error rule was used to select the best tree (Thang *et al.*, 2008; Protopopoff *et al.*, 2009). CART also provides a score indicating the importance of the different variables. This discriminatory power is reported relatively to the most important variable (which is given a score of 100). Uni- and multivariate logistic regression analysis was done in Stata 10

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(http://www.stata.com). The Pearson chi square test was used to check for differences between the two districts.

5.3 Results

5.3.1 Population baseline characteristics

The study population baseline characteristics are shown in Table 5.2. A total of 680 households (HH) from the two districts (425 HH, 33 villages in Katete; 255 HH, 20 villages in Petauke) participated in the study. The age ranged between 1-96 years with an overall median age of 15 years. A significantly higher number of HH kept pigs in Katete (67.5%) than in Petauke (32.9%). The main source of income for most HH (99.3%) in both areas was farming while the highest education level attained in the participating HH was 10.4%, 55.7%, 33.15% and 0.7% for no education, primary, secondary and tertiary respectively. Pork was consumed by at least one individual in 95.6% of the HH in the two areas and was consumed in a variety of ways including boiling, frying, roasting and in various combinations of these. Over 60% of the HH in both areas reported having slaughtered a pig in their backyards with only 0.5% having had the meat inspected.

Table 5.2: Study population and household (HH) baseline characteristics in Katete (n = 969), Petauke (n = 712) areas and overall (n = 1681)

	Katete No (%)	Petauke No (%)	Total No (%)
Number of HH	425	255	680
Sex			
-Male	403 (41.6)	290 (40.7)	693 (41.2)
-Female	566 (58.4)	422 (59.3)	988 (58.8)
Age groups			
-< 10 y	306 (31.6)	258 (36.2)	568 (33.6)
-11-20y	224 (23.1)	219 (30.8)	443 (26.4)
-21-40y	250 (25.8)	109 (15.3)	359 (21.4)
-41-60y	141 (14.6)	73 (10.3)	214 (12.7)
-> 60y	48 (5.0)	53 (7.4)	101 (6.0)
Ethnic groups			
-Nsenga	2 (0.2)	710 (99.7)	712 (42.4)
-Chewa	958 (98.9)	0.0 (0.0)	958 (57.0)
-Others	9 (0.9)	2 (0.3)	11 (0.6)
Education level in HH			
-None	36 (8.5)	35 (13.7)	71 (10.4)
-Primary	269 (63.3)	110 (43.1)	379 (55.7)
-Secondary or higher	120 (28.2)	110 (43.1)	230 (33.8)
Farming as main HH income	425 (100.0)	250 (98.0)	675 (99.3)
Pig keeping HH*	287 (67.5)	84 (32.9)	371 (54.6)
HH without latrines	198 (46.6)	118 (46.3)	316 (46.5)
Pork consumption in HH	409 (96.2)	241 (94.5)	650 (95.6)
Slaughtered a pig at HH*	309 (72.7)	112 (49.8)	421 (61.9)
Slaughtered pig inspected	2 (0.6)	0 (0.0)	2 (0.5)
Observed cysts in pork meat*	960 (99.1)	692 (97.2)	1652 (98.3)
Did not know what cysts were	767 (78.1)	459 (64.5)	1216 (72.3)
Consumed pork with cysts*	272 (29.1)	145 (21.7)	417 (26.0)
Sold infected pork*	149 (36.3)	46 (19.4)	195 (30.1)
Threw away infected pork*	167 (39.3)	181 (71.0)	417 (51.2)

*Statistically significant differences ($P < 0.05$) observed between the two districts. n = number of people sampled

5.3.2 Disease prevalences

Table 5.3 is a summary of taeniosis and cysticercosis prevalence in the two districts highlighting the differences. Katete district recorded a significantly higher prevalence of parasitic infections than Petauke (taeniosis, $\chi^2 = 7.66$, $P = 0.006$; cysticercosis, $\chi^2 = 19.44$, $P = 0.000$).

Table 5.3: The prevalence by gender of human taeniosis after copro-Ag ELISA and human cysticercosis using sero-Ag ELISA in the study areas of Katete and Petauke districts (Chapter 3 and 4)

Disease	Katete			Petauke		
	No	%	(95%CI)	No	%	(95%CI)
Taeniosis*	(n = 227)			(n = 708)		
Male	13	16.1	(7.9-24.2)	16	7.9	(4.8-11.0)
Female	14	9.7	(4.8-14.5)	25	5.2	(3.1-7.3)
Overall	27	12.0	(7.7-16.2)	45	6.3	(4.5-8.1)
Cysticercosis *	(n = 969)			(n = 712)		
Male	54	13.4	(10.1-16.7)	16	5.6	(2.9-8.3)
Female	64	9.7	(8.7-13.9)	25	5.9	(3.7-8.2)
Overall	118	12.2	(10.1-14.2)	41	5.8	(4.1-7.5)

*Statistically significant differences ($P < 0.05$) observed between the two districts using the Pearson Chi square test

5.3.3 Analysis of risk factors by CART

5.3.3.1 Cysticercosis

Katete area

According to the discriminatory power in the analysis, the number of inhabitants in a HH emerged as the strongest overall discriminating determinant for cysticercosis infection followed by age. The other important determinants listed as important are shown in Table 5.4.

Table 5.4: Ranking of cysticercosis risk factors by overall discriminatory power in Katete district

Variable	Power
Number of inhabitants in household (HH)	100
Age	58.88
Slaughtering a pig in the backyard of the HH	49.44
Gender	40.77
Knowledge of someone with madness	40.12
Keeping pigs	40.06
Not boiling of pork before consumption	38.90

The corresponding classification tree showed that number of inhabitants in a HH was the first splitter (Figure 5.2) with cysticercosis prevalence being higher in HHs with less than 10 inhabitants (12.7%) compared to HH with more than 10 (2.0%). In the former group, prevalence was higher for those without knowledge of a mad person (16.6%) compared to those who had (10.5%). For those with knowledge of a mad person, age was the best discriminator with a threshold of 28.5 years; those above this threshold had a higher prevalence (15.2%) especially so if they were male (23.3%) as compared to females (9.7%). For females above the age threshold, prevalence was higher if they did not boil pork before consumption (29.4%) than if they did (6.5%). In the group less than the 28.5 years threshold, they had a lower prevalence if they came from a HH with less than 6 inhabitants (4.1%) than a HH with more than this number of inhabitants (11.9%). For the latter group, prevalence was higher if they did not slaughter a pig at home (36.8% versus 9.0% if they did). Each terminal node is categorized as 1 (positive) and 0 (negative) depending on whether the proportion of 1's exceeds the proportion of 1's in the population (12.2%). From all negative individuals, 57% (485/851) were properly classified as negative and from all positives, 74% (87/118) were properly classified as positive.
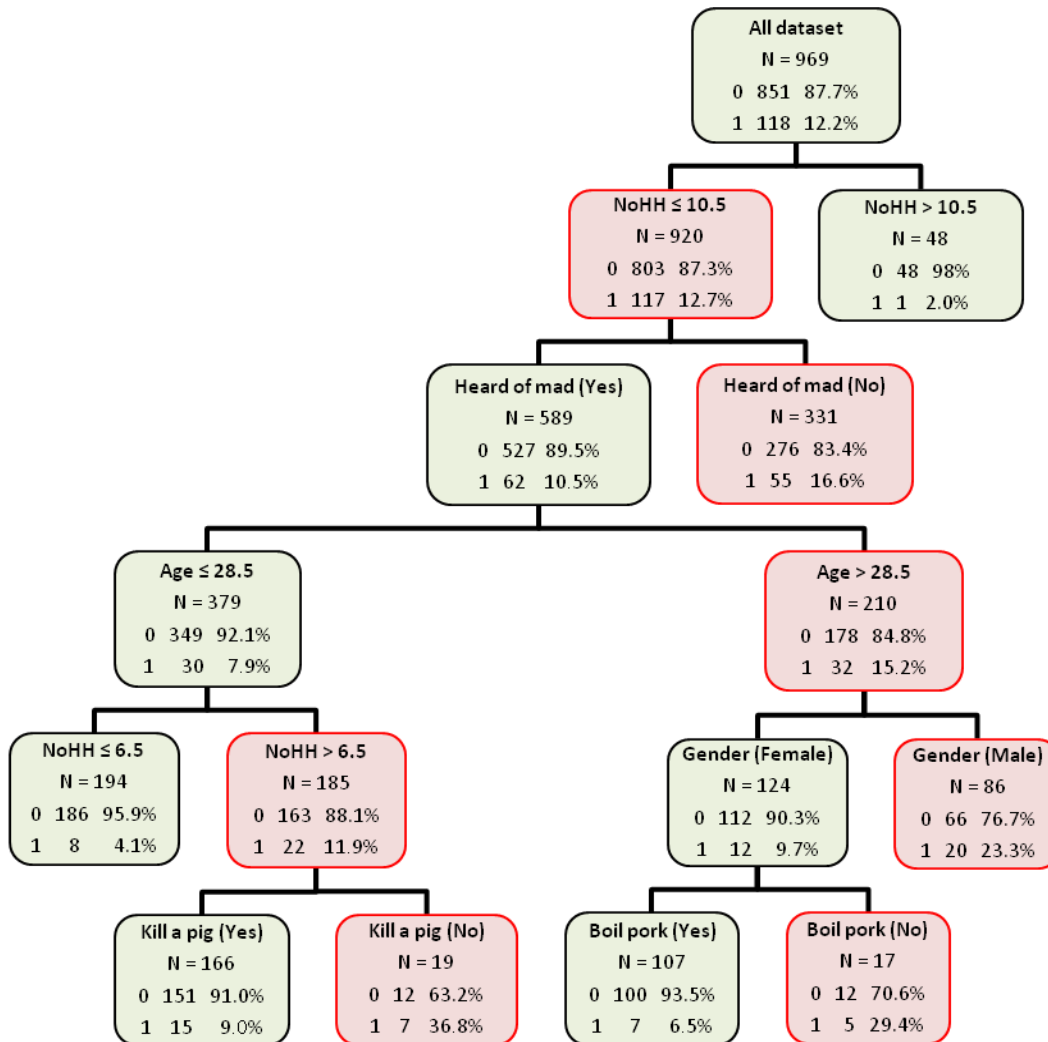
Figure 5.2: Classification tree of the risk factors for cysticercosis infection in the Katete study area. The high risk groups are represented by a red outline. In each node 0 stands for a negative sero-Ag ELISA results and 1 for a positive result. NoHH stands for number of inhabitants in a household

Petauke area

The important determinant for cysticercosis infection in the Petauke area, according to the discriminatory power included age and not having heard of helminth infections with powers of 100 and 23.89, respectively. The corresponding tree for the district revealed only one important splitter, which was age. Cysticercosis prevalence was higher in people above the age of 32.5

(14.0%, n = 164) than those equal to or less than this age (3.3%, n = 544). The analysis properly classified 79% (526/667) of the negatives as negative and 56% (23/41) of the positives as positive.

5.3.3.2 Taeniosis

Katete area

The overall discriminatory determinants for taeniosis infection in the Katete area are listed in Table 5.5 with number of inhabitants in a HH being the most important followed by having heard of someone with chronic headache and history of not having taken an antihelmintic one year prior to the study.

Table 5.5: Ranking of taeniosis risk factors by overall discriminatory power in Katete district

Variable	Power
Number of inhabitants in a household	100
Knowledge of someone with chronic headache	64.91
Not having taken an antihelmintic in the past one year	55.47
Free range keeping of pigs	51.02
Knowledge of someone with epilepsy	22.14

The best splitter in the corresponding tree was number of inhabitants in the HH with a threshold of 6. Groups above this threshold had a higher taeniosis prevalence (17.3%, n = 110) than those below it (6.9%, n = 116). For the group above the 6 inhabitants in a HH, prevalence was even higher for those with a history of not having taken any antihelmintic in the year prior to the study (18.8% versus 0.0% for those that did). For all negative individuals the tree classified 59% (177/199) properly as negatives and 70% (19/27) positives were properly classified as positives.

Petauke area

Table 5.6 shows the determinants and their discriminatory powers determined as being important for taeniosis infection in Petauke district. Age was the most important factor followed by not boiling the pork meat before eating and consumption of pork.

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Table 5.6: Ranking of taeniosis risk factors by overall discriminatory power in Petauke district

Variable	Power
Age	100.00
Not boiling pork before consumption	71.50
Pork consumption by member(s) of the household (HH)	53.64
Highest education level in HH	46.25
Number of inhabitants in HH	45.52
Positive for other helminth infections	31.54
Observed cysts in pork	27.85

The important splitter in the corresponding tree was consumption of pork by at least one member of the HH (Figure 5.3). No cases were recorded in those that did not consume pork while prevalence was highest in the group that consumed pork (6.7%). In the latter group prevalence was higher for those with secondary or tertiary level of education in the household (9.2%) than those with primary or no education at all (5.0%). Among those with secondary and tertiary education, belonging to the group with age equal to or below 54.5 years but being older than 9.5 years were important determinants for taeniosis infection (prevalence 12.2%). For those below the age of 9.5 years, prevalence was higher in the group that did not boil the pork before consumption (21.4% versus 1.4% for those that did). The tree correctly classified 76% (505/667) of all negatives as negative and 52% (24/45) positives as positive.
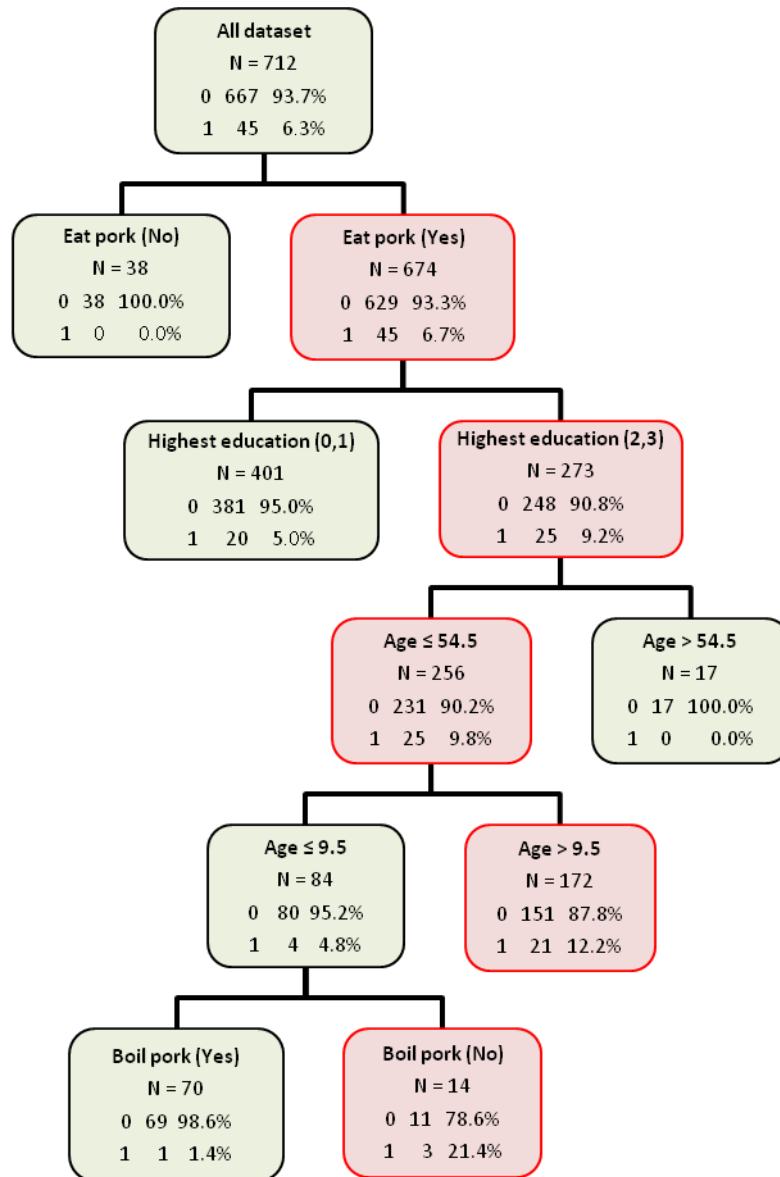
Figure 5.3: Classification tree of the risk factors for taeniosis infection in the Petauke study area. The high risk groups are represented by a red outline. In each node 0 stands for a negative sero-Ag ELISA results and 1 for a positive result

5.3.4 Analysis of risk factors by logistic regression

Katete area

Uni- and multivariate logistic regression analysis of the determinants listed as important (power above 20) in the CART analysis revealed that age and knowledge of a mad person in the village were significant ($P < 0.05$) for cysticercosis (Table 5.7). None of the factors listed as important by the CART analysis for taeniosis were significant on logistic regression analysis (Table 5.7).

Table 5.7: Risk factor analysis for cysticercosis and taeniosis infection for the Katete study area: uni- and multivariate analysis using logistic regression

Risk factors	Cysticercosis		Taeniosis	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Age	1.01 (1.00-1.02)	0.02	-	-
Gender	1.22 (0.82-1.81)	0.32	-	-
Number of inhabitants in HH	0.97 (0.89-1.06)	0.57	1.08 (0.87-1.35)	0.45
Not boiling of pork before consumption	0.67 (0.37-1.22)	0.19	-	-
Keeping pigs	1.15 (0.65-2.03)	0.63	-	-
Backyard slaughter of pigs	1.04 (0.56-1.94)	0.90	1.33 (0.26-6.73)	0.71
Heard of someone with madness	1.68 (1.12-2.51)	0.01	-	-
Heard of someone with chronic headache	-	-	0.37 (0.07-3.03)	0.36
Taken an anthelmintic in past one year	-	-	0.55 (0.07-4.62)	0.56
Heard of someone with epilepsy Know how pigs acquired cysts*	-	-	0.82 (0.09-7.120	0.86

*Variable dropped by the model as it predicted failure. HH = household

Petauke area

The uni- and multivariate analysis results are shown in Table 5.8; age (on both uni- and multivariate analysis) and having heard of helminth infection (univariate only) were positively related ($P < 0.05$) to cysticercosis. None of the determinants for taeniosis were significant.

Table 5.8: Risk factor analysis for cysticercosis and taeniosis infection for the Petauke study area: uni- and multivariate analysis using logistic regression

Risk factors	Cysticercosis		Taeniosis	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Age	1.03 (1.01-1.04)	0.00	1.00 (0.99-1.02)	0.69
Ate pork	-	-	*	
Positive for other helminths	-	-	1.34 (0.68-2.64)	0.40
Number of inhabitants in HH	-	-	0.97 (0.84-1.12)	0.66
Highest education in HH				
-Primary	-	-	0.95 (0.30-3.00)	0.93
-Secondary	-	-	2.04 (0.66-6.31)	0.22
-Tertiary	-	-	2.96 (0.29-30.61)	0.36
Not Boiling of pork before consumption	-	-	0.55 (0.28-1.08)	0.08
Not heard of helminth infections	2.60 (0.97-7.00)	0.06**	-	-
Observed cysts in pork	-	-	*	

*Variable dropped by the model as it predicted failure. OR = Odds ratio. **$P = 0.01$ on univariate analysis. HH= household

5.4   Discussion

The use of both parametric and non-parametric methods to study determinants of human *T. solium* infections can be useful in providing insights about this complex disease and to rank all the potential factors related to the disease prevalence. CART, which expresses its results in the form of a decision tree, differs from the classical regression analyses, which use linear

combinations to express relationships between variables. It does not need relationships to be linear or additive and interactions do not need to be pre-specified or of a particular multiplicative form (Speybroeck, 2012). The overall discriminatory power of each variable can be determined and the tree allows the exploration of relationships between variables and their relative importance (Thang *et al.*, 2008). This study is to our knowledge the first to use the CART method to rank factors that are associated with both human taeniosis and cysticercosis, and the first in Zambia to quantitatively investigate the determinants of human *T. solium* infections.

The number of inhabitants in a HH and age were determined by the CART analyses as the most important factors in the Katete and Petauke study areas respectively. Change point analyses in earlier studies have indicated that the number of people with active infections (cysticercosis) increases from a certain age onwards. In Ecuador, this change point was determined to be at 60 years (Praet *et al.*, 2010c) while in Zambia (Petauke study area) this change point was observed at 30 years (Chapter 3). The analyses from this study corroborate the latter finding, indicating an increased risk for cysticercosis after the age of 32.5 for the Petauke area and 28.5 for the Katete area.

This is the first time that the number of HH members is identified as a possible determinant of *T. solium* infection. Households with between 6 and 10 members seem to be related to higher infection rates for both taeniosis and cysticercosis, probably due to factors such as bulk food preparation and hence chances of undercooking, more people consuming pork, low levels of hygiene and therefore increased risk of infection. The presence of a tapeworm carrier in a HH has been reported to be a major risk factor for human cysticercosis (Schantz *et al.*, 1992; Garcia-Garcia *et al.*, 1999). The aforementioned factors could also pertain to HHs with more than 10 members. However, these HHs were determined to have lower infection rates, probably due to a possible less consumption of pork as they could not afford to buy large quantities of pork.

Other important factors identified were knowledge of someone with chronic headache, not having taken an anthelmintic one year prior to the study, free range pig keeping, not boiling pork, consumption of pork (for taeniosis); backyard slaughter of pigs, not having heard of helminth infections, gender and having heard of madness (for cysticercosis). Most of these have been described in other studies (Sanchez *et al.*, 1997; Sarti, 1997; Garcia *et al.*, 2003a; Sikasunge

et al., 2007); however, they were never ranked in order of importance. The reduced risk for taeniosis in individuals who had taken an anthelmintic during the last year could be explained by the common use of albendazole (for soil transmitted helminths) and praziquantel (for schistosomiasis) in the local health centers. Praziquantel has been reported to be very effective against taeniosis (Diaz Camacho *et al.*, 1991; Allan *et al.*, 1997; Sarti *et al.*, 2000) while albendazole has a rather poor efficacy (Chung *et al.*, 1991; Steinmann *et al.*, 2008).

The higher taeniosis prevalence in HH with high education level was unexpected and contrary to results described by Sanchez *et al.* (1998) in a study in Honduras. However, in our study area, people, whether educated or not, were not aware of what the cysts in pork were (Table 5.2) and assumed that the meat was "harmless", as reported in studies in Kenya and Tanzania (Ngowi *et al.*, 2004; Kagira *et al.*, 2010). As higher education generally means more HH income and hence increased consumption of (infected) pork, this can entail higher risk of acquisition of the infection (Rodriguez-Canul *et al.*, 1999).

Absence of latrines, one of the most commonly described risk factors (Ngowi *et al.*, 2004; Sikasunge *et al.*, 2007), was not identified as an important factor in this study. Almost 50% of HH had no latrines, and therefore, the environment may have been contaminated, even for the HH with latrines. Living with a carrier in a household has been reported to be associated with increased cysticercosis risk (Sarti-Gutierrez *et al.*, 1988; Garcia & Del Brutto, 1999) and cases tend to aggregate in neighboring HH (Diaz Camacho *et al.*, 1990; Flisser, 2002). Recent reports state that human cysticercosis cases significantly surround tapeworm carriers (Lescano *et al.*, 2009) indicating contamination of the community environment. In future, it may be interesting to define a population level threshold of HH needing to have latrines in order to observe the effect on cysticercosis prevalence.

The finding that most people had heard of someone suffering from chronic headaches (87.9%), epilepsy (95.6%) and madness (73.0%) (Data not shown) in their communities is alarming, indicating a possible high level of NCC. Recent studies indicate that in endemic areas, NCC accounts for almost 30% of acquired epilepsy (Ndimubanzi *et al.*, 2010). We have determined high cysticercosis prevalences in both pigs (Sikasunge *et al.*, 2008b) and humans (Chapters 3 and

4) in our study area, indicating an urgent need to investigate the prevalence of NCC and its association with epilepsy in these areas.

In conclusion, the CART approach has given insights in the important factors at play with regards to *T. solium* infections and has shown that its control remains complex. This study has confirmed the importance of most commonly described factors and has ranked them. It has also recorded obvious differences in the presence and ranking of factors between the two study areas which significantly differ in disease prevalence as well, hence describing two different epidemiological situations. This raises the question of whether control programmes should be area specific focusing on a few identified risk factors or rather be created for an entire multi-country region, addressing multiple human and animal host related factors in a multidisciplinary way; and which approach would be the most cost effective. The ranking of disease determinants will help prioritize control efforts targeting specific important factors. The identification of specific age groups (older than 30 years) and also specific HH (with number of inhabitants between 6 and 10) as people with a higher risk of infection can help identify the primary target group of control programmes.

CHAPTER 6

Field evaluation of urine antigen detection for the diagnosis of *Taenia solium* cysticercosis

Adapted from:

6.1   Introduction

Diagnosis of (neuro)cysticercosis depends primarily on imaging techniques and on immunodiagnostic methods that detect either antibodies or circulating antigens in serum or cerebrospinal fluid (CSF) (Deckers & Dorny, 2010). Imaging techniques are expensive and often not available in endemic areas. The detection of antibodies in serum may indicate exposure to infection and not necessarily the presence of established viable infection (Parija *et al.*, 2004). Also, antibodies may persist long after the parasite has been eliminated, resulting in false positivity and unnecessary anti-parasitic therapy. In contrast, antigen positivity indicates an infection with viable cysticerci. A monoclonal antibody-based ELISA (B158/B60 Ag-ELISA) (Dorny *et al.*, 2004b) was estimated to have a sensitivity of 90% and a specificity of 98% for the detection of infection in serum (Praet *et al.*, 2010b).

However, CSF or blood collection is an invasive procedure and associated with the risk of acquiring blood–borne infections such as hepatitis B and HIV if the method is not carried out under stringent conditions. Urine collection is non invasive, relatively easily accepted by the

community and very convenient if multiple samplings would be required per day. It can thus be of help in both clinical and epidemiological settings.

Tests to detect antigenuria have been developed for several other parasitic diseases such as leishmaniasis (Kohanteb *et al*., 1987), malaria (Katzin *et al*., 1991), schistosomiasis (Kremsner *et al*., 1993), Chagas' disease (Freilij *et al*., 1987), filariasis (Zheng *et al.*, 1987b), cystic echinoccocosis (Parija *et al*., 1997). These have proven to be a good alternative to the common tests that utilize serum as specimen. They all have been reported to work well in clinical settings; however their performance in field conditions is not well described. The same holds for the urine Ag-ELISA for the diagnosis of human NCC that has so far only been evaluated in clinical settings (Castillo *et al*., 2009; Parija *et al*., 2004). Therefore, the aim of this study is to evaluate the performance of the urine B158/B60 antigen ELISA for the detection of human cysticercosis under field conditions.

6.2   Materials and methods

6.2.1   Study areas

Community based studies were carried out in *T. solium* endemic rural areas in the parish of Cazaderos, situated in the southern Andean province of Loja, Ecuador (Praet *et al.*, 2010c) and in the Eastern Province of Zambia (Phiri *et al.*, 2002; Dorny *et al.*, 2004b; Sikasunge *et al.*, 2008b). Paired urine and blood samples were collected between September and November 2007 from 748 participants in Ecuador and between July and August 2009 from 690 participants in Zambia (Chapter 3). Samples were collected from all people from the community that volunteered to participate in the study and this included both sexes and ages 1 to 98 years.

6.2.2   Urine and blood sample collection and storage

All participants were provided with a disposable plastic container in which to place the urine. Upon submission of a urine sample, 5 ml of blood (2 ml from children) was then collected in plain blood tubes. The urine was aliquoted in duplicates of 1.8 ml vials and stored at -20°C until use. Blood was allowed to clot and kept overnight at 4°C, after which it was centrifuged at 3000 g for 15 minutes. The serum obtained was aliquoted in 1.8 ml vials and stored at -20°C until use.

6.2.3 Detection of cysticercal antigens in the urine and serum

The B158/B60 Ag-ELISA to detect cysticercal antigens in serum and urine was done as described by Dorny *et al*. (2004) and Castillo *et al*. (2009), respectively. Briefly, some modifications in the serum protocol were carried out for the analyses of the urine samples: ELISA plates were coated with the capture monoclonal antibody (MoAb) B158C11A10 in bicarbonate buffer at 5µg/ml, washed, and blocked with Phosphate Buffered Saline (PBS) to which Tween 20 and 1% new born calf serum (NBCS) were added. Unlike serum samples, urine samples were not pre-treated using trichloroacetic acid (TCA) however, were diluted 1:2 in blocking buffer and pre-incubated with the biotinylated MoAb B60H8A4 for 1 hour. Next, the pre-treated urine samples were added to the wells and incubated at 37ºC on a shaker for 15 min. The plates were emptied, dried and without washing, another 100 µl of the urine-MoAb mixture was added. The plates were then incubated overnight at 4°C and without shaking. Afterwards, the same procedure as for serum samples was followed, that is washing, incubation with streptavidin-horseradish peroxidase diluted at 1/10,000 in blocking buffer, washing and incubation with orthophenylene diamine (in distilled water with hydrogen peroxide). Finally, to stop the reaction, 50µl of 4N H_2SO_4 was added to each well. Eight negative and 2 positive control serum samples were run on each plate. The plates were read using an automated spectrophotometer at 490 nm with a reference of 655 nm. The optical density of each serum/urine sample was compared with a sample of negative serum/urine samples (n = 8) at a probability level of $P = 0.001$ to determine the result in the test (Sokal & Rohlf, 1981).

6.2.4 Data analyses

The agreement between the serum and urine Ag-ELISA results was determined calculating the positive and negative agreement indices (AI) (Erhart *et al*., 2002; Bhattarai *et al*., 2009). Credibility intervals estimates (95%) were calculated using the Bayesian method proposed by Graham and Bull (Graham & Bull, 1998).

Moreover, a multinomial Bayesian model adapted from Berkvens *et al*. (2006) was used (see appendix, section 6.5) to estimate the characteristics (sensitivity and specificity) of the serum and urine Ag-ELISA's to detect infected individuals. A Bayesian Latent Class Analysis was selected as method of choice, because none of the tests described in this study is a gold standard. The

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Bayesian approach allows prior information on test sensitivity and test specificity to be combined with the diagnostic test results at hand. Assuming good test result reproducibility, various prior information scenarios can be evaluated. Prior information on the test characteristics was obtained from the available literature (Castillo *et al.* (2009) for the urine Ag-ELISA and Praet *et al.* (2010b) for the serum Ag-ELISA) and adapted by experts of the Institute of Tropical Medicine of Antwerp (Belgium) to be expressed as conditional probabilities (Table 6.1). The model allows estimating the credibility intervals for differences between the estimated characteristics of the same test between countries and between tests. A credibility interval with both limits having the same sign (zero not included in the interval) can be interpreted as the equivalent of a significant result in a frequentist approach (Adel *et al.*, 2010; Praet *et al.*, 2010b). The analysis was conducted in WinBUGS and R. Criteria assessing the fit between prior information and test results were evaluated [i.e., the Bayesian *P* value (Bayesp), the Deviance Information Criterion [DIC], and the number of parameters effectively estimated by the model (pD)] (Berkvens *et al.*, 2006).

Table 6.1: Prior information for the detection of infected individuals in Ecuador and Zambia (uniform distributions)*

Sensitivity of the Ag-ELISA for the detection of infected individuals (th1[2] and th2[2] in the model; see appendix, section 6.5)	[0.8 - 1]
Specificity of the Ag-ELISA for the detection of infected individuals (th1[3] and th2[3]in the model; see appendix, section 6.5)	[0.97 - 1]
Probability to have a positive result for the urine Ag-ELISA if the individual is infected and positive for the serum Ag-ELISA (th1[4] and th2[4] in the model; see appendix, section 6.5)	[0.82 - 1]
Probability to have a negative result for the urine Ag-ELISA if the individual is not infected and negative for the serum Ag-ELISA (th1[6] and th2[6] in the model; see appendix, section 6.5)	[0.99 - 1]

* the other probabilities are not constrained and left as uniform distributions [0 - 1] (see appendix, section 6.5)

6.3 Results

6.3.1 Antigen detection in serum and urine

Of the 748 samples collected in Ecuador, 21 (2.8%) were positive on serum Ag-ELISA and 90 (12.0%) on urine Ag-ELISA. Eight samples (1.1%) were positive on both serum and urine Ag-ELISA (Table 6.2).

Table 6.2: Serum and urine Ag-ELISA results for the samples from Ecuador

		Serum		
		+ ve	- ve	Total
Urine	+ ve	8	82	90
	- ve	13	645	658
	Total	21	727	748

In Zambia, 690 paired samples were collected, 41 samples (5.9%) were positive on serum Ag-ELISA and 168 samples (24.3%) were positive on urine Ag-ELISA (Table 6.3).

Table 6.3: Serum and urine Ag-ELISA results for the samples from Zambia

		Serum		
		+ ve	- ve	Total
Urine	+ve	27	141	168
	-ve	14	508	522
	Total	41	649	690

6.3.2 Agreement between the serum and urine Ag-ELISA results

A better agreement was observed in the negative direction (AI of 93.1 and 86.8 for Ecuador and Zambia, respectively) than in the positive direction (AI of 14.4 and 25.8 for Ecuador and Zambia, respectively) (Table 6.4).

Table 6.4: Positive and negative agreement indices (AI) between serum and urine Ag-ELISA with 95% credibility interval estimates for Zambia and Ecuador

Country	Positive AI (%)	95% Credibility Interval	Negative AI (%)	95% Credibility Interval
Ecuador	14.4	7.3-23.3	93.1	92.0-94.0
Zambia	25.8	19.0-33.0	86.8	85.0-89.0

6.3.3 Sensitivity and specificity of the serum and urine Ag-ELISA's

Prior information used in the model was based on Castillo *et al*. (2009) for the urine Ag-ELISA and Praet *et al*. (2010b) for the serum Ag-ELISA. However, when running the model, the prior on the specificity of the urine Ag-ELISA was not in agreement with the Ecuadorian and Zambian test results. Therefore, th1[6] and th2[6] in the model (see appendix, section 6.5) were relaxed from [0.99 - 1] to [0.7 - 1].

The sensitivity for the serum and urine Ag-ELISA was not statistically different for both tests in both countries (Table 6.5). The specificity of the serum Ag-ELISA was not statistically different in both countries, and statistically higher than the specificity of the urine Ag-ELISA in both countries. The specificity for the urine Ag-ELISA was statistically higher in Ecuador than in Zambia (Table 6.5).

Table 6.5: Sensitivity (Se) and specificity (Sp) and 95% credibility interval estimates of the serum and urine Ag-ELISA for samples from Zambia and Ecuador (expressed as percentages)

	Serum Ag-ELISA	Urine Ag-ELISA
Se Ecuador	90 (80-99)	86 (74-98)
Se Zambia	90 (80-99)	85 (73-98)
Sp Ecuador	98 (97-100)	88 (86-91)
Sp Zambia	98 (98-99)	78 (75-81)

6.4   Discussion

We aimed to explore the usefulness of urine as a sample for the diagnosis of human cysticercosis. Detecting specific antigen in urine is a non invasive approach in the diagnosis of parasitic infections and the excretion of cysticercal antigens in urine has already been demonstrated by other researchers (Parija *et al*., 2004; Castillo *et al.*, 2009). Up to now studies have been conducted in clinical settings with very encouraging results and therefore, the current study aimed to evaluate the use of urine as a specimen in field settings.

As a gold standard diagnosis was not available in this study, a Bayesian analyses was used to estimate characteristics of both tests. This approach consists of combining data and prior information (knowledge) to estimate posterior parameters (sensitivity, specificity of the diagnostic tests and true prevalence of the disease). Prior information on the parameters can be fixed on a deterministic way or be given a probability distribution. Experts having experience on the diagnosis of the disease can provide this knowledge or information can be found in publications reporting the results of similar experiments conducted elsewhere (Praet *et al.*, 2010c). As this model allows estimating the credibility intervals for differences between the estimated characteristics of the same test between countries and between tests, it was considered as a convenient methodology for this study in which different populations are compared.

Results from this study determined similar sensitivities for both urine and serum in both countries, with an estimated sensitivity of the urine Ag-ELISA of 86%, which is slightly lower

than the 91% reported by Castillo *et al*. (2009). However, the estimates for specificity of the urine Ag-ELISA are considerably lower in this study compared to the 100% reported by Castillo *et al*. (2009) using the same B158/B60 Ag-ELISA and Parija *et al*. (2004) using the staphylococcal Co-agglutination test. However, those two studies were hospital-based with a lower number of samples (one group of NCC patients and one control group), and the methodology used by Parija *et al*. (2004) is different. In addition, the high estimates for test performances recorded were based on a subgroup of individuals with viable NCC, and reduced sensitivities and specificities were noted for the other subgroups. As our research was field-based, a mix of infected and non-infected individuals is expected in our sample. The infected group is likely to comprise individuals with NCC and with extra-neural cysticercosis. In addition, cysticerci may be in different stages of development and degeneration. All these factors may impact on the absence or presence and on the level of circulating antigens. In a recent paper from Praet *et al*. (2010b) differences in test characteristics have been determined depending on the infection status of the individuals, highlighting the importance of a case definition when determining test sensitivity and specificity.

The lower specificity of the urine test calls for further clarification. In a different study where NCC patients were followed up, it was noticed that after treatment (praziquantel on the first day followed by a two weeks course of albendazole) urine levels remained positive more than a month after serum levels became negative (personal communication P. Dorny). This could be due to the delayed clearance of cysticercal antigens in the urinary system, resulting in negative serum results while still positive urine ELISA's. This phenomenon needs further research and could explain the lower specificity estimates of the urine Ag-ELISA in this study. Another factor could be the TCA treatment included in the serum protocol and cause of an extra dilution of the sample (TCA treatment and subsequent neutralization results in a 1:4 dilution). This step is not included in the urine Ag-ELISA, as such the more concentrated urine samples can turn out positive in the urine test while the more diluted serum samples remain under the cut off. A revision of the cut off calculation should perhaps be envisaged. Dehydration occurs fairly often in hot climates (but not under hospital conditions) and causes a concentration of the urine, which could again explain higher levels of antigen if present. The lower estimate on specificity for the Zambian samples in comparison with the samples from Ecuador could be indicative for the occurrence of more cross reactions in Zambia. High prevalences of other parasites such as soil

transmitted helminths and *Schistosoma* spp. were recorded in the Zambian study area (publication in preparation; personal communication, K. E. Mwape and G. Zulu). In Ecuador, *Hymenolepis nana*, *Trichuris trichiura*, *Strongyloides stercoralis*, hookworms and *Ascaris lumbricoides* were detected in only four, two, five, two and one individuals, respectively (Praet *et al.*, 2010c). Even though no cross reactions have been reported for helminths in the serum Ag-ELISA, (Erhart *et al*., 2002; Dorny *et al*., 2003) this hasn't been investigated for the urine Ag-ELISA, and as such cannot be excluded. Cross reactions with *Trypanosoma* spp. were observed before the inclusion of the TCA treatment in the serum protocol. As this step is not included in the urine test, this could be a possible interfering factor. However, a recent study indicated the occurrence of only 82 new cases of sleeping sickness in Zambia during the last ten years (Simarro *et al*., 2010).

The current study was a large scale field study and compared results from two geographically different endemic areas on different continents and therefore gives an improved understanding of the suitability of the urine Ag-ELISA as described by Castillo *et al.* (2009) in cysticercosis diagnosis. Results indicate that in field-based surveys the performance of the urine Ag-ELISA is inferior to the serum Ag-ELISA, more specifically regarding its specificity. The urine Ag-ELISA could possibly be an excellent clinical tool in the case of diagnosis/follow up of individuals with viable NCC, (Parija *et al*., 2004; Castillo *et al*., 2009) however under field conditions the use of serum is preferential at this moment. Alternatively, the urine Ag-ELISA can be used as a first screening tool, followed by confirmatory serum Ag-ELISA. The advantages of urine as a sample specimen, such as the non-invasiveness and the easy acceptability by the community justify further research to address the poorer specificity of urine Ag detection.

6.5   Appendix: Bayesian model run in WinBUGS

```
Model
{
res1[1:4] ~ dmulti( p1[1:4], n1)
res2[1:4] ~ dmulti( p2[1:4], n2)

p1[1] <- th1[1]*th1[2]*th1[4] + (1-th1[1])*(1-th1[3])*(1-th1[7])
p1[2] <- th1[1]*th1[2]*(1-th1[4]) + (1-th1[1])*(1-th1[3])*th1[7]
```

```
p1[3] <- th1[1]*(1-th1[2])*th1[5] + (1-th1[1])*th1[3]*(1-th1[6])
p1[4] <- th1[1]*(1-th1[2])*(1-th1[5]) + (1-th1[1])*th1[3]*th1[6]

p2[1] <- th2[1]*th2[2]*th2[4] + (1-th2[1])*(1-th2[3])*(1-th2[7])
p2[2] <- th2[1]*th2[2]*(1-th2[4]) + (1-th2[1])*(1-th2[3])*th2[7]
p2[3] <- th2[1]*(1-th2[2])*th2[5] + (1-th2[1])*th2[3]*(1-th2[6])
p2[4] <- th2[1]*(1-th2[2])*(1-th2[5]) + (1-th2[1])*th2[3]*th2[6]

th1[1] ~ dunif(0,1)
th1[2] ~ dunif(0.8,1)
th1[3] ~ dunif(0.97,1)
th1[4] ~ dunif(0.82,1)
th1[5] ~ dunif(0,1)
th1[6] ~ dunif(0.7,1)
th1[7] ~ dunif(0,1)

th2[1] ~ dunif(0,1)
th2[2] ~ dunif(0.8,1)
th2[3] ~ dunif(0.97,1)
th2[4] ~ dunif(0.82,1)
th2[5] ~ dunif(0,1)
th2[6] ~ dunif(0.7,1)
th2[7] ~ dunif(0,1)

se1[1] <- th1[2]
sp1[1] <- th1[3]
se1[2] <- th1[2]*th1[4] + (1-th1[2])*th1[5]
sp1[2] <- th1[3]*th1[6] + (1-th1[3])*th1[7]

se2[1] <- th2[2]
sp2[1] <- th2[3]
se2[2] <- th2[2]*th2[4] + (1-th2[2])*th2[5]
sp2[2] <- th2[3]*th2[6] + (1-th2[3])*th2[7]

for ( i in 1:4)
{
d1[i] <- res1[i]*log(max(res1[i],1)/(p1[i]*n1))
}
G0 <- 2*sum(d1[])
res11[1:4] ~ dmulti(p1[1:4],n1)

for (i in 1:4)
{
d11[i] <- res11[i]*log(max(res11[i],1)/(p1[i]*n1))
}
Gt <- 2*sum(d11[])
```

```
bayesp1 <- step(G0 - Gt)

for ( i in 1:4)
{
d2[i] <- res2[i]*log(max(res2[i],1)/(p2[i]*n2))
}
G10 <- 2*sum(d2[])
res22[1:4] ~ dmulti(p2[1:4],n2)

for (i in 1:4)
{
d22[i] <- res22[i]*log(max(res22[i],1)/(p2[i]*n2))
}
G1t <- 2*sum(d22[])
bayesp2 <- step(G10 - G1t)

for ( i in 1:2)
{
sediff[i]<-se1[i]-se2[i]
spdiff[i]<-sp1[i]-sp2[i]
}
prevdiff<-th1[1]-th2[1]

for ( i in 1:1)
{
sediffer112[i]<-se1[1]-se1[2]
sediffer212[i]<-se1[1]-se1[2]
spdiffer112[i]<-sp1[1]-sp1[2]
spdiffer212[i]<-sp1[1]-sp1[2]
}
}
list(res1=c(8,13,82,645), n1=748, res2=c(27,14,141,508), n2=690)
```

CHAPTER 7

Improving the diagnosis of taeniosis, a worldwide parasitic disease of public health and economic importance

7.1   Introduction

Even though taeniosis is a worldwide parasitic disease, detection of tapeworm carriers remains a public health issue (Flisser, 2006). In humans, this zoonotic disease is caused by three *Taenia* species i.e. *Taenia saginata*, *Taenia solium* and *Taenia saginata asiatica* (Murrell, 2005). While the life cycle differs from one species to another, clinical signs in infected individuals are quite similar since most carriers remain asymptomatic but gastrointestinal dysfunctions, loss of appetite and weight loss may be observed. Unlike taeniosis where symptoms are not of major clinical importance, the pathology caused by the establishment of the larval stage of *T. solium* in the central nervous system of accidental hosts (human), neurocysticercosis, may be responsible for a high disease burden and morbidity in endemic areas (Murrell, 2005). On the other hand, the presence of cysticerci in the specific intermediate hosts i.e. cattle for *T. saginata*, pigs for *T. solium* and *T. s. asiatica*, may be of economical importance due to carcass condemnation in countries where carcass inspection at abattoir level is applied (Murrell, 2005; Carabin *et al.*, 2006; Praet *et al.*, 2009).

The coproscopic examination of stool samples has remained the routine method for the diagnosis and identification of *Taenia* spp. eggs to date. Although coproscopy as a diagnostic method shows a high specificity, the sensitivity, however, is rather low (Allan *et al.*, 1996b; Flisser, 2006; Somers *et al.*, 2006). This is primarily due to the intermittent nature of egg/proglottid excretion by the tapeworms (Garcia *et al.*, 2003a). In addition, the technique does not allow differentiating between *Taenia* species, which is essential for the clinical follow-up of patients living in areas where the parasites are sympatric (Ito *et al*., 2003).

As an alternative, an immunological technique, the copro-antigen Enzyme-Linked Immunosorbent Assay (copro-Ag ELISA) was developed over 20 years ago (Allan *et al.*, 1990).

The method aims at detecting tapeworm antigens in faeces using polyclonal antibodies directed against the parasite. Some studies demonstrated a higher sensitivity of the assay compared to coproscopy (Allan *et al.*, 1996b) but this higher test performance has been questioned elsewhere (Somers *et al.*, 2006). Although the test has proven to be very useful for the diagnosis of taeniosis, it is only genus specific and as such, cannot differentiate among *T. solium, T. saginata* and *T. s. asiatica* infections (Allan *et al.*, 2003; Ito & Craig, 2003). Furthermore, this assay is not commercially available. A modification of the copro-Ag ELISA originally described by Allan *et al.* (1990) has been reported to be *T. solium* species specific with a specificity of 100% (Guezala *et al.*, 2009). This is a hybrid assay because it combines the use of polyclonal antibodies against *Taenia* adult tapeworm somatic extracts and an enzyme conjugated rabbit IgG against *T. solium* adult excretory-secretory antigen; however, this test was only validated on a small number of samples.

The possibility of serological diagnosis has been described using *T. solium* specific antigens to detect antibodies against adult *T. solium* in serum by Western blot. The test is reported to have sensitivity and specificity rates of 95% and 100%, respectively (Wilkins *et al.*, 1999). The serological diagnosis of taeniosis, however, has the disadvantage of residual antibodies from past exposure of individuals that might result in false positives (Allan *et al.*, 2003; Ito & Craig, 2003; Meza-Lucas *et al*., 2003). The test is also currently only used for research purposes owing to the cost and accessibility of the test as the purification process of the antigens requires expensive equipment (Allan *et al*., 2003) and the commercial strips are very expensive. Hendali *et al.*, 2010, described a rapid test method using recombinant proteins for the immunodetection of taeniosis which could be affordable, reliable, rapid and easy to perform. Though feasible, the test however, requires field evaluation and improvements on its sensitivity for taeniosis detection in endemic areas.

Molecular techniques have also been developed allowing species-specific tapeworm detection in faeces (Nunes *et al*., 2003; Yamasaki *et al*., 2004; Nunes *et al*., 2005; Mayta *et al*., 2008; Nkouawa *et al*., 2009; Jeon *et al.*, 2011). However, these methods have not yet been properly validated in the field.

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This study aims at estimating and comparing the test characteristics such as sensitivity and specificity of coproscopy, copro-Ag ELISA and a newly developed real-time polymerase chain reaction assay (PCR) specific for the detection of *T. solium* and *T. saginata* DNA in faeces (copro-PCR). The three diagnostic tests have been applied on stool samples collected in two Zambian communities where taeniosis is endemic. Because none of the tests included in the study design is a gold standard method and because no gold standard test for the diagnosis of taeniosis exists, a Bayesian approach was used to allow estimation.

7.2 Materials and methods

7.2.1 Study design

7.2.1.1 *Faecal samples and tests*

The sampling protocol of the present study has been described in Chapters 3 and 4. Samples were collected in the two community-based studies conducted in the rural communities of Kakwiya and Vulamkoko, in Petauke and Katete districts, respectively, of the Eastern province of Zambia. The collected faecal samples were tested using 3 diagnostic methods, namely coproscopy, microscopic identification of *Taenia* spp. eggs in faeces according to Ritchie (1948), the copro-Ag ELISA (Allan *et al*. 1990; Chapter 3) and the copro-PCR.

7.2.1.2 *DNA isolation*

DNA isolation for the copro-PCR test was carried out in Lusaka and further analyses at the Leiden University Medical Centre. DNA isolation from the ethanol stored samples was conducted as described by Verweij *et al*. (2009). Briefly, approximately 200 µg faeces were suspended in 200 µl PBS containing 2% polyvinylpolypyrolidone (PVPP; Sigma, Steinheim, Germany) and heated for 10 minutes at 100 °C. After sodium-dodecyl sulphate-proteinase K treatment (2 hours at 55 °C), DNA was isolated with the QIAamp Tissue Kit spin columns (QIAgen, Hilden, Germany). In each sample, 103 PFU/ml Phocin Herpes Virus 1 (PhHV-1) was added within the isolation lysis buffer, to serve as an internal control (Niesters, 2002).

7.2.1.3 *DNA amplification and detection*

T. solium and *T. saginata*-specific primers and detection probes were designed using Primer Express software (Applied Biosystems, Foster City, CA, USA) from the Internal Transcribed Spacer 1 (ITS1) sequences for *T. solium* and *T. saginata*, respectively (GenBank accession nos. EU747662 and AY392045). The *T. solium*-specific primers, TsolITS_145F and TsolITS_230R (Biolegio, The Netherlands) were chosen such that an 86-bp fragment inside the ITS1 sequence should be amplified. The Tsol_ITS_169Tq_FAM double labelled probe (Biolegio) was used to detect *T. solium*-specific amplification. *T. saginata*-specific PCR primers and a detection probe were chosen such that a 79-bp fragment within the ITS1 sequence should be amplified and detected for *T. saginata* specifically. The *T. saginata*-specific primers and probe set consisted of forward primer Tsag_ITS_F529, reverse primer Tsag_ITS_R607, and the *T. saginata*-specific double labelled probe Tsag_ITS_581Tq_Quasar705 (Biolegio). PhHV-1-specific primers and probe (Niesters, 2002) set consisted of forward primer PhHV-267s, reverse primer PhHV-337as and the double-labelled probe PhHV-305tq (Biolegio). National Center for Biotechnology Information (NCBI) BLAST search was used to test the theoretical specificity of the primers and probes.

To establish the PCR assays, genomic DNA was isolated using the QIAamp Tissue Kit (QIAgen, Hilden, Germany) from individual proglottids of *T. solium* and *T. saginata*. Serial 10-fold dilution series of DNA extracted from each cestode were tested with and without the presence of internal control DNA to estimate the influence of the internal control. Each dilution series was also tested with and without the other target to assess the ability to detect mixed infections. The PCR and the DNA isolation protocol used was further evaluated using DNA extracts of 25 stool samples from individual patients from a *T. solium* and *T. saginata* endemic area in Ecuador, in which microscopy revealed *Taenia* eggs. The specificity of the PCR was tested against 150 DNA controls derived from people infected with a wide range of intestinal microorganisms (ten Hove *et al*., 2008).

Amplification reactions were performed in white PCR plates in a volume of 25 µl with PCR buffer (HotstarTaq master mix, QIAgen, Germany), 5 mM $MgCl_2$, 2.5 µg Bovine Serum Albumin (Roche Diagnostics Nederland B.V., Almere, the Netherlands), 1 pmol of each *T.*

solium-specific primer, 2 pmol of each *T. saginata*-specific primer, 3.75 pmol of each PhHV-1-specific primer, 1.25 pmol of each specific detection probe and 5 µl of the DNA sample. Amplification consisted of 15 minutes at 95ºC followed by 50 cycles of 15 seconds at 95ºC, 30 seconds at 60ºC. Negative and positive control samples were included in each amplification run. Amplification, detection, and analysis was performed with the CFX real-time detection system (Bio-Rad Laboratories). The PCR output consists of a cycle-threshold (Ct) value, representing the amplification cycle in which the level of fluorescent signal exceeds the background fluorescence, and indicating the parasite-specific DNA load in the faecal sample tested. All primers and detection probes are described in Table 7.1.

Table 7.1: Oligonucleotide primers and detection probes for real-time PCR on different targets for the detection of *T. solium* and *T. saginata* DNA.

Target organism Oligonucleotide name	Oligonucleotide sequence
Taenia solium	
Tsol_ITS_145F	5'-ATGGATCAATCTGGGTGGAGTT-3'
Tsol_ITS_230R	5'-ATCGCAGGGTAAGAAAAGAAGGT-3'
Tsol_ITS_169Tq	FAM 5'-TGGTACTGCTGTGGCGGCGG-3'-BHQ 1
Taenia saginata	
Tsag_ITS_F529	5'-GCGTCGTCTTTGCGTTACAC-3'
Tsag_ITS_R607	5'-TGACACAACCGCGCTCTG-3'
Tsag_ITS_581Tq	Quasar705 5'-CCACAGCACCAGCGACAGCAGCAA-3'-BHQ2
Phocin Herpes Virus 1	
PhHV-267s	5'-GGGCGAATCACAGATTGAATC-3'
PhHV-337as	5'-GCGGTTCCAAACGTACCAA-3'
PhHV-305tq	Cy5 5'-TTTTTATGTGTCCGCCACCATCTGGATC-3'-BHQ2

BHQ; Black Hole Quencher

7.2.2 Bayesian analysis

Because none of the diagnostic tests included in this study is a gold standard, a Bayesian analysis was used to estimate the prevalence of taeniosis in this population and the characteristics of the tests. A multinomial Bayesian model adapted from Berkvens *et al.* (2006) was used (appendix, section 7.5). Prior information (Table 7.2) on the test characteristics was extracted from available literature or obtained through expert elicitation (experts of the Institute of Tropical Medicine of Antwerp (Belgium) and of the Leiden University Medical Centre, Leiden (The Netherlands) and expressed as conditional probabilities (Tables 7.2; Allan *et al.*, 1990; Murrell, 2005; Flisser, 2006). The analysis was conducted in WinBUGS and R (Ihaka & Gentlemen, 1996; Lunn *et al.*, 2000). The Markov chain Monte Carlo model was run for 25000 iterations and the first 5000 iterations were discarded as the burn-in phase. Criteria assessing the fit between prior information and test results were evaluated, i.e. the Bayesian *P*-value (Bayesp), the Deviance Information Criterion (DIC) and the number of parameters effectively estimated by the model (pD) (Lunn *et al.*, 2000; Spiegelhalter *et al.*, 2002; Gelman *et al.*, 2003; Berkvens *et al.*, 2006).

Table 7.2: Prior information included in the Bayesian model (uniform distributions)*

Probability to have a positive result for copro-Ag ELISA if the individual is infected and positive for coproscopy (th[4] in the model; see appendix, section 7.5)	[0.9 - 1]
Probability to have a positive result for copro-Ag ELISA if the individual is infected and negative for coproscopy (th[5] in the model; see appendix, section 7.5)	[0.5 - 1]
Probability to have a positive result for copro-PCR if the individual is infected, positive for coproscopy and positive for copro-Ag ELISA (th[8] in the model; see appendix, section 7.5)	[0.95 - 1]
Probability to have a positive result for copro-PCR if the individual is infected, positive for coproscopy and negative for copro-Ag ELISA (th[9] in the model; see appendix, section 7.5)	[0.95 - 1]
Probability to have a positive result for copro-PCR if the individual is infected, negative for coproscopy and positive for copro-Ag ELISA (th[10] in the model; see appendix, section 7.5)	[0.5 - 1]
Probability to have a negative result for copro-PCR if the individual is not infected, negative for coproscopy and negative for copro-Ag ELISA (th[12] in the model; see appendix, section 7.5)	[0.95 - 1]
Probability to have a negative result for copro-PCR if the individual is not infected, negative for coproscopy and positive for copro-Ag ELISA (th[13] in the model; see appendix, section 7.5)	[0.95 - 1]
Probability to have a negative result for copro-PCR if the individual is not infected, positive for coproscopy and negative for copro-Ag ELISA (th[14] in the model; see appendix, section 7.5)	[0.95 - 1]
Probability to have a negative result for copro-PCR if the individual is not infected, positive for coproscopy and positive for copro-Ag ELISA (th[15] in the model; see appendix, section 7.5)	[0.95 - 1]

* the other probabilities are not constrained and left as uniform distributions [0 - 1]

Coproscopy = microscopic identification of *Taenia* spp. eggs in faeces; copro-Ag ELISA = Enzyme-Linked Immunosorbent Assay for the detection of *Taenia* spp. antigens in faeces; copro-PCR = Polymerase Chain Reaction assay for the detection of *T. solium* DNA in faeces.

7.3   Results

A total of 934 faecal samples (718 from Petauke and 226 from Katete) were collected. Of these, 817 had quantities enough for the three tests and the results of these are presented herein. The results for the three tests are shown in Table 7.3. Presence of other helminthic infections during coproscopy was also noted and the results are presented in Chapters 3 and 4.

Table 7.3: Results of the three diagnostic tests applied on 817 faecal samples of the same number of individuals.

Coproscopy	Copro-Ag ELISA	Copro-PCR	No. of individuals
0	0	0	744
0	0	1	6
0	1	0	63
0	1	1	2
1	0	0	0
1	0	1	0
1	1	0	0
1	1	1	2

0 = negative test result; 1 = positive test result; No. of individuals = number of individuals for each result category. Coproscopy = microscopic identification of *Taenia* spp. eggs in faeces; copro-Ag ELISA = Enzyme-Linked Immunosorbent Assay for the detection of *Taenia* spp. antigens in faeces; copro-PCR = Polymerase Chain Reaction assay for the detection of *T. solium* DNA in faeces.

Bayesian sensitivity and specificity estimates of the three diagnostic tests for the detection of taeniosis are presented in Table 7.4. There was no disagreement between the prior information and the test results as indicated by the Bayesp, the DIC and the pD.

Table 7.4: Estimates of the sensitivity and specificity of the three diagnostic tests for the detection of taeniosis

Diagnostic test	Sensitivity (95% CI)	Specificity (95% CI)
Coproscopy	0.525 (0.111-0.965)	0.999 (0.995-1.000)
Copro-antigen ELISA	0.845 (0.619-0.980)	0.920 (0.900-0.938)
Copro-PCR	0.827 (0.570-0.976)	0.990 (0.982-0.996)

CI = credibility interval; Coproscopy = microscopic identification of *Taenia* spp. eggs in faeces; copro-Ag ELISA = Enzyme-Linked Immunosorbent Assay for the detection of *Taenia* spp. antigens in faeces; copro-PCR = Polymerase Chain Reaction assay for the detection of *T. solium* DNA in faeces.

Eleven cases of taeniosis were diagnosed by copro-PCR, with ten or 91.0% being due to *T. solium* and one or 9.0% due to *T. saginata*. The taeniosis due to *T. solium* crude prevalence was estimated at 1.2% while the Bayesian analysis estimated the prevalence at 0.6% (95% Credibility Interval: 0.1-1.7) in the selected population.

7.4   Discussion

This study gives an estimation of the characteristics of three diagnostic tests to detect *T. solium* taeniosis in individuals living in an endemic area. Bayesian analysis shows high specificities for the three tests (between 90 and 100%). However, specificity of the copro-Ag ELISA is lower than specificity estimates for the two other tests, and lower than reported in the literature. In a study in Peru, a specificity of 98% was reported for the detection of *T. solium* (Zamora *et al.*, 2004). In our study, 63 individuals are positive for copro-Ag ELISA but negative for the other tests. Allan and colleagues (1990) demonstrated that antigen detection in faeces is genus specific with *T. saginata* and *T. solium* both reacting in the assay but with no cross-reactions with other parasite infections. Ninety one percent of the taeniosis cases diagnosed by copro-PCR were due to *T. solium*, indicating that the pork tapeworm is much more prevalent in the study area. This

study reports for the first time the presence of *T. saginata* in this part of Zambia and the sympatricity with *T. solium* may explain the difference in specificity among the tests. However, the two positive individuals on coproscopy were both confirmed as being infected with *T. solium* using copro-PCR. Moreover, beef is rarely consumed in the area and when consumed, it is boiled, suggesting a low *T. saginata* prevalence.

Though Allan and colleagues (1996b) already pointed out the presence of false positive results with the copro-Ag in a field study conducted in Guatemalan communities, cross-reactions with other parasites other than *Taenia* spp. have not been reported (Allan *et al*., 2003). Nevertheless, potential non-specific reactions of the polyclonal antibodies should be further investigated. In this study, the *T. saginata* positive sample by copro-PCR which was also copro-Ag positive highlights the non-specificity of the copro-Ag test using polyclonal antibodies against adult *T. solium*. This calls for further improvements in the copro-Ag ELISA test as the differential diagnosis of taeniosis has public health implications.

The ability of the copro-Ag ELISA to detect immature tapeworm stages may also explain the higher number of copro-Ag ELISA positive cases compared to coproscopy (only detecting eggs and so adult, gravid tapeworms). The copro-PCR is also thought to be less efficient for the detection of immature tapeworms (Verweij J., personal communication). To which level the copro-PCR is dependent on the presence of reproductive material (eggs) or on high amounts of DNA liberated by long worms, requires further investigation.

The rate at which tapeworms establish following ingestion of cysticerci is not well known. It is generally assumed that only one tapeworm develops in a host (solitary worm). Competition between tapeworms, of the same or different species, influencing their establishment has been suggested by Conlan *et al*. (2009). Since people may consume pork infected with many cysts, potentially many of these can develop into adult worms within one host. Studies have demonstrated that an important proportion of infected individuals can harbour multiple tapeworms, i.e. 8.2% and 20% in studies by Bustos *et al*. (2012) and Jeri *et al*. (2004), respectively. We may also speculate that some juvenile tapeworms are expelled before they reach maturity.

We report a sensitivity and specificity of 82.7 % and 99.0%, respectively, for the copro-PCR. These figures are lower than what was reported by Mayta *et al.* (2008) who used a nested PCR assay on archived samples giving sensitivity and specificity of 97% (32/32) and 100% (123/123), respectively. Their assay is further reported to have 100% sensitivity and specificity under field conditions using coproscopy and copro-Ag ELISA as gold standards. However, since these are not gold standard tests for the diagnosis of taeniosis, these characteristics could have been over-estimated

On the other hand, knowing that coproscopy is genus- and not species-specific, the specificity of this test to detect *T. solium* taeniosis as estimated here is particularly high probably because the infection is practically mono-specific. This can be explained by a low prevalence of *T. saginata* infections in the region even though reaching solid conclusions is impossible since apparent prevalence based on coproscopy is very low (only 2 individuals tested positive for coproscopy). Using such insensitive tests, a higher sample size has to be included in order to allow interpreting the estimated test characteristics.

Several authors have questioned the sensitivity of coproscopy mainly because, even in high prevalent *T. solium* pig and human cysticercosis areas, the apparent prevalence of taeniosis, as estimated using egg detection is usually very low. The discrepancy between high cysticercosis and low taeniosis prevalence remains a scientific issue (Flisser, 2006). Even though the results of the present study support the hypothesis of false negative results using coproscopy, the true prevalence of taeniosis estimated here remains very low (0.6%) compared to a prevalence of active cysticercosis, based on detection of circulating antigens of the metacestode of *T. solium* (Dorny *et al.*, 2004a) of 5.5% (Chapter 3) and 12.2% (Chapter 4) estimated in the same communities. Moreover, some recent epidemiological studies indicate a high exposure to *T. solium* eggs, based on detection of antibodies directed against the metacestode of *T. solium* (Tsang *et al.*, 1989) in endemic areas suggesting a high level of environmental contamination with the eggs (Garcia *et al.*, 2001; Praet *et al.*, 2010b; Chapter 3; Chapter 4).

In conclusion, this study compares for the first time the characteristics of three tests for the diagnosis of taeniosis. Although cross reactions have been demonstrated not to occur with the copro-Ag ELISA, additional studies to improve the test are required and the use of monoclonal

antibodies to detect antigens in stool is suggested. Further, the copro-PCR test requires improvements in its sensitivity as it has the added advantage of being highly specific. Improving the performance of the available tests for the detection of tapeworm carriers, remains a key factor in controlling the parasite in endemic areas.

7.5 Appendix: Bayesian model run in WinBUGS

```
model
{
result[1:8] ~ dmulti( pr[1:8], n)
pr[1] <- th[1]*th[2]*th[4]*th[8] + (1-th[1])*(1-th[3])*(1-th[7])*(1-th[15])
pr[2] <- th[1]*th[2]*th[4]*(1-th[8]) + (1-th[1])*(1-th[3])*(1-th[7])*th[15]
pr[3] <- th[1]*th[2]*(1-th[4])*th[9] + (1-th[1])*(1-th[3])*th[7]*(1-th[14])
pr[4] <- th[1]*th[2]*(1-th[4])*(1-th[9]) + (1-th[1])*(1-th[3])*th[7]*th[14]
pr[5] <- th[1]*(1-th[2])*th[5]*th[10] + (1-th[1])*th[3]*(1-th[6])*(1-th[13])
pr[6] <- th[1]*(1-th[2])*th[5]*(1-th[10]) + (1-th[1])*th[3]*(1-th[6])*th[13]
pr[7] <- th[1]*(1-th[2])*(1-th[5])*th[11] + (1-th[1])*th[3]*th[6]*(1-th[12])
pr[8] <- th[1]*(1-th[2])*(1-th[5])*(1-th[11]) + (1-th[1])*th[3]*th[6]*th[12]

th[1] ~ dunif(0,1)
th[2] ~ dunif(0,1)
th[3] ~ dunif(0,1)
th[4] ~ dunif(0.9,1)
th[5] ~ dunif(0.5,1)
th[6] ~ dunif(0,1)
th[7] ~ dunif(0,1)
th[8] ~ dunif(0.95,1)
th[9] ~ dunif(0.95,1)
th[10] ~ dunif(0.5,1)
th[11] ~ dunif(0,1)
th[12] ~ dunif(0.95,1)
th[13] ~ dunif(0.95,1)
th[14] ~ dunif(0.95,1)
th[15] ~ dunif(0.95,1)

se[1] <- th[2]
sp[1] <- th[3]
se[2] <- th[2]*th[4] + (1-th[2])*th[5]
sp[2] <- th[3]*th[6] + (1-th[3])*th[7]
se[3] <- th[2]*(th[4]*th[8]+(1-th[4])*th[9]) + (1-th[2])*(th[5]*th[10]+(1-th[5])*th[11])
sp[3] <- th[3]*(th[6]*th[12]+(1-th[6])*th[13]) + (1-th[3])*(th[7]*th[14]+(1-th[7])*th[15])
```

```
for ( i in 1:8)
{
d[i] <- result[i]*log(max(result[i],1)/(pr[i]*n))
}
G0 <- 2*sum(d[])
result2[1:8] ~ dmulti(pr[1:8],n)
for (i in 1:8)
{
d2[i] <- result2[i]*log(max(result2[i],1)/(pr[i]*n))
}
Gt<- 2*sum(d2[])
bayesp<- step(G0 - Gt)
}
list(result=c(2,0,0,0,2,63,6,744), n=817)
```

CHAPTER 8

General discussion and conclusions

In this chapter, the contributions of the present work to the understanding of the epidemiology of human *T. solium* infections, important shortcomings that still exist in transmission dynamics including diagnostic tools, and the possible options for control in eastern Zambia are discussed.

8.1   Transmission dynamics of human *T. solium* infections

The transmission dynamics of *T. solium* infections in endemic regions have not been fully studied. Some reports have demonstrated exposure levels, age and immunity, and also the trading of infected pigs as important factors influencing transmission (Rodriguez-Hidalgo *et al.*, 2006; Praet *et al.*, 2010a; Praet *et al.*, 2010c). Other studies have demonstrated highly varying infection levels/infection establishments indicating that, although many people are exposed, as expressed by not only short-term antibody, but also short term antigen presence (Table 4.3, Chapter 4), few develop active infections as reported by Meza-Lucas *et al.*, (2003), Fleury *et al.* (2004), Praet *et al.* (2010b) and Jayaraman *et al.* (2011) as well as our own observations in this study in Katete district (Chapter 4). Longitudinal studies would give insights into the transmission dynamics of *T. solium* infections in man. Such studies could provide estimates of the rate of exposure to the parasite, the rate and duration of newly established infections, detection and appearance of clinical signs, the viability of the adult tapeworms in terms of how long they live in the host and how much a carrier contaminates his/her environment and the duration of this contamination. However, such studies are constrained by logistical and ethical issues making their implementation very difficult/impossible, both on an individual and a large scale. Further, many sero-prevalence studies utilize antibody detection, which has the limitation of not specifically detecting viable infections (Garcia *et al.*, 2001; Dorny *et al.*, 2003). The use of both sero-Ab and Ag detection assays has the advantage of detecting not only exposure but also active infections in a population and has, hence, been recommended in epidemiological studies (Deckers *et al.*, 2008; Praet *et al.*, 2010c).

Animal models have been used to provide a better understanding of the disease dynamics. Studies with *T. saginata* in cattle have revealed that in highly endemic zones, animals get infected when they are young and gradually acquire resistance as they continuously get exposed (Murrell, 2005). It has been demonstrated, in pigs, that cysts develop faster and remain viable for a longer period in young than in older animals in which an effective innate immune response leads to reduced cyst numbers (Deckers *et al.*, 2008). The pig model is useful in understanding the infection dynamics in man, but it has its own limitations. In man, a decline in immunity is observed in the elderly people (Praet *et al.*, 2010a), but pigs are usually slaughtered before such a phenomenon may be observed. Further, the coprophagic nature of pigs tends to expose them to a more massive infection dose than in man (Garcia *et al.*, 2003c). It is, therefore, difficult to conclusively extrapolate the findings in the pig models to the situation in man.

8.2   Epidemiology of human *T. solium* infections in eastern Zambia.

The main aim of this study was to obtain a better understanding of the epidemiology of the human *T. solium* taeniosis/cysticercosis disease complex in the eastern part of Zambia. This was achieved through taeniosis and cysticercosis prevalence studies as well as determination of the incidence of cysticercosis, with the later study enabling a better understanding of the transmission dynamics of the disease in the study area.

According to the global distribution of *T. solium* infections reported by the World Health Organization (WHO, 2010), Zambia is described as endemic for the parasite despite it being identified in only some parts of the country. Since data in humans was largely lacking, this status was arrived at following extensive studies carried out in pigs (Phiri *et al.*, 2002; Dorny *et al.*, 2004b; Phiri *et al.*, 2006; Sikasunge *et al.*, 2007; Sikasunge *et al.*, 2008b). The estimated high prevalences of taeniosis and cysticercosis in the current study give a better picture of the situation in the eastern part of Zambia, confirming the endemicity of the disease in this area. *T. solium* taeniosis tends to have a low prevalence (typically of ≤ 1%, even in endemic communities) (Allan *et al.*, 1996a), and a community with a prevalence of ≥ 1% is considered hyper-endemic (Cruz *et al.*, 1989). Prevalence rates of 6.3% (Petauke) and 11.9% (Katete), determined in the current study, therefore, indicates that the communities in the study area are even hyperendemic. Since the general demography, environment, livestock management systems

and habits such as culinary traditions, open air defaecation and lack of hygiene are similar in all parts of each district, it could be assumed that the levels of infection were also similar. However, the differences observed in disease prevalences in the two neighbouring districts indicate a very different epidemiological situation. Therefore, the extrapolation of data to other areas is difficult and a generalization on country level (as done by the WHO) is not recommended.

The cysticercosis prevalence in the current study, as determined by the Ag-ELISA is higher than in most other countries not only on the African continent (Table 2.1, Chapter 2) but also in some countries in Latin America (Rodriguez-Hidalgo, 2007) and Asia (Murrell, 2005); though lower than in the Democratic Republic of Congo (Kanobana *et al.*, 2011). However, the prevalence based on sero-Ab analysis is comparable to most endemic areas in other continents (Rodriguez-Hidalgo, 2007).

In a study in the southern part of Ecuador, Praet *et al*. (2010c) demonstrated that age related decline in the human host's immunity was associated with increasing frequency of viable infections. In our study in Petauke district, age was strongly related to cysticercosis positivity on sero-Ag detection, however, this was not so in Katete. Unlike the study in Ecuador, the increase in infections in our Petauke study occurred at an earlier age due probably to an earlier decrease in immunity resulting from high levels of undernourishment and co-infections with other parasitic and pathogens. Since many other factors play a role in the establishment of infection, age-related host immunity could have been a less determining factor in the Katete area. It is possible that the Katete area has a highly contaminated environment, which is related to the number of tapeworm carriers. However, prevalence of taeniosis did not differ significantly in the two areas. The disposal of human stool and the survival of the egg in the environment can be affected by other factors, such as presence and use of latrines. The number of households with latrines was the same for the two areas; however, this does not imply that latrine use was the same for both areas. Indeed availability of latrines does not automatically mean that the people use them (Raja'a *et al.*, 2001). Also, physical environmental factors such as rainfall and landscape, as identified in studies on soil transmitted helminths (Weaver *et al.*, 2010) could also influence the spread and maintenance of *T. solium* eggs in the environment. The possible highly contaminated environment in the Katete area could account for the significantly higher human cysticercosis prevalence and also the high prevalence in pigs as reported by Sikasunge *et al*. (2008).

Unfortunately, antibody detection could not be done on samples from Petauke as this could have given insights on the levels of people's exposure to cysticercosis infection.

The longitudinal study in Katete using both antigen and antibody detection has provided insights in the transmission dynamics of human *T. solium* cysticercosis in a porcine cysticercosis endemic area. The high sero-Ab prevalence compared to sero-Ag prevalence probably indicates exposure rather than active infection (Garcia *et al.*, 2001). This is the first study to report sero-Ag incidence revealing an incidence rate of 6.2% over a period of 12 months. High rates of sero-Ab conversion of cysticercosis in humans and reversion in pigs and humans have been reported in Latin America highlighting the importance of this parameter in indicating levels of environmental contamination (Garcia *et al.*, 2001; Garcia *et al.*, 2003c; Meza-Lucas *et al.*, 2003). Interestingly, our study reveals that significantly higher seroreversion rates (Figure 4.3, Chapter 4) do also occur with sero-Ag analysis. This entails that in an endemic area, despite the constant exposure to infection, many individuals are either able to self-cure or the infection establishes itself but is quickly eliminated. But since they are constantly exposed, they still get re-infected (as can be seen by the 0.5% infected after an Ag positive test, at baseline, becoming negative after six months and positive after the next six months). Praet *et al*. (2010c) used rule-based simulation models and reported that in a continually exposed community, the rate of seroreversion (sero-Ab based) after one year decreases from 60% after first exposure to 20% after second and subsequent exposures. Our study shows higher sero-Ab reversion rate of 32.7% indicating a community with people having multiple exposures.

The taeniosis prevalence recorded in this study is higher than what has been reported in other parts of Africa (Table 2.1, Chapter 2). This could probably primarily be attributed to the improved diagnostic test used since our prevalence was based on coproantigen ELISA, which has better sensitivity than microscopic examination (Allan *et al.*, 2003). The prevalence of 0.3% in Petauke determined by microscopic examination compares very well with other reports in Africa and underscores the gross underestimation of the levels of infection by this test, which unfortunately, is the only one applied in many parts of the continent. Adult worms could however, not be collected from individuals positive on coproantigen ELISA after niclosamide treatment and purgation. Even though the assay has been reported not to cross react with other parasites and has been said to detect antigens not associated with reproductive material (i.e. from

immature tapeworms), it still remains to be re-evaluated. It has been proposed that instead of the polyclonal antibodies that are used in the assay, monoclonal antibodies be used as this could not only increase sensitivity but also specificity since the current assay is not able to differentiate between *T. solium* and *T. saginata*. It is possible that the high taeniosis prevalence could have been due to immature worms resulting from recent infection as a result of increased infected and undercooked pork consumption. People consume pork with many cysts that have the potential to establish into adult worms in the intestines of the same host but it is possible that many might die off and never reach maturity as observed with cysticerci infection. A number of people can harbour multiple tapeworms (Bustos *et al.*, 2012) indicating the possibility of many cysts developing into adult worms within one host. Interspecies competition among the taeniid tapeworms has also been described (Conlan *et al.*, 2009) and therefore, it is possible that intraspecies competition could also occur resulting in the development of only one tapeworm. The confirmation by PCR of the presence of *T. solium* in the two districts entails a high risk of contracting cysticercosis and ultimately neurocysticercosis from the carriers.

8.3   Important determinants of taeniosis and cysticercosis infection

Important disease determinants playing a role in the maintenance of the parasite in the local communities were studied using novel as well as existing methods of analyzing disease determinants.

Our study reveals clear differences in results when using different methodologies that study disease determinants. Classical logistic regression analyses express relationships between variables whereas CART gives greater insights into which of the determinants are important (Speybroeck, 2012). In the current study, the CART analyses revealed a different order of importance of disease determinants between two areas and that the usually reported determinants such as pork consumption and latrine use were not determined as important. Whether this new information could lead to improvements in some of the control programmes, such as health education (Sarti *et al.*, 1997; Ngowi *et al.*, 2008) and mass chemotherapy of pigs or humans (Allan *et al.*, 1997; Sarti *et al.*, 2000; Garcia *et al.*, 2007), that have been devised and implemented in the past is not clear. Some of the factors reported to affect strategies such as mass chemotherapy include population coverage and cost (Allan *et al.*, 1997). Given such

factors, older individuals and those from large families could be targeted. This could increase the success rates in projects aiming to improve sanitation in rural communities (Murrell, 2005) and changing people's risk behaviour (Ngowi *et al.*, 2008). The CART probably gives insights as to what factors in a population need to be targeted when implementing a control programme. This, therefore, questions the methodology used to analyze these factors leading to the proposal that perhaps a multi-methodological approach should be taken. The question that remains is, should the formulation of control measures be based on one or both methods? The CART has highlighted the fact that the control of *T. solium* remains a complex task as what might be an important determinant in one area may be less important in another area.

Aspects of people's knowledge of a member of the community suffering from NCC related symptoms were also determined to be important. This could provide confirmation of the probable presence of symptomatic cysticercosis in the areas under study and requires quantification.

The importance of the tapeworm carrier in a community has been highlighted by many reports. Proper disposal of faeces would play a major role in the control of the infection in both pigs and humans. However, the number of latrines in endemic areas is reported to be low leading to open-air defaecation and hence access of pigs to human faeces (Murrell, 2005). Further, latrines may be available but still people don't use them. Through conversations with the local people, it was revealed that there are traditional taboos that contribute to the lack of use of latrines, suggesting a huge task with regards to control measures. Anthropological studies are therefore recommended to evaluate and understand these taboos which could be greatly contributing to the maintenance of the parasite in communities. Though dependent on the eliminating of these taboos and change of people's attitudes, increased latrine use would have a positive impact on the control of the tapeworm in the long term and should still be recommended. Also, sanitation improvements by use of latrines would have an impact not only on *T. solium* infections but also on other sanitation related diarrhoea causing pathogens, soil transmitted helminths (STHs) and schistosomiasis. Studies have shown that improved sanitation would have a far more positive impact on the control of STHs than the currently used approach of mass chemotherapy (de Silva *et al.*, 2003; Bethony *et al.*, 2006) and also on the control of diarrhoea in poor communities (Kakakhel *et al.*, 2011).

It is clear from the present study that identifying an efficient control programme targeting a disease determinant is one thing; however, implementation is a completely different issue, as the perception of the local community plays a critical role and has to be taken into consideration.

8.4 Diagnostic tools

Taenia solium taeniosis/cysticercosis remain neglected diseases due to the lack of information and awareness of the extent of the problem mainly because of the absence of suitable sensitive and specific diagnostic tools which can be applied at low cost and large scale in endemic areas (Eddi *et al.*, 2003; Budke *et al.*, 2009). As such, in this thesis, tools for the diagnosis of cysticercosis and taeniosis were evaluated.Given the cultural problems associated with the collection of blood in epidemiological studies, the potential use of urine for the diagnosis of cysticercosis in field conditions was evaluated.

The collection of blood samples for diagnosis of cysticercosis has generally not been accepted by many communities, especially in the Zambian setting, where it is commonly associated with Satanism. One of the alternatives to blood samples is urine, which has been used for the diagnosis of other parasitic infections and tends to be better accepted by the communities, even when repeated collection is requested (Parija *et al.*, 2004). The high sensitivity and specificity of antigen detection in urine, recorded in hospital settings in NCC studies (Parija *et al.*, 2004; Castillo *et al.*, 2009) could however, not be replicated under field conditions due to a loss in specificity revealing a need for further research to improve the test. Pig models can probably be used to investigate how long cysticercus antigens remain in urine after clearance of the infection and serum becoming negative. The properties of the antigens in urine, and other body fluids require, however, further investigation to establish the nature of the protein. Also requiring further investigations are the differences that may exist between the human and porcine urinary systems, especially in terms of antigen clearance from the blood circulation and the period it remains in urine. Field applicability of the urine antigen detection would be greatly be enhanced if such a test would be in a dipstick format to allow for a quick diagnosis. Another alternative to blood sampling is saliva which has been evaluated for the diagnosis of NCC (Feldman *et al*., 1990) and applied for systemic infections such as immunodeficiency syndrome and hepatitis A (Archibald *et al*., 1986; Parry *et al*, 1987) by detecting specific antibodies. Again, further studies

are required to evaluate the possibility of using saliva as a sample for the diagnosis of cysticercosis in field studies. Again, pig models could be used in these studies.

Finally, due to the lack of a gold standard test for the diagnosis of taeniosis, the performance of three currently available tests namely, coproscopic examination, copro-Ag ELISA and copro-PCR were compared.

The lack of a reliable test for taeniosis that is both highly sensitive and specific has resulted in the underestimation of the true disease status in endemic areas. It has further made the screening of tapeworm carriers, and their treatment, complicated. Effective diagnosis of taeniosis is one of the main problems in the choice of selective treatment after testing as a control option. Due to the low prevalence of taeniosis in endemic areas, selective treatment would be cheaper and more cost effective than mass drug administration (Alexander *et al.*, 2011); but without a proper diagnostic test this would be a futile option. Self detection has been evaluated to be effective for the diagnosis of taeniosis (Flisser *et al.*, 2005), however, unlike *T. saginata*/*T. s. asiatica*, the proglottids of *T. solium* are passively passed with faeces (Murrell, 2005) and are usually notnoticed by the carrier and this could make the test ineffective. Microscopic examination of stool samples after concentration, though widely applied and genus specific has a very low sensitivity. Furthermore, the copro-Ag ELISA, though sensitive, cannot distinguish between *T. solium* and *T. saginata.* The use of PCR on DNA extracted from stool samples has a twofold advantage, i.e. diagnosis and differentiation of the tapeworms. We have presented the first report of *T. saginata* in Eastern Zambia, which had until now only been described in cattle (cysticercosis) in the Central and Western province of Zambia. Despite *T. solium* occurring more frequently than *T. saginata,* the detection and differentiation of the *Taenia* species is of high medical importance since presence of adult *T. solium* carriers are the only cause of cysticercosis not only to family members but also to the community (Garcia-Noval *et al.*, 1996; Carrique-Mas *et al.*, 2001; Flisser, 2006).

A comparison of microscopy, coproantigen ELISA and copro-PCR in this study showed that PCR is a better test for the diagnosis of taeniosis with a sensitivity of 97.6% and specificity of 99.6% as compared to microscopy and copro-Ag ELISA. The possibility to extract DNA from faecal samples allows the use of PCR, not only for differentiation but also for diagnosis, which is

a valuable tool towards the elimination of the parasite in endemic areas. However, the main obstacles for the use of such a test in poor endemic areas are the cost and specialized equipment required. There is still need for cheaper tests that can be used routinely in field conditions and in endemic areas.

8.5   Options for control of *T. solium* infections in Eastern Zambia

The fact that cysticercosis usually has no overt disease-specific manifestations, neither in pigs nor in humans, has made it difficult to sensitize responsible authorities, both in the veterinary and medical sectors. It is for this reason that the impact of cysticercosis/NCC needs to be determined so that these authorities can evaluate the need for control and allocate the required resources. It has been estimated that about 30% of epileptic cases in endemic areas are due to NCC (Ndimubanzi *et al.*, 2010), resulting in considerable burden, in terms of DALYs and monetary impact, on the affected people and communities (Carabin *et al*., 2006; Praet *et al*., 2009; Bhattarai *et al*., 2012). Studies have further indicated cognitive deficits in NCC patients accompanied by a marked decrease in quality of life (Wallin *et al.*, 2012) and when severe, it results in hormonal changes, especially in women, that could have repercussions on their reproductive health (Cardenas *et al.*, 2012). These factors highlight the need for control measures against *T. solium* infections in endemic poor rural communities so as to contribute to the improvement of people's quality of life.

From our field experience it was evident that there is lack of knowledge of the parasite not just among the common people but also among professionals, such as veterinary and health officials. There is a saying in Zambia that "what you don't know won't harm you and you'll do nothing to prevent it". This is exactly what is happening on the ground. People consume infected pork because they consider it harmless. There are many local theories as to what the cysts in the pork are, some think it's just contamination of the pork with maize bran while others state that it's the "bad hands" of the person that feeds the pigs and most, if not all, do not understand the link between the cysticerci and epilepsy. Thus for any *T. solium* control measures to succeed, there is need to educate not only the common people but also the veterinary and health officials. The health education should be integrated into the already existing primary health care system and directed not exclusively at taeniosis/cysticercosis but also towards changing people's attitudes

and practices with regards to sanitation and personal hygiene (Sarti & Rajshekhar, 2003). This will have spill over effects on the other sanitation related diseases prevalent in the areas. Specifically for cysticercosis, a study in Tanzania demonstrated that, although, education campaigns have been shown not to change people's eating habits within one year, a reduction in pig infection was observed (Ngowi *et al.*, 2008). And this reduction in pig infection would contribute greatly towards the control of the infection in communities. Health education can provide an entry point into communities and will not only have positive long-term effects but also greatly determine the success of other control options that can be proposed.

The proper disposal of human faeces has been highlighted as being sufficient to break the life cycle of the tapeworm (Murrell, 2005). This would mean the construction and use of latrines. The traditional taboos associated with the use of latrines prevailing in eastern Zambia would, however, complicate this approach. Moreover, poor hygienic standards, mostly related to poverty and ignorance are still prevailing and remain a key factor for the presence of a multitude of parasites, including *T. solium*. The health education could in the long-term change peoples' attitudes and begin to see the added benefits of improved sanitation. The use of new and less costly innovative methods of improving sanitation in communities such as Community-Led Total Sanitation could be utilized as it allows communities to conduct their own appraisal and analysis of open-air defecation and take their own action to become open defecation free (Kar & Chambers, 2008). Even though the effect of this approach on sanitation related diseases has not yet been determined, it has led to marked improvements in sanitary conditions in communities where it has been implemented. It is therefore hypothesized that this improvement in sanitation could lead to a reduction in *T solium* infections in endemic communities.Other proposed control measures that include improved pig husbandry, improved veterinary control, treatment of pigs and treatment of tapeworm carriers have their own pros and cons in our study area and are discussed below.

Improved pig husbandry would entail the construction of pig pens. However, these are poor communities than cannot afford it, let alone provide the pigs with feed (Lekule & Kyvsgaard, 2003). Therefore such an approach, even with health education would be a huge challenge in our study area. Though veterinary control of pigs seems appropriate in preventing people from consuming infected pork, and hence reduces transmission to humans, it remains a futile approach

in the short term. The control would consist of inspection of pigs before slaughter and of carcasses after slaughter. Information collected through this would rely on methods that are not very sensitive (Dorny *et al.*, 2004b) and hence some infected pork would still be passed off as safe for consumption. In fact some kind of screening does take place in the field by traders from the nearby towns that go to the villages to buy pigs. The traders, however, only check the tongues for the presence of cysts before buying the pig and since lightly infected pigs rarely show cysts on the tongue; and the poor sensitivity of tongue palpation as a diagnostic method (Dorny *et al.*, 2004b; Phiri *et al.*, 2006), infected pork is still passed on for consumption. Moreover, most of the pigs are slaughtered in backyards with no veterinary inspection at all.

Our interactions with the local people revealed that interventions through treatment and/or vaccination of both the intermediate and definitive hosts would be welcomed by the communities. Thus, the option of vaccinating pigs against cysticercosis would be an alternative to preventing pigs from contracting the infection in light of their contact with human faeces. The reports of 100% protection (Flisser *et al*., 2004; Gonzalez *et al*., 2005; Assana *et al.*, 2010) are a very big milestone towards the control of *T. solium* infections. This approach, however, would have to be applied on a very large scale and will greatly be determined by economic parameters as it may be too costly for the veterinary authorities. It is likely that the availability of vaccines to resource poor communities would have to be highly subsidized or given at no cost to the pig keepers. The treatment of adult tapeworm carriers after detection has the disadvantage of the unavailability of an effective diagnostic test and facilities for the analysis of samples. In such a scenario, therefore, mass treatment as is currently done for STHs as well as schistosomiasis in children could be the best approach and is proposed. An integrated approach could be adopted that targets multiple parasites as this would be less costly for the health authorities. These mass treatment campaigns should target the entire population. The use of mass treatment has resulted in decreases in taeniosis and porcine cysticercosis prevalences in endemic areas (Cruz *et al.*, 1989; Sarti & Rajshekhar, 2003). However, since its effects last for only up to two years (Garcia *et al.*, 2002) it should be done for a number of years.

A factor that also needs to be taken into account is the evident endemic stability existing in the two study areas. The drastic reduction of the presence of the parasite might, therefore, upset this situation and total eradication would put the local community at increased risk of quicker and

higher infection rates should a tapeworm carrier enter the community. The question therefore, would be whether to adopt a quick total eradication of the tapeworm by involving the entire population (human and pigs) and a large scale control programme, or it should be aimed for a more gradual reduction in levels of infection. Total eradication would in reality be impossible to achieve as it may have to be applied to all areas in the country that are endemic and for which information is still lacking. Zambia, being a developing country, control of the "big three" diseases namely malaria, HIV and tuberculosis always receive priority with no resources left for the other diseases, especially neglected ones. Garcia *et al.* (1999) reported that the economical and geographical constraints for most developing countries makes eradicating the disease complex, as occurred in Europe in the early 1900s though improved sanitation, almost impossible. Moreover, as the world is now a global village, movements of people from endemic regions of the world would easily re-introduce the infection (Eddi *et al.*, 2003; Broglia & Kapel, 2011). Control measures would therefore, have to be in line with the international community so as to eliminate the parasite globally. These would have to be those that are efficient and cost effective under the prevailing local conditions.

We have established that households with many inhabitants seem to at higher risk of infection as well as older individuals. This should therefore provide an entry point into communities with regards to control programmes as they would be the part of the population to be targeted. The question remains; do similar studies involving CART have to be done for each and every area or alternatively large scale studies covering a province, country or even a region be conducted to have a wider picture that guides a control programme on a wider scale?. With the obvious differences in epidemiology of the diseases between two neighbouring districts, the economic viability of formulating area or region specific control programmes requires assessment. The most cost effective approach would probably be the regional approach.

As stated by Lightowlers, (2003), the future control of *T. solium* infections lies in an integrated approach, because a single control measure is unlikely to achieve effective and long lasting control. But for an integrated control strategy of cysticercosis to work, there must be recognition of the interrelationships among the risk factors that play a major role in the infection (Widdowson *et al.*, 2000).

8.6   Conclusions and prospects

It is evident that the understanding of the epidemiology of the pork tapeworm requires continued research. Differences in transmission dynamics from one area to another makes this even more complicated and difficult to apply the findings of the results found in one area to other areas. This work has provided insights into the local epidemiology of the infection in a highly exposed community. The simultaneous use of Ag-ELISA and EITB has provided useful information and is recommended to be used in future studies. Studies are also required to determine the levels of NCC in these cysticercosis endemic areas so as to determine the disease burden on the local people. The traditional taboos that lead to open air defaecation present in eastern Zambia need to be explored through anthropological studies as they may have implications on control measures. The use of the coproantigen ELISA is a useful tool but requires fine-tuning while the copro-PCR is a promising tool if it is made available to endemic countries and issues of cost are addressed. The use of urine in epidemiological studies should be investigated further as well as other bodily fluids that could be of diagnostic value.

Finally, given the complexity of the disease determinants at play in maintaining the parasite in communities, it is of utmost importance that the medical and veterinary sectors work in close collaboration towards the elimination of the disease.

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