PREVALENCE OF PORCINE CYSTICERCOSIS IN MBOZI AND MBEYA RURAL DISTRICTS, MBEYA TANZANIA

BY

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ABSTRACT

An epidemiological survey was conducted between November and December 2007 in 30 randomly selected villages and four slaughterslabs in Mbozi and Mbeya Rural districts, southern Tanzania, to determine the prevalence of porcine cysticercosis. Fifteen villages and three slaughterslabs were from Mbozi and fifteen villages and one slaughterslab were from Mbeya Rural. A total of 600 live pigs (300 in each district) of different sex and age categories were randomly selected from smallholder pig-keeping households and subjected to lingual examination and Antigen-ELISA tests. Postmortem examination was performed in pigs slaughtered in official slaughterslabs and local brew clubs. Questionnaire survey and direct observations were used to investigate potential factors related to transmission of *T. solium*. The overall prevalence of porcine cysticercosis in Mbozi district was 11.7% (95% CI = 8.5-15.8%) and 32% (95% CI: 27-37.5%) based on lingual examination and Antigen-ELISA, respectively. In Mbeya Rural district, the prevalences were 6% (95% CI: 3.8-9.3%) and 30.7% (95% CI: 25.8-36.1%), by lingual examination and Antigen-ELISA tests, respectively. The agreement between the two tests was poor ($\kappa < 40\%$). There were no significant differences in the prevalence of porcine cysticercosis in different age and sex categories of pigs. None of the 805 pigs slaughtered at official slaughterslabs was infected with cysticercosis based on post-mortem inspection. However, of those slaughtered at local brew clubs, 8.2% (n=437) in Mbozi district and 10.8% (n=74) in Mbeya Rural were positive for cysticercosis. Potential risk factors for porcine cysticercosis in the districts included poor pig management, poor sanitary practices, lack of knowledge on the transmission of *T. solium*, and lack of meat inspection services. This study recommends educational campaigns in the study communities on the epidemiology of the disease, and subsequent revision of the current regulatory framework for pig trade and pork inspection to safeguard public health and improve livelihoods.

DECLARATION

I, Erick Vitus Gabriel Komba, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work and has not been submitted for a degree award in any other University.

Signature: …… Date: ……...

The above declaration is confirmed by supervisor:

Name	Signature	Date
Dr. H.A. Ngowi		

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DEDICATION

To Mourine Alfreda and Nourine Agatha, my beloved twin daughters; and Bernadetha my wife.

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LIST OF ABBREVIATIONS AND SYMBOLS

^{o}C	Degrees Celcius
%	Percentage
#	Number
µl	Microliter
µM	Micromole
CESA	Cysticercosis in Eastern and Southern Africa Project
CI	Confidence interval
cm	Centimeter
CT	Computerized tomography
DANIDA	Danish International Development Agency
DNA	Deoxyribonucleic acid
g	Gram
kg	Kilogram
M	Molar
mg	Milligram
ml	Milliliter
min	Minutes
mm	Millimetre
MoAb	Monoclonal antibody
MRI	Magnetic Resonance Imaging
NBCS	New born calf serum
H_2SO_4	Sulphuric acid
PBS	Phosphate buffered saline
pH	Hydrogen ion concentration
r.p.m	Revolutions per minute
SUA	Sokoine University of Agriculture

WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Taenia solium cysticercosis in pigs is a growing problem in many areas of Africa, Asia and Latin America where traditional pig husbandry is practised (Engels *et al*., 2003). On the other hand human cysticercosis caused by this parasite is now a global problem because of tourism and increasing migration of people all over the world. The best-documented evidence for this comes from the United States where cysticercosis is most frequently diagnosed in immigrants from or visitors to Latin America but where autochthonous cases have also been detected (Shandera *et al*., 2002). Nevertheless, only few studies have been conducted in developing countries, which are presumed to be the areas of high endemicity of the parasite. These studies are important to guide the planning of appropriate measures to control the parasite.

Tanzania is currently estimated to have 1.2 million pigs (MWLD, 2006). Most of the pigs in the country are raised in rural areas by small scale farmers (Ngowi, 1999). Unfortunately, due to conditions related to poverty, such as inadequate sanitation, poor pig management practices and lack or absence of meat inspection and control, porcine cysticercosis has emerged as an important constraint to the nutritional and economic wellbeing of these smallholder farming communities as well as a serious public health risk to the rural and urban areas where many infected pigs are transported and consumed (Phiri *et al*., 2003). Porcine cysticercosis was first documented in Tanzania in the mid 1980s. Cases of the disease were detected when a group of pigs exported from Arusha, Tanzania to Nairobi, Kenya was condemned due to massive cysticercosis infection in most of the pigs. The pigs were later found to have originated from Mbulu District (Nsengwa and Mbise, 1995). Subsequent studies conducted in Mbulu district in

northern highlands (Boa *et al*., 1995; Ngowi *et al*., 2004a), Chunya, Iringa Rural and Songea districts in the southern highlands (Boa *et al*., 2001) revealed that porcine cysticercosis was prevalent in these areas. A health education package developed, which involved community participation in Mbulu district was able to reduce the incidence rate of porcine cysticercosis by 43% (Ngowi *et al.*, 2008).

1.2 Problem statement and Justification

In order to develop appropriate intervention strategies for *T. solium* infections, baseline information on the prevalence, distribution, and transmission risk factors is necessary. There was no study that had determined the prevalence of and transmission risk factors for porcine cysticercosis in Mbozi and Mbeya Rural districts of Mbeya, the second region in terms of pig population, and the region bordering the porcine cysticercosis endemic districts of Iringa and Songea.

The present epidemiological survey was thus conducted in Mbozi and Mbeya Rural districts with the aim of establishing the presence and magnitude of porcine cysticercosis, in order to provide baseline data for designing and implementing appropriate surveillance and control strategies for the parasite.

1.3 Objectives

1.3.1 Overall objective

The overall objective of this study was to determine the prevalence of porcine cysticercosis in Mbozi and Mbeya Rural districts of Mbeya region as a foundation for planning relevant measures to control *T. solium* infections.

1.3.2 Specific objectives

i. To determine the prevalence of porcine cysticercosis in live pigs in Mbozi and Mbeya Rural districts;

ii. To establish the prevalence of porcine cysticercosis in pigs slaughtered in Mbozi and Mbeya Rural districts.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Aetiology

Porcine cysticercosis is an important parasitic infection of pigs caused by the larval stage of a zoonotic tapeworm, *Taenia solium*. Together with other Taenia species such as *Taenia saginata*, *Taenia hydatigena* and *Taenia crassiceps*, *Taenia solium* is a member of the family *Taeniidae* and genus *Taenia*. The adult worm is flat with a tape-like shape. Its body (strobila) can measure between 2 to 4 m in length with a total of 800 to 1000 segments (proglottids). The larval stage of *T. solium* is commonly known as *Cysticercus cellulosae* (Soulsby, 1982). The morphology of the cysticercus is that of a vesicle, but at times, in the brain, this morphology can vary to irregular (racemose) forms (White, 2000). Embryonated eggs of *T. solium* are typical taeniid eggs. They are brown, measuring 26-34 µm in diameter and have a double walled membrane with radiating striae (Schantz, 1996).

2.2 Life Cycle and Mode of Transmission

The life cycle of *T. solium* includes the human as the only natural definitive host, harbouring the adult tapeworm, and the pig as the common intermediate host, harbouring the larval stage of the parasite (Soulsby, 1982). A pig gets infected when it eats human faeces or feed containing viable *T. solium* eggs which develop to cysticerci in the body. The cysticerci (*C. cellulosae*) develop primarily in skeletal and cardiac muscles. The fully developed cysticercus measures up to 20 by 10 mm and becomes infective within nine to ten weeks (Soulsby, 1982). Human is infected when he consumes raw or undercooked, infected (measly) pork. Human being may also act as an intermediate host by digesting eggs created by the adult tapeworm directly or regurgitating gravid proglottids from the human gut to the stomach (Cai *et al*., 2006).

2.3 Epidemiology

T. solium infection is widely endemic in rural areas of developing countries in Latin America, Asia, and Africa, where poverty conditions such as poor sanitation and intimate contact between humans and their livestock are common place (Pawlowski *et al.*, 2005). In Africa, porcine cysticercosis has been reported in several countries including Cameroon, Zaire, South Africa, Nigeria, Kenya, Zimbabwe, Madagascar, Rwanda, Burundi, Zambia and Tanzania (Phiri *et al*., 2003; Zoli *et al*., 2003).

In Tanzania, community based studies on porcine cysticercosis based on lingual examination indicate a prevalence of 17.4% in northern highlands district of Mbulu and a prevalence range of 5.1-16.9% in the southern highlands (Ngowi *et al*., 2004a; Boa *et al*., 2001). Domesticated pigs are the most common intermediate hosts, but, bush pigs, man, dogs, cats, rats and monkeys may also harbour the cystic stage (Gracey, 1986). Man is the only natural definitive host but it has been reported that taeniosis may be established in lar gibbon (Cadigan *et al*., 1967), chachma baboon (Vester, 1967), golden hamster (Vester, 1974), and chinchilla (Maravilla *et al*., 1998).

2.4 Clinical Signs

Porcine cysticercosis is usually without conspicuous signs but intracranial involvement is not uncommon (Gonzalez *et al*., 2003). A pig with numerous cysts (over 400) in the brain from Mbulu district was observed to be frequently circling (Boa *et al*., 2002) although the study design used could not ascertain the cause-effect relationship. In man the cysts can lodge in the brain (neurocysticercosis), muscles, subcutaneous tissue and/or the eye. Cysts in the eye may lead to visual loss. Neurocysticercosis has been shown to cause arachnoiditis, hydrocephalus, stroke, dementia and numerous other neurological problems (Del Bruto *et al*., 2001). Seizures are more common with multiple lesions (Kramer *et al*., 1989).

In human, symptoms such as abdominal pain, distension, diarrhoea and nausea have been attributed to the adult tapeworm infestation, but, there are no controlled studies that have demonstrated their association (Schantz *et al*., 1998).

2.5 Diagnosis of *Taenia solium* Infections

2.5.1 Diagnosis of porcine cysticercosis

In endemic countries porcine cysticercosis is commonly diagnosed by tongue examination by pig traders. Although the method is very specific, it has low sensitivity (Gonzalez *et al*., 1990). According to Dorny *et al*. (2004), tongue examination has a sensitivity of 21% and specificity of 100%. Meat inspectors usually use post-mortem examination of pig carcasses, the method that has accuracy similar to that of tongue examination. Different immunodiagnostic techniques are available for diagnosis of porcine cysticercosis. They include detection methods for specific antibodies or circulating parasite antigens in serum or cerebrospinal fluid (Dorny *et al*., 2003). Today the Enzyme-linked imunoelectrotransfer blot assay (EITB) and Enzyme-linked immunosorbent assay (ELISA) are the antibody-detection test formats that are most frequently used for diagnosis (Wilkins *et al*., 2002). Antigen detection techniques have been developed and provide a useful tool in identifying individuals with active infections and therefore a tool for serological monitoring of anti-parasitic therapy (Dorny *et al.*, 2003). The commonly used is Antigen–ELISA with a sensitivity and specificity of 86.7% and 94.7%, respectively (Dorny *et al*., 2004). Many studies have reported shortfalls with antibody detection (Ab-ELISA) in animals (Pinto *et al.*, 2000; Garcia *et al.*, 2001; Dorny *et al.*, 2003). Ag-ELISA has been shown to have a high sensitivity for detecting a pig with even a single cyst (Nguekam *et al.*, 2003), and it has the advantage of differentiating between recent infections with live metacestodes and older infections with degenerated metacestodes, which are no longer infective

(Harrison *et al.,* 1989). Due to their high cost, immunodiagnostic techniques are however not readily available for routine or field use in developing countries but rather used in research projects.

2.5.2 Diagnosis of human cysticercosis

A number of serological techniques are being developed for the detection of circulating host antibodies or parasite antigens in cysticercotic individuals (Tsang *et al.,* 1989; Ito *et al.,* 1998; Dorny *et al.,* 2003). However the validation of these techniques in human is hindered by the lack of a gold standard. In addition, no research group has yet used a Bayesian approach to estimate the sensitivity and specificity of different approaches in the absence of a gold standard. The only gold standard would be pathological confirmation through biopsy or autopsy, procedures that have ethical limitations (Carpio *et al.,* 1998).

Invariably, previous antibody-detection assays had moderate sensitivities and poor specificity because they used crude antigens (Dorny *et al.,* 2003). However, recent developments have led into the production of highly purified antigens that has improved the accuracy of the serodiagnostic tests (Dorny *et al.,* 2003). The most specific test developed is the enzyme-linked immunoelectrotransfer blot (EITB), an immunoblot of seven cysticercus glycoproteins, purified by lentil lectin-purified chromatography, which gives about 100% specificity and 70-90% sensitivity (Tsang *et al.,* 1989). However, a sensitivity of 28% has been found in cases with single cyst in the brain (Wilson *et al.,* 1991). Ito *et al.* (1998) prepared a highly species specific antigen from cyst fluid using single-step preparative isoelectric focusing. This assay can be used in pigs as well as humans.

Several attempts have been done to produce recombinant antigens that can be used in immunoblot and ELISA (Chung *et al.,* 1999; Sako *et al.,* 2000). These synthetic polypeptides have been reported to have high specificity but lower sensitivity than that of native antigens (Dorny *et al.,* 2003). The drawback of the antibody-detection methods is that they indicate exposure to infection, and therefore the tests can detect maternal-transferred antibodies or presence of antibodies without the viable parasite (such as shortly after treatment or exposure without parasite establishment). The assays are not good for clinical purposes as they may lead into unnecessary use of anti-parasitic drugs where the parasites are not viable. However, the antibody-detection techniques have been useful in identifying “hot spots” of the infection where control measures should be directed (Dorny *et al.,* 2003).

Several assays have been developed to detect parasite antigens but only the monoclonal antibody-based tests directed at defined parasite antigens may ensure reproducibility (Correa *et al.,* 1989; Harrison *et al.,* 1989; Brandt *et al.,* 1992; Erhart *et al.,* 2002). Antigen detection may be done on serum as well as on cerebrospinal fluid. The sensitivity and specificity of antigen ELISA in detecting human cysticercosis is thought to be high although there are no proper studies that have evaluated this (Dorny *et al.,* 2003).

Greater attention has been directed to the diagnosis of human neurocysticercosis due to its greater impact on public health. Computerised tomographic (CT) scans and magnetic resonance imaging (MRI) are two neuroimaging techniques that have been used to diagnose human neurocysticercosis. The sensitivity of MRI for the detection of calcified lesions is poor, and thus CT remains the best screening neuroimaging procedure for patients with suspected neurocysticercosis (Garcia and Del Brutto, 2003).

MRI is the imaging modality of choice for the evaluation of patients with intraventricular cysticercosis, brainstem cysts and small cysts located over the convexity of the cerebral hemispheres and it can be the technique of choice in the follow-up of patients following treatment (Garcia and Del Brutto, 2003). While some CT and MRI findings in neurocysticercosis are highly suggestive of this disease, the differential diagnosis with other infectious or neoplastic diseases of the CNS may be difficult (Garcia and Del Brutto, 2003). In such cases, a combination of clinical diagnosis, immunodiagnostic tests, and epidemiological data may improve proper interpretation of the neuroimaging findings (Garcia and Del Brutto, 2003). The big drawback of the use of neuroimaging techniques is their availability in the field and their high cost. This hinders their application especially in poor countries like Tanzania as it costs an individual approximately US $ 200 to undergo CT scanning. This amount of money can hardly be afforded by the rural poor, most of who earn below the poverty line of US $1 per day.

2.5.3 Diagnosis of human taeniosis

Until early 1990s, stool microscopy to visualise Taenia eggs was the only diagnostic method available for the diagnosis of human taeniosis (Garcia *et al.*, 2003). This method is still the only one available for clinical use in many developing countries including Tanzania. Stool microscopy has two major disadvantages, the first being its inability to differentiate between *T. solium* and *T. saginata* eggs (Soulsby, 1982). In rare occasions, when the scolex or mature proglottids are found in the human stool, differentiation of the two species can be possible based on their morphological characteristics. The second disadvantage of stool microscopy is its low sensitivity of between 30-40% (Garcia *et al.,* 2003; Allan *et al.,* 2003), probably due to the fact that Taenia eggs are excreted intermittently (Allan *et al*., 1996).

Progress has been made towards the development of immunodiagnostic tests for the detection of human taeniosis. The most commonly used test in some endemic areas is coproantigen test (Garcia *et al.,* 2003; Allan *et al.,* 2003). Parasite coproantigens constitute parasite specific products in the faeces of the host that are amenable to immunological detection. These products are a result of parasite metabolic activities and are thus present independently of parasite reproductive material such as eggs and proglottids, and they disappear within a week after treatment (Allan *et al.,* 2003).The antigen detection is genus specific with *T. solium* and *T. saginata* both reacting in the assay but with no cross-reactions with other parasites (Allan *et al.,* 2003). The true coproantigen test sensitivity and specificity is likely to be greater than 90% and 99%, respectively based on micro-plate formats (Garcia *et al.,* 2003; Allan *et al.,* 2003). With dipstick ELISA formats the test has shown sensitivity and specificity of 76% and 99.9%, respectively (Allan *et al.,* 2003).

Wilkins *et al.* (1999) demonstrated the possibility of diagnosing *T. solium* taeniosis by the detection of species-specific circulating antibodies using the EITB method. Based on studies conducted elsewhere, the test has revealed the sensitivity and specificity of 95% and 100%, respectively (Allan *et al.,* 2003). Being an antibody-detection method it also has a drawback of indicating exposure to infection, and therefore detecting maternal-transferred antibodies or presence of antibodies without the viable parasite. One area that remains to be investigated is the rate at which sera, following removal of the intestinal infection, become negative for circulating antibodies to the diagnostic antigens (Allan *et al.,* 2003).

2.6 Prevention and Control

The International Task Force for Disease Eradication in its meeting in 1992 listed *T. solium* as among the six diseases (poliomyelitis, mumps, rubella, dracunculiasis, lymphatic filariasis, and cysticercosis) that were candidates for eradication. The elimination of *T. solium* from most European and North American countries is an important evidence of potential eradicability of this parasite (Sarti and Rajshekhar, 2003; Gonzalez *et al.,* 2003). The elimination of this zoonosis in those countries resulted from the process of economic development, which in turn improved the environmental sanitation, pig husbandry and marketing procedures. The measures are however not feasible in most developing countries. For these countries control measures such as treatment of tapeworm carriers, treatment of infected pigs, vaccination of pigs, and health education should be relied on, at times in combination.

2.6.1 Treatment of tapeworm carriers

Historically, chemotherapy of human taeniosis was both toxic and relatively ineffective, making large-scale chemotherapeutic interventions impossible. It was only in 1960 that the first effective, safe, synthetic taeniacide, niclosamide, was introduced. Niclosamide is a broad-spectrum taenicide with 85% efficacy in the treatment of taeniosis at a single dose; however, some generically produced and/or long stored batches may be less effective due to time-dependent polymerisation of the active particles. There are no reported contraindications to niclosamide other than concomitant use of alcohol, pregnancy or an age below 2 years (WHO, 1995). Large-scale use of niclosamide is limited by the relatively high cost of treatment compared with praziquantel (PZQ), a safe and highly effective drug against a wide spectrum of cestodes and flukes that was introduced in 1972. However, PZQ may provoke an intensive inflammatory reaction around the damaged cysticerci and thus sporadically cause neurological symptoms

when used against taeniosis in individuals with asymptomatic neurocysticercosis. On a cost-benefit basis this adverse effect is not a reason to exclude using PZQ in large-scale treatment interventions, however, appropriate precautions should be taken to deal with such an adverse reaction. On the other hand, there have been no published reports of serious neurological sequelae during population treatment of schistosomiasis with high doses of PZQ (40 mg/kg) in areas endemic for cysticercosis (Pawlowski *et al.,* 2005). The efficacy of the therapy in human taeniosis with PZQ is around 95% and, at 10 US cents per cure (Pawlowski *et al.,* 2005).

With the availability of the two well known synthetic, safe and effective drugs against tapeworms in humans, (niclosamide and praziquantel), both now on the WHO Essential Drugs list (WHO, 1995), the use of traditional treatments, such as Areca nuts or Kosso flowers, should be discouraged because of their high toxicity and/or potential carcinogenicity (Pawlowski 2005, in press).

2.6.2 Treatment of infected pigs

Antiparasitic treatment of pigs with cysticidal drugs would allow pig producers to remove established infection identifiable through simple techniques like tongue examination and hence minimize economic losses at slaughter (Gonzalez *et al.,* 2003). Trials have been conducted to determine the efficacy of a range of molecules including albendazole, albendazole sulphoxide, PZQ, flubendazole and oxfendazole against the parasite in pigs (Gonzalez *et al.,* 2001). Several studies have involved administration of the drug to pigs over several days, which would be unpopular with producers. Furthermore, most regimens tested have not resulted in complete parasite resolution although they have often significantly reduced parasite viability. The continued presence of cysticerci in the carcass would lead to condemnation at slaughter. Perhaps

the most promising approach is the administration of oxfendazole in a single oral dose of 30 mg/kg. There is a delay of several weeks before cyst death occurs, thought to be due to the drug damaging the parasite and exposing it to immunological attack. Up to 12 weeks are necessary for resolution of the cystic lesions to small calcified scars and pigs treated in this way appear to be resistant to further infection for several months following treatment (Gonzalez *et al.,* 2001). Gonzalez *et al*. (1998) found that twelve weeks after treatment of twelve pigs with oxfendazole in a single oral dose of 30 mg/kg, all meat appeared clear and only minuscule scars remained except in one animal that had viable brain cysts. This approach may therefore present an effective mechanism for parasite control, although there remain a number of issues to be resolved such as determining whether the drug is safe in this regimen for pigs and establishing a suitable withdrawal period prior to slaughter and human consumption to minimize consumer exposure to drug residues

2.6.3 Vaccination of pigs

There has been considerable work conducted on developing prophylactic vaccines for *T. solium* cysticercosis in pigs and a number of candidate vaccines now exist. Oncosphere excretory/secretory antigens elicit a strong protective immune response across several taeniid species. For example, *T. saginata* oncosphere-derived antigens elicit a protective immune response in cattle and excretory/secretory of *T. solium* oncospheres are similarly protective in pigs (Plancarte *et al.,* 2001). One limitation of this approach is that the amounts of antigen that can be produced from oncospheres are extremely limited. For this reason recombinant peptide antigens derived from *Taenia ovis* oncospheres were developed and subsequently shown to give very high (nearly 100%) protection against development of cysticerci. These recombinant *T. ovis* antigens provided high levels of cross-specific immunity against *T. solium* in pigs (Plancarte *et*

al., 2001). Homologues of these peptides were identified in *T. solium* (Lightowlers *et al.,* 2003) and two *T. solium* oncosphere recombinant antigens have now been shown to prevent development of cysticercosis in pigs by up to 100% (Flisser *et al.,* 2003). These molecules may therefore provide the basis for the development of a practical vaccine for pigs. However, there remains much work to do to turn these into practical and widely available vaccines. Some remaining issues are: (i) ensuring appropriate formulation; (ii) production of a stable vaccine suitable for use in the endemic zone; (iii) better understanding of efficacy (including duration of protective immunity) and safety; and (iv) establishment of production of a vaccine in a suitable facility and at an acceptable end user friendly price, especially for small scale producers.

A number of vaccine studies have been conducted using antigens derived from related parasites like *Taenia crassiceps*. These studies have generally shown good levels of protection and have in some cases involved field trials of the vaccine in endemic villages (Huerta *et al.,* 2001). However, there are problems with the methods for data interpretation (Lightowlers *et al.,* 2003). Work with the synthetic or recombinant antigens identified from *T. crassiceps* have shown particular promise in pigs (Huerta *et al.,* 2001).

In addition to work on peptide-based vaccines, work has also recently been reported on DNA type vaccines using DNA as a priming vaccine followed by a peptide booster (Cai *et al.,* 2001; Guo *et al.,* 2004). Such an approach offers an additional avenue to *T. solium* vaccination, which may overcome some of issues with peptide-only vaccines such as duration of immunity, for instance by inducing a longer period of protective immunity (Guo *et al.,* 2004). However, DNA based prime boost type vaccines suffer from being expensive.

2.6.4 Health education

For several years, improvements in sanitary infrastructure and health education have been proposed as alternative solutions to control of *T. solium* infections. However, the impact of such approaches, which are often multi-factorial, can be difficult to measure. A study in China demonstrated a strong association between cysticercosis and some social practices such as unsanitary rearing of pigs, inability to recognize infected pork and insufficient knowledge on transmission. This study suggested that education could play a critical role in control (Cao *et al.,* 1997). Another series of studies that demonstrated that health education about *T. solium* could be a feasible alternative to control of *T. solium* infections was conducted in Mexico (Sarti *et al.,* 1997). This study was performed in two rural communities of Mexico of around 3000 inhabitants each. One community had only health education and the other health education and mass treatment of human carriers of the tapeworm with PZQ (5 mg/kg single dose).

The health education campaign in both communities promoted knowledge of the transmission of taeniosis/cysticercosis and improved hygiene and sanitation. Its impact was evaluated by measuring changes in practices and knowledge and estimating human taeniosis and swine cysticercosis prevalence before and after the campaign. In the community with health education only, the prevalence of porcine cysticercosis before the campaign were 2.6, 5.2 and 4.8% by lingual examination, antibody detection (immunoblot assay) and postmortem inspection, respectively. Four years later, the rates were 0, 2, and 0%, respectively. Prevalences of taeniosis in humans by coproantigen detection before, one year and 42 months after the campaign were 0.78, 0.51 and 0.41%, respectively. The conclusion was that health education had substantially

reduced the opportunities for *T. solium* transmission but had not caused its eradication (Sarti *et al.,* 1997).

In the other community with both a health education campaign and mass treatment, the prevalence of taeniosis by coproantigen detection were 0.79, 0.97 and 0.70%, respectively, before the intervention strategy, one year later and 42 months later. This represents no significant reduction in taeniasis prevalence over time ($P>0.05$). However, porcine cysticercosis prevalence detected by tongue palpation before, one year and 42 months after the intervention strategy was applied were 4.1, 2.1 and 0.7% ($P<0.001$); positive serology rates in pigs were 7.5, 8.7 and 3.2% ($P<0.001$), respectively, and postmortem detection before the campaign was 9.3% (56/600) and after the campaign, 0.9% (2/227) ($P<0.002$). These results demonstrated that health education was a successful intervention strategy, at least in reducing porcine cysticercosis prevalence (Sarti *et al.,* 1997). In Tanzania, Ngowi *et al.* (2008) conducted a health education trial in Mbulu district. The trial led to an important reduction in the incidence rate of porcine cysticercosis (incidence rate ratio 0.57) despite minimal improvement in behaviours related to its transmission. However, the intervention had a significant reduction in the reported cases of household consumption of infected pork (a reduction by 20%).

2.6.5 Integrated approach

It is thought that none of the strategies for *T. solium* control can stand as a sole strategy. Therefore, combined interventions are recommended (Gonzalez *et al.,* 2003; Lightowlers, 2003; Sarti and Rajshekhar, 2003). Intervention programmes combining treatment of human and pig populations have been tried in Peru with little success (Gonzalez *et al.,* 2003). A practical, cost-effective combination of interventions needs

therefore to be defined from reliable data, including trials in diverse geographical regions to ensure its potential applicability in other zones. Besides being culturally acceptable this combination of interventions needs to take advantage of the economic factors that drive domestic pig rearing. It is essential to incorporate health education in any of the control strategy combinations in order to sustainably prevent re-infection or further transmission of the parasite.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

This study was carried out in Mbozi and Mbeya Rural Districts of Mbeya region. Mbozi District is located in the southwest of Mbeya region between latitudes 7° and 9° 12' south of the equator and longitudes 32° 7' 30" and 33° 2' 0" east of Greenwich Meridian. Mbeya Rural District lies between latitudes 8° and 9° south of equator and between longitudes 33° and 35° east of Greenwich Meridian. Figure 1 shows the study area (the two districts and their corresponding villages).

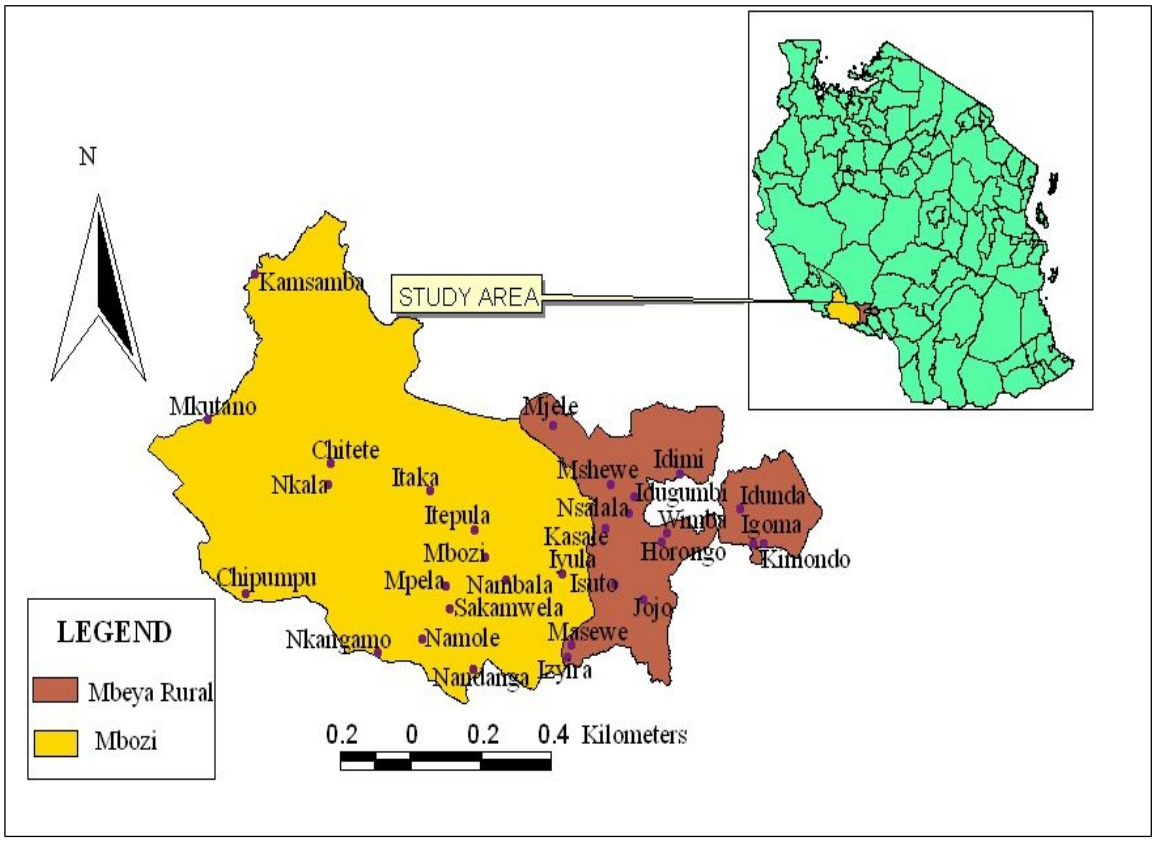

Figure 1 : Study villages in Mbozi and Mbeya Rural Districts, Mbeya-Tanzania

3.2 Study Design

A Cross-sectional study design was employed in this study whereby the prevalence of porcine cysticercosis and related knowledge and practices were assessed at a single point in time. The field study was carried out between November and December 2007.

3.3 Sample Size

The sample size was calculated using the formula for random sampling developed by Martin *et al*. (1987) as follows:

$n=Z^2PQ/L^2$; where n = required sample size, Z is the Z value for a given confidence level, P is a known or estimated prevalence, Q= (1-P), and L=allowable error of estimation. For the purpose of this study a confidence level was assumed at 95% with an allowable error of estimation of 5%. The average prevalence of porcine cysticercosis in the study areas was estimated at 11% based on a study previously conducted in a neighbouring district (Chunya) where a prevalence of 5.5% was obtained by lingual examination (Boa, 2005). This prevalence was doubled in the present study because a more sensitive technique of Antigen-ELISA was to be employed, which could detect two or more times that by lingual method (Dorny *et al*., 2004). Therefore, $n = (1.96^2 \times 0.11 \times 0.89)/0.05^2 = 150$ pigs. Adjusting for multistage sampling design used, the sample size was multiplied by two, and, therefore, 300 pigs were examined in each of the two districts.

For post-mortem examination of pig carcasses the sample size depended on the number of pigs slaughtered during the study period (November 2007 - December 2007). The post-mortem inspection included pigs slaughtered in official and non-official slaughter places if the meat inspector was called for inspection.

3.4 Sample Selection

A multistage sampling design was adopted. Fifteen villages were randomly selected from each of the two districts, followed by random selection of ten households in each village. The sampling frames comprised of lists of pig keeping villages and pig keeping households within the villages. In households with one or two pigs all the pigs were examined. In households with more than two pigs, two to four pigs were randomly selected and examined. Examination of more than two pigs in some households was done so as to cover for the deficiency created by selected households with only one pig. Sows that had recently farrowed or heavily pregnant, and piglets less than two months old were excluded from the study. For the questionnaire survey the target respondents were the household heads though in cases of their absence other family members who could deliver the required information were involved.

3.5 Data Collection

In this study data collection involved ante-mortem lingual examination and blood sampling for antigen-ELISA in live pigs. In addition, post-mortem inspection was done in pigs slaughtered in slaughter facilities or local brew clubs. A handheld global positioning system (GPS) instrument was used to locate each study village office.

3.5.1 Lingual examination

A pig was firmly restrained in lateral recumbency and the head stabilized by the use of a pig snare. The mouth was opened and maintained open using a wooden stick. The tongue was then gripped using a cloth and gently pulled out, visually examined and palpated all along its ventral side for the presence of cysticerci. A pig was considered positive if cyst-like nodules were either seen or felt (Gonzalez *et al*., 1990).

3.5.2 Blood sample collection

A pig was restrained in dorsal recumbency. About 5 ml of blood sample was collected from either the external jugular vein or the cranial vena cava into a plain vacutainer tube. The right side of the neck of the pig was preferred over the left side where phrenic nerve occupies a more vulnerable position, while the unpaired thoracic duct is also more to the left (Dyce *et al*., 1996). The blood sample was centrifuged within eight hours, sera collected in Eppendorf tubes and deep frozen at -20°C till when sent to Zambia for Antigen-ELISA.

3.5.3 Antigen-ELISA

The Ag-ELISA was performed according to Pouedet *et al.* (2002) and slightly modified as described by Sikasunge *et al.* (2007). Incubation steps were reduced from 1 h to 30 min (coating) or 15 min (other steps); all incubations were done on a shaking plate except for the last step (substrate); streptavidin–horseradish peroxidase (Jackson Immunoresearch lab Inc.) diluted 1/10,000 was used as the conjugate. The optical density of each serum sample was compared with negative pig serum samples (n=8) at a probability due to chance ($p < 0.001$) to determine the result of the test (Sokal and Rohlf, 1981). The detailed procedure is described in Appendix 1.

3.5.4 Questionnaire survey

A study complementary to this was conducted simultaneously to determine risk factors for porcine cysticercosis in the two districts. The study involved questionnaire survey, whereby a structured questionnaire was administered to one person (preferably the household head) in each pig-keeping household involved in prevalence study. The detailed questionnaire is presented in Appendix 2. Nevertheless, the questionnaire as presented in the appendix is very detailed as it also investigated other issues not presented in this dissertation.

3.5.5 Post-mortem examination of pig carcasses

For post-mortem examination of pig carcasses, meat inspectors were involved in the collection of data. Official slaughterslabs, three in Mbozi (Tunduma, Mlowo and Vwawa) and one in Mbeya Rural (Mbalizi) were predetermined. Examination of pork, involving village/ward livestock field officers, was also done at village level in 121 local brew clubs (59 in Mbozi, 62 in Mbeya rural) where pigs were also slaughtered. Since the Tanzania Meat Inspection Act of 1993 does not include guidelines for pork inspection, pork inspectors abide by the guidelines for inspection of beef. Majority of them mentioned masseter muscles, tongue, hamstring muscles, diaphragm, liver, lungs and the heart as the areas inspected.

3.6 Data Analysis

Data were entered in Microsoft Office Excel 2003 and analysed in statistical package for social sciences (SPSS®) and MedCalc® software. Descriptive statistics were computed to determine village and overall district prevalence of porcine cysticercosis and their 95% CI intervals.

Statistical significance in differences of prevalence by lingual examination and Ag-ELISA tests were determined using Chi-square test. Also analyses were done to examine associations between the prevalence and various factors such age and sex of pigs.

Kappa statistic was computed to determine the agreement between the lingual examination and Ag-ELISA tests for the detection of porcine cysticercosis. Kappa statistic is given by the formula below (Woodward, 2004):

$$K = \frac{(P0 - Pe)}{(1 - Pe)}$$

Where:

P0: Is observed proportion of agreement

Pe: Is proportion expected by chance

The interpretation of the kappa values was as follows;

if		
K=1	:	perfect agreement
K≥0.75	:	excellent agreement
0.4<K<0.75	:	fair to good agreement
K≤0.4	:	poor agreement
K=0	:	No agreement

Descriptive statistics were computed for the questionnaire responses to establish the prevalence of various factors.

CHAPTER FOUR

4.0 RESULTS

4.1 General Results

A total of 30 villages were visited (15 in Mbozi District and 15 in Mbeya Rural District). A total of 150 households were visited per district (total 300 households). Of the households visited, three refused participation without giving out reasons for their unwillingness to participate. The participation proportion was therefore 99%. This study examined a total of 600 pigs, 300 in each district. Of the examined pigs 35.7 % were males and 64.3% were females. The age of the examined pigs ranged from 2 months to 6 years. The aging was based on farmers' records/memory. Of them 151 were weaners, 125 growers and 324 adults. The pigs were of the exotic, mixed and local breeds.

During the study period a total of 337 pig carcasses were inspected in officially known slaughter slabs in Mbozi District (Tunduma 101, Mlowo 108, Vwawa 128). In the official slaughter slab of Mbeya Rural District (Mbalizi) a total of 468 pig carcasses were inspected. On the other hand 511 pig carcasses were inspected in non-official slaughter places, mostly local brew clubs.

4.2 Prevalence of Porcine Cysticercosis in Live Pigs

The prevalence of porcine cysticercosis in Mbozi and Mbeya Rural Districts by lingual and Ag-ELISA are presented in Table 1 and Table 2, respectively. The overall prevalence in Mbozi District was 11.7% (95% CI = 8.5-15.8%) by lingual examination and 32% (95% CI = 27-37.5%) by Ag-ELISA. Mbeya Rural District had an overall prevalence of 6% (95% CI = 3.8-9.3%) by lingual examination, and 30.7% (95% CI = 25.8-36.1%) by Ag-ELISA. The Ag-ELISA results indicated that all villages were

infected. The difference in prevalence between the two districts was statistically not significant ($p>0.05$) Plate 1 shows a pig with multiple cysticerci under the tongue found in the study area.

Table 1: Prevalence of porcine cysticercosis by lingual examination and Ag-ELISA in Mbozi District, Mbeya -Tanzania (November 2007)

Village	Number of pigs examined	Pigs positive for cysticercosis			
		Lingual examination		Ag-ELISA	
		Number infected	Prevalence (percentage)	Number infected	Prevalence (percentage)
Nandanga	20	3	15	10	50
Iyula	20	3	15	10	50
Itepula	20	2	10	6	30
Kamsamba	20	2	10	5	25
Itaka	20	2	10	5	25
Chitete	20	2	10	8	40
Nkala	20	1	5	10	50
Namole	20	5	25	7	35
Nambala	20	1	5	2	10
Sakamwela	20	5	25	8	40
Mbozi	20	1	5	1	5
Chipumpu	20	3	15	5	25
Mkutano	20	2	10	4	20
Nkangamo	20	1	5	7	35
Mpela	20	2	10	8	40
TOTAL	**300**	**35**	**11.7**	**96**	**32**

Table 2: Prevalence of porcine cysticercosis by lingual examination and Ag-ELISA in Mbeya Rural District, Mbeya -Tanzania (December 2007)

Village	Number of pigs examined	Pigs positive for cysticercosis			
		Lingual examination		Ag-ELISA	
		Number infected	Prevalence (percentage)	Number infected	Prevalence (percentage)
Horongo	20	2	10	9	45
Jojo	20	2	10	8	40
Idimi	20	4	20	12	60
Nsalala	20	0	0	2	10
Idugumbi	20	1	5	2	10
Idunda	20	0	0	1	5
Wimba	20	1	5	3	15
Masewe	20	1	5	8	40
Izyira	20	2	10	10	50
Kasale	20	0	0	1	5
Igoma	20	2	10	7	35
Mjele	20	3	15	12	60
Mshewe	20	0	0	5	25
Kimondo	20	0	0	8	40
Isuto	20	0	0	4	20
TOTAL	**300**	**18**	**6**	**92**	**30.7**

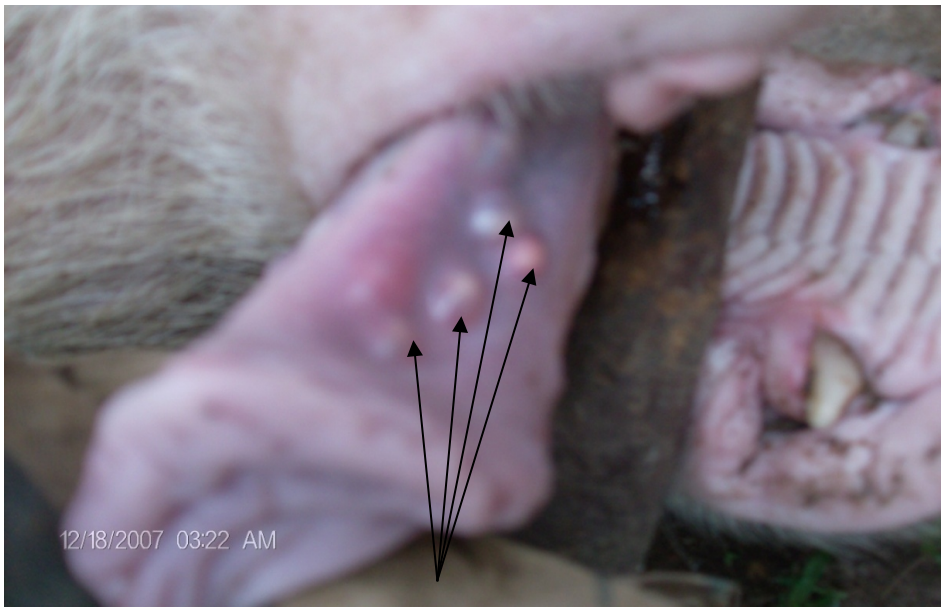

Plate 1: Multiple cysts under the tongue of a pig (arrow) examined in Mbeya Rural District, Mbeya -Tanzania (December 2007)

4.3 Comparison of Lingual Examination and Ag-ELISA Tests in Detection of Porcine Cysticercosis

There were significant differences in the prevalence of porcine cysticercosis between lingual examination and Ag-ELISA methods. The difference was 20.3% (95% CI=18.8-30.6) in Mbozi District ($p<0.0001$) and 24.7% (95% CI=13.8-26.6) in Mbeya Rural District ($p<0.0001$), with the Ag-ELISA detecting more cases. Kappa statistic test revealed poor agreement between the two methods. In both Districts the agreement was below 40% (95% CI: 31.8 – 51.5%).

Table 3: Agreement between lingual examination and Ag-ELISA tests for detection of porcine cysticercosis in Mbozi District

		Ag-ELISA			
	Status	Positive	Negative	Total	Apparent prevalence
Lingual	Positive	35	0	35	11.7%
examination	Negative	61	204	265	
	Total	96	204	300	
	Apparent prevalence	32%			
	κ = 0.39				

Table 4: Agreement between lingual examination and Ag-ELISA tests for detection of porcine cysticercosis in Mbeya Rural District

		Ag-ELISA			
	Status	Positive	Negative	Total	Apparent prevalence
Lingual	Positive	18	0	18	6%
examination	Negative	74	208	282	
	Total	92	208	300	
	Apparent prevalence	30.7%			
	κ = 0.25				

4.4 Prevalence of Porcine Cysticercosis in Slaughter Pigs

Post-mortem meat inspection revealed no cysticercosis in all the 805 pigs slaughtered in official slaughterslabs during the two months of the study in both districts. However, prevalence of 8.3% (n=437) and 10.8% (n=74) were obtained for pigs slaughtered at local brew clubs in villages in Mbozi (Table 5) and Mbeya Rural (Table 6) Districts, respectively. The individual village prevalence ranged between 0 and 58% in Mbozi District and between 0 and 35.71% in Mbeya Rural District.

Table 5: Prevalence of porcine cysticercosis in pigs slaughtered in village local brew clubs in Mbozi District (November-December, 2007)

Village	**Pigs positive for porcine cysticercosis**

	Number of pigs	Number	Prevalence (%)
Itaka	22	0	0
Chitete	109	12	11
Nambala	26	0	0
Kamsamba	5	0	0
Nkangamo	200	0	0
Igale	31	18	58
Ipyana	18	1	5
Nambizo	26	5	19.2
TOTAL	**437**	**36**	**8.2**

Table 6 : Prevalence of porcine cysticercosis in pigs slaughtered in village local brew clubs in Mbeya Rural District (November-December, 2007)

Village	Number of pigs examined	Pigs positive for porcine cysticercosis	
		Number	Prevalence (%)
Horongo	14	5	35.7
Wimba	9	3	33.3
Idimi	5	0	0
Igoma	19	0	0
Isuto	3	0	0
Idugumbi	9	0	0
Mshewe	15	0	0
TOTAL	**74**	**8**	**10.81**

4.5 Prevalence of Porcine Cysticercosis by Age

The prevalence of porcine cysticercosis by Ag-ELISA in Mbozi District was 28.2% in weaners (n=78), 35.4% in growers (n=48), and 32.7% in adult pigs (n=174). In Mbeya Rural District, prevalences of 22% (n=73), 21% (n=77), and 40% (n=150) were observed by the technique in weaner, grower, and adult pigs respectively. The differences in prevalence were not statistically significant ($P>0.05$). For the purpose of this study, a weaner pig was defined as a pig between 2-4 months old, a grower as the one between 5-8 months and an adult as one aged 9 months and above.

4.6 Prevalence of Porcine Cysticercosis by Sex

Out of 92 male pigs examined in Mbozi District 33.7% were positive for cysticercosis while 31.2% of the 208 females examined were positive. In Mbeya Rural District, 31.1% of 122 male pigs examined by Ag-ELISA had cysticercosis while 30.3% of the

178 females had cysticercosis. Thus, in both districts the differences in the prevalence between male and female pigs were not statistically significant (P>0.05).

4.7 Results of the Questionnaire Survey and Structured Observations in Mbozi and Mbeya Rural Districts

4.7.1 Respondents general characteristics

A total of 100 males and 50 females were interviewed in Mbozi District; and 93 males and 57 females were interviewed in Mbeya Rural District. Their ages ranged from 20–88 years (Mbozi) and 23–90 years (Mbeya Rural). The level of education of the respondents were such that in Mbozi District, 75.6% had primary school education, 7.2% secondary school education, 0.7% college or university education, and 16.5% had no formal education. In Mbeya Rural District, 72.5% had primary school education, 6% secondary school education, 4% adult education, and 17.4% had no formal education. All the visited pig keepers practiced mixed farming. A few of the respondents were in addition employed in paid jobs or other businesses.

4.7.2 Possible risk factors for the transmission of porcine cysticercosis

Practices and knowledge related to the transmission of .porcine cysticercosi in Mbozi and Mbeya Rural Districts are presented in Table 7 and Table 8, respectively. Plates 2 to 6 illustrate various practices related to the transmission of porcine cysticercosis in the study areas.

Table 7: Practices related to transmission of porcine cysticercosis in Mbozi and Mbeya Rural Districts (November and December 2007)

Factor	Positive responses	
	Mbozi (n=150)	**Mbeya Rural (n=150)**
Practices		
Respondents consuming pork	127 (84.7%)	134 (89.3%)
Preferred form of pork preparation		
• frying	41 (27.3%)	57 (38%)
• boiling	108 (72%)	91 (60.7%)
• barbecuing	0 (0%)	1 (0.7%)
Home slaughtering of pigs	37 (24.7%)	14 (9.3%)
Home slaughtered pig not inspected	2 (1.3%)	3 (2%)
Households lacking latrine	14 (9.3%)	3 (2%)
Latrines without closing doors	130 (86.7%)	105 (70%)
Faeces in the toilet surroundings	30 (20%)	26 (17.3%)
Free range pig management system	79 (52.7%)	26 (17.3%)
Pens allowing pigs to escape	60 (40%)	56 (37.3%)

Table 8: Knowledge on porcine cysticercosis in Mbozi and Mbeya Rural Districts, Mbeya-Tanzania (November and December 2007)

Factor	Positive responses	
	Mbozi (n=150)	**Mbeya Rural (n=150)**
Practices		
Respondents who were not aware of porcine cysticercosis	15 (10%)	9 (6%)
Respondents who didn't know how people get taeniosis	135 (90%)	141 (94%)
Respondents who were not aware of *T. solium* cysticercosis in people	148 (98.7%)	146 (97.3%)
Respondents who didn't know how a pig get cysts	136 (90.7%)	138 (92%)
Respondents who didn't know what to do with a pig with cysticercosis	50 (33.3%)	57 (38%)

Plate 2: A free ranging pig in Mbozi District -Tanzania (November, 2007)

Plate 3: Pit latrines without closing doors in Mbozi District, Mbeya, Tanzania (November, 2007)

(a) **(b)**

Plate 4: A poorly constructed pig pen (a) and a well constructed pig pen (b), both made from local materials in Mbeya Rural District, Mbeya – Tanzania (December 2007)

Plate 5: Closeness between a pig pen (right arrow) and a pit latrine (left arrow), a positive pig was detected in this household

Plate 6: A pork trader in Mbeya Rural District with utensils used in pork frying.

CHAPTER FIVE

5.0 DISCUSSION

The present study is original in the sense that it has been conducted in naïve districts of Mbeya region in respect to porcine cysticercosis surveys. In addition, the study has employed the geographical positioning system (GPS) to map the study area.

The prevalence of porcine cysticercosis of 11.7% and 6% observed by lingual examination in Mbozi and Mbeya Rural Districts, respectively, were higher than that of 5.5% (n = 692) obtained by Boa (2002) in Chunya District of the same region. The prevalence were however lower than that of 17.4% (n = 770) observed in Mbulu District, northern Tanzania (Ngowi *et al.*, 2004a). Nevertheless, the lingual examination method has shown to have a very low sensitivity compared to Ag-ELISA, which detected about two point seven (2.7) times more cases in Mbozi and about five times more cases in Mbeya Rural Districts. It has been observed in this study that the differences in prevalences of porcine cysticercosis by lingual examination and Ag-ELISA are statistically significant and that the two tests are in poor agreement. Some studies elsewhere have found that Ag-ELISA can detect more cases than lingual examination (Pouedet *et al.*, 2002; Nguekam *et al.*, 2003).

The findings of the present study however caution the generalisation of the relative ability of the Ag-ELISA in detecting porcine cysticercosis and call for further studies to investigate reasons for the variations in the relative ability of the test in detecting porcine cysticercosis such as the big difference that was observed between Mbozi and Mbeya Rural Districts. Sarti *et al.* (1992) observed that not all positive pigs necessarily have cysts on the tongue and that probably infection intensity could be the most important factor determining whether cysts are discernible by visual inspection of the

tongue or not. Gonzalez *et al.* (1990) observed that application of lingual examination in detecting porcine cysticercosis could detect up to 70% of infected pigs whereas a study conducted by Phiri *et al.* (2006) reported that lingual examination could only detect 61.3% of *T. solium* infected pigs although it exhibited a high specificity of 100%. On the other hand Dorny *et al.* (2004) found through Bayesian analysis that lingual examination could only detect 21% of truly infected pigs.

Antigen detection by ELISA test is known to detect only living cysticerci both in cattle (Brandt *et al*., 1992) and in pigs (Nguekam *et al*., 2003). The number of seropositive pigs is a good indication of animals which present a risk to the consumer. The results of the test in the present study therefore suggest that pork consumers in the study areas and anywhere pigs are transported for consumption are at high risk of infection with taeniosis given the high prevalence of porcine cysticercosis. According to Pouedet *et al*. (2002) the sensitivity and specificity of the Ag-ELISA for the detection of porcine cysticercosis as derived from Gibbs sampling analysis is 85.8–87.2 and 98.1–98.9%, respectively; suggesting that the prevalence figure may underestimate or slightly overestimate the real situation. This may however be counteracted by a potential cross-reactivity of the test with other parasites harboured by the population such as metacestodes of *Taenia hydatigena*. Data on the prevalence of *T. hydatigena* in Tanzanian pigs are scant. Only one study in northern Tanzania found a prevalence of 1.4% (n = 70) in slaughtered pigs (Ngowi *et al.,* 2004b), indicating a low prevalence.

Though no positive case for porcine cysticercosis was detected in pigs that were slaughtered in official slaughter slabs in the study area during the study period, cases were detected in pigs slaughtered at local brew clubs. Butchers are aware of the relationship between the presence of cysts under the tongue and condemnation of the

carcass during meat inspection at the abattoir and so they are hesitant to purchase pigs with cystic lesions under the tongue for fear of loosing money. Due to this, therefore, infected pigs are rarely encountered in abattoirs (Ngowi, 1999). Though the sensitivity of lingual examination test is fairly low, pre-selection based on the test may partly serve as a reason why in this study no positive case of porcine cysticercosis was detected in slaughtered pigs in slaughter facilities, considering also that the lingual examination and meat inspection have similar sensitivity (Dorny *et al.*, 2004). In the present study it was learnt that pig traders were using an additional method of screening whereby water is poured over the back of the pig and a positive animal reacts by bending. This may have something to do with the presence of cysts in psoas muscles. This technique may have an additional value to pre-selection. Further studies are however needed to establish the science behind and the accuracy of the test. It was reported by Sarti *et al.* (1992) and Carrique-Mas *et al.* (2001) that abattoir surveys appear to underestimate the real prevalence of the disease. Data obtained from abattoirs therefore do not reflect the true disease picture in rural communities as it is the case in the present study because known infected pigs are rejected at the farm level.

While pre-selection of the pigs prior to taking them to slaughter facilities may have had some bearing on the post-mortem results in the slaughterslabs, it may also have a bearing on the results of post-mortem inspection at local brew clubs where positive cases were detected. The traders from town are of first priority to the pig keepers as they pay higher prices. Most of the pigs bought by the local brew clubs butchers are probably rejects of the town traders, many of which have been manipulated by the pig keepers by removing the lingual cysticerci after being detected. The results in official slaughterslabs could also be contributed by the tendency of slaughter facility workers to hide carcasses found to be positive for porcine cysticercosis (those pre-selected false

negative pigs making their way to the slaughterslab) during the inspection. This was explained by the pork inspectors who reported to always show up at the facilities when carcass dressing is over. One of the inspectors reported that, upon surprise visits, it was common to find butchers selling infected pork. Another reason is that porcine cysticercosis is neglected in the national meat inspection regulations of Tanzania since there are no special instructions as to the method of examination for porcine cysticercosis although special provisions have been given for bovine cysticercosis (Anonymous, 1978). A study conducted in Mbulu District found that the chance of detecting porcine cysticercosis in slaughter pigs when the current pork inspection regulations are followed is low (Boa *et al*., 2002). Differences in relative cyst densities observed in that study suggest the need for revising the current national pork inspection regulations (Boa *et al*., 2002).

This study revealed several factors related to the prevalence of *T. solium* infections, which include poor pig husbandry, poor sanitary practices such as presence of faeces on toilet floor, lack of knowledge on the transmission of *T. solium* taeniosis/cysticercosis, lack of pork inspection at slaughter and selling of pork infected with *T. solium* cysticerci.

Infection rate with porcine cysticercosis is known to increase with free-ranging type of pig management (Sarti *et al.,* 1992). Free range results into free access by scavenging pigs to human faeces. About half of the respondents in Mbozi and one fifth in Mbeya Rural Districts admitted to allow their pigs to roam freely especially during the dry (post harvesting) season. It was however observed that a significant proportion of the available pig pens were of poor structure, allowing pigs, especially piglets, to escape (plate 4). In a village where free range management of pigs was not practised (revealed

from questionnaire survey and onsite observation) porcine cysticercosis was not detected except on one imported weaner pig from a village where free range management system was a feature.

Studies have demonstrated that in endemic areas, *T. solium* porcine infections have been associated with poverty, absence of latrines and free access by scavenging pigs to human faeces (Diaz *et al.,* 1992; Schantz *et al.,* 1992; Sarti *et al.,* 1997). In this study it was observed that many households had pit latrines, most of them without closing doors in a way giving a chance for pigs especially escaping weaners to gain access to human faeces which at times may contain *T. solium* eggs. Closeness of latrines and pig pens was a common feature in most of the visited households as illustrated in Plate 5. During the data collection period presence of faeces was detected on the floor of a good number of pit latrines.

Apart from poor sanitary and pig husbandry practices, occurrence and prevalence of *T. solium*, is associated with certain community behavioural and environmental practices that must be modified in order to prevent the continued spread of this zoonosis. Such practices include consumption of infected pork. It was learnt from some respondents that clandestine trade in infected pigs was common in the study area because infected pigs and carcasses were cheaper than health ones. Some traders slaughter infected pigs at home and eventually sell the uninspected pork at night in local brew clubs where frying, which at times might not be adequate to kill *C. cellulosae*, is a major preparation method. Sometimes barter trade was practised whereby infected pork was exchanged with other farm products such as maize, groundnuts, maize, rice and fingermillet. It was reported that most consumers of infected pork salted or cooked the pork at high temperatures for a longer time than would be for non-infected pork.

Although high temperature boiling is considered to be effective in destroying the cysts, pork consumed during most traditional ceremonies is usually not adequately cooked because of large amounts of meat that have to be prepared during a short period of time.

Lack of knowledge on the association between porcine cysticercosis and taeniosis in man, as was a feature in most of the respondents in the study area, is likely to contribute to high incidence of the conditions. Sanchez *et al.* (1997) found that the less the population knew about the existence of the parasite, the greater the risk they had of being seropositive. These will in turn serve as a source of infection to pigs thereby maintaining the cycle.

The present study has revealed that pigs of both sex and different age groups are equally susceptible to *T. solium* cysticercosis provided they are exposed to the source of infection. This is consistent with what was observed in a study done in Mbulu District, northern Tanzania by Ngowi (1999).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

It is clearly shown in this study that in low-input pig farming, as is the case in the selected villages of Mbozi and Mbeya Rural Districts of Mbeya region, southern Tanzania, all the conditions for an effective transmission of *T. solium* from man to pigs and vice versa are present. The results of the study show that porcine cysticercosis is highly prevalent in the districts confirming that the disease is potentially a serious problem in a typical rural resource poor pig farming setting. As observed in studies done in other areas endemic for the parasite, this study also gives an indication of the potential link between poor pig management and sanitary practices, poor knowledge and lack of meat inspection, the indicators of low socio-economic status, and the disease in pigs. Since human is the only source of porcine infection with *T. solium*, the results furthermore suggest the presence of tapeworm carriers in the study areas, providing the epidemiological link between human taeniosis and porcine cysticercosis. This eventually results into endemicity of *T. solium* taeniosis/cysticercosis complex in the area.

It is therefore recommended that the best way of reducing the prevalence of the porcine cysticercosis is to provide effective education campaigns aimed at clearly explaining the life cycle of the parasite, pointing out the epidemiological link between the human taeniosis and porcine cysticercosis and improving sanitary practices at the household and personal level. Awareness on the economic and public health impact of the parasite should also be provided. Coupled with these should be diagnosis and treatment of human tapeworm carriers. Since the Tanzanian Meat Inspection Act of 1993 does not include comprehensive instructions concerning detection of porcine cysticercosis, their

inclusion and ensuring availability of quality pork inspection services is also recommended.

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APPENDICES

Appendix 1: Enzyme – linked immmunosorbent assay for the detection of circulating *Taenia solium* cysticerci antigens (Ag-ELISA) in serum

The monoclonal antibodies used are B158C11A10 used as first MoAb and a biotinylated MoAb B60H8A4 used as a detector antibody (second MoAb)

The sera were pre-treated using freshly prepared 5% trichloroacetic acid (TCA) (Sigma, Chemical Co.) w/v dissolved in distilled water. Pre-treatment was done in order to remove non-specific immune-complexes to increase the specificity and sensitivity of the assay. A 5% TCA solution is prepared by dissolving 1 g of TCA in 20 ml of distilled water. The serum samples are thus pre-treated by mixing an equal volume of serum and 5% TCA. For the negative control sera, 75µl of serum is used while 150µl of serum is used for the pre-treatment of positive control and the test sera. These mixtures of sera and 5% TCA solution are incubated for 20 minutes at room temperature.

After incubation, the mixture was centrifuged at 12,000 r.p.m for nine minutes and the supernatant of the same volume of the added sera removed and aliquoted into microtitre tubes. The pH of the collected supernatant was raised by adding an equal volume of 75µl sodium carbonate/bicarbonate buffer (0.610 M) at pH of 10.0 (neutralization buffer) to the supernatant of the negative control sera and 150µl neutralization buffer to the supernatant of positive control and the test sera. 100µl of this mixture at final serum dilution of 1:4 was used in the Ag–ELISA protocol.

The plate was coated with 100µl of MoAb B158C11A10 diluted at 5 µg/ml in carbonate buffer (0.06M, pH 9.6) and incubated at 37ºC on a shaker for 30 min. After coating the plates were washed once with PBS-T20 and drained by beating the plate

vigorously on blotting paper. Blocking to avoid non-specific reactive sites was done by adding 150µl per well of PBS-T20/1% NBCS and then the plates incubated on a shaker for 15 minutes at 37ºC. Thereafter, the plates were drained. Without washing the plate, 100µl of pre-treated sera at a dilution of ¼ was added and incubated at 37ºC on a shaker for 15 minutes. The plate was drained and washed five times. 100µl of biotinylated MoAb B60H8A4 diluted at 1.25 µg/ml in PBS-T20/1% NBCS was added and the plate incubated at 37ºC on a shaker for 15 minutes. After this the plate was drained and washed five times with PBS-T20 as above. 100µl of streptavidin-horseradish peroxidase (Jackson Immunoresearch Lab, Inc.) diluted at 1/10,000 in PBS-T20/1% NBCS was added to act as conjugate after which the plate was incubated at 37ºC on a shaker for 15 minutes. One tablet of the chromogen/substrate, orthophenylene diamine (OPD) (SIGMA, #P-8412) is added to 180 ml of distilled water. Then 100µl of this solution was added to the wells and incubated at room temperature for 15 minutes in the dark without shaking. To stop the reaction, 50µl of $4NH_2SO_4$ was added to each well. The plates are read using an ELISA reader (Labsystem Multiskan RC) at 492 nm.

Appendix 2 : Questionnaire for cross - sectional survey to determine risk factors for porcine cysticercosis: pig keepers

A. General information

i. District_____Ward_____Village_____

ii. Agro-ecological zone_____ Farming system _____

iii. Date of interview _____Name of enumerator _____

B. Household characteristics

i. Name of respondent _____ Gender 1 = male, 2 = female _____

ii. Respondent's position in the household

1 = household head, 2 = wife of household head, 3 = child of household

4 = others (specify) _____

iii. Age of the household head _____ (yrs)

iv. Gender of the household head: 1 = male 2 = female _____

v. Ethnic group/affiliation of the household head_____

vi. Marital status: 1 = married, 2 = single, 3 = widowed, 4 = divorced

vii. Education level of the household head

1 = No formal education, 2 = Adult education, 3 = primary: standard 1 – 4,

4 = primary: standard 5 – 7, 5 = secondary: O - level, 6 = secondary, A-level,

7 = College/ university, 8 = others (specify) _____

viii. How many persons live in your household _____

ix. Household composition (household members who live in the household majority of days in the week)

Age group	Number (size of age group)
Below 7 years	
7 – 14 years	
15 – 21 years	
22 – 55 years	
Above 55 years	
Total	

C. General information regarding farming

i. What is your main economical activities

S/N	Type of economical activities	Indicate (tick)	Order of importance (rank)
1.	Crop farming		
2.	Livestock keeping		
3.	Fishing		
4.	Salary employment		
5.	Business		
6.	Artisan		
7.	Charcoal making		
8.	Others (specify)		
9.			
10.			

ii. Do you have land? 1 = Yes, 2 = No **(If no go to question vi**)

iii. If yes, what type of land ownership do you have? 1 = your personal own land, 2 = hired/rented land,3 = your friend/relative land, 4= others (specify)_____

iv. If it is your own personal land, how did you acquire it? 1 = inheritance, 2 = provided by village government, 3 = purchased, 4 = others (specify) _____

v. What is your total land holding? _____ (acres)

vi. If no, where do you keep your pigs? _____

vii. What are the main types crops do you grow?

Type of crop →				
Order of importance (rank)				

viii. What types of livestock do you keep?

Type of livestock	Total number	Order of importance	No sold in the last 12 months
1.			
2.			
3.			
4.			
5.			
6.			

D. Commencement and trend of pig production

i. When did you start keeping pigs? (year) _____

ii. How is the trend of your pig numbers for the past ten years?

Year	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007
Pig No										

iii. What is your purpose of keeping pigs?

Purpose	Income generation	Domestic meat production	Manure production	Cultural (i.e. dowry)	Others (specify)	
Indicate (tick)						
Order of importance						

v. What is your current pig flock size (number) _____ (crosscheck with question **C viii**)

vi. What is your current herd structure

Type	Total Number	Type of pig (ecotype/breed)*
Breeding females (Sows)		
Breeding males (boar)		
Adult non castrated males (not for breeding)		
Adult castrated males		
Adult females (not for breeding)		
Pre – weaned male piglets		
Pre – weaned female piglets		
Weaned female piglets (2 – 4 months)		
Weaned non castrated male piglets (2 – 4 months)		
Weaned castrated male piglets (2 – 4 months)		
Grower females (5 – 8 months)		
Grower males non castrated (5 – 8 months)		
Grower males castrated (5 – 8 months)		
Total		

* Type of pig (ecotype/breed): 1 = local, 2 = exotic, 3 = mixed (local & exotic), 4 = mixed (exotic & exotic), 5 = not known

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vi. Who is the owner of pig enterprise

1 = father, 2 = Mother, 3 = Children, 4 = father and mother, 3 = whole family,

5 = others (specify) _____

vii. In your household, who is mainly responsible for the following pig production activities

Type of activity	Who is responsible (father = 1, mother = 2, children = 3, hired labor = 4, others (specify))
1. Erecting and repair of pig structures	
2. Collection of pig feeds	
3. Processing of feeds and feeding of pigs	
4. Cleaning of the pig structure	
5. Health monitoring	
6. Decision on pig treatment	
7. Disposing off the pigs (selling, slaughtering, gifts, etc)	

E. Pig acquisition

i. Usually from which locations do you acquire/purchase your pigs?

1 = within the village, 2 = neighbouring villages, 3 = far villages, 4 = other districts within region, 5 = other region (specify) _____

ii. For the past 12 months (one year) how many pig did you acquire and how?

Means of acquisitions	Tick	Number acquired	From which location(s)[1]	Which is the source(s)[2]	Place(s) of acquisition [3]	Purpose of acquisition [4]
1. Buying						
2. Gift from relatives/friends						
3. Inheritance						
4. Others (Specify)						
Total						

[1] From which location(s): 1 = within village, 2 = neighboring village, 3 = far villages, 4 = other districts within region, 5 = other region (specify).

[2] Which are the sources: 1 = pig keepers, 2 = pig traders, 3 = institute (indicate name and place), 4 = others (specify)

[3]Place of acquisition: 1 = pig keepers households, 2 = markets, 3 = others (specify)

[4]Purpose of acquisition: 1 = fattening, 2 = breeding, 3 = slaughter, 4 = others (specify)

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iii. What period (month) and particulars (age group, weight, and sex) for the pigs purchased in the past 12 months?

Pig particulars	Month											
	Jan	Fe	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
No purchased												
Age group*												
Estimated weight (kg)												
Sex: 1 = male, 2 = female												
Price paid (Tsh)												

Age group* = 1 = weaned piglets, 2 = grower (4 – 8 months), 3 = adult

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iv. Which are the important examinations you normally do to a pig before buying it

Examination	Tick	Rank	Explain your preference criteria
Presence of cyst(s)			
Body condition characteristic			
Length of body			
Size of the body			
Colour			
Other health status (specify at D)			
Background history/records (i.e. reproductive & productivity (specify at D)			
Others (specify)			

v. What are the main determinants of purchasing price for pigs you have purchased?

Main price determinants	Tick	Rank	Explain your preference criteria
Breed/ecotype of pig			
Colour of the pig			
Health condition			
Sex of the pig			
Body condition status			
a. Fat status of the pig			
b. Size/weight			
c. Length of body			
1. Season of the year			
2. location of origin			
3. Others (specify)			

F. Pig production systems and management practices

i. How do you keep your pigs during different period of the year

Period of the year	Pig production system (Tick)						How long have you practiced (years)
	Total confinement	Semi confinement	Free range	Tethering	Herding/ grazing	Others (specify)	
Planting season							
Growing season							
Harvesting season							
Off season (dry period)							

ii. What factors/reasons motivated you to use indicated pig keeping system(s) in different period of year

Period of the year	Production system(s) used	Motivating factors/reasons for using	Advantages experienced for using the system	Disadvantages/problems experienced for using the system
Planting season				
Growing season				
Harvesting season				
Off season/dry period				

iii. What are the main feed resources do you use during different period of the year

Period of the year	Production system(s) used	Main feed resources used	Rank	Sources of feed
Planting season				
Growing season				
Harvesting season				
Off season/dry period				

G. Pig shelter (enumerator to combine physical observation of shelter and interview)

i. Do you have specific shelter for your pigs

1 = Yes, 2 = No (**if no go to question iii – iv**)

ii. If yes, what type of pig shelter are you using

1 = earthed floor, 2 = slated raised floor, 3 = slatted earthed level floor, 4 = concreted floor

5 = others (specify) _____

iii. If no, which factors made you not to erect shelter for your pig (s)

1._____ 2_____

iv. If no, where do you keep pigs during the day?_____, during the night?_____

v. How do you rate the importance of pig shelter

1 = very important, 2 = important, 3 = less important, 4 = not important

vi. Where do you get building material for your pig shelter? 1 = free from my farm, 2 = free within village,

3 = buying within village, 4 = free outside villages, 5 = buying outside villages

vii. Condition of pig shelter (enumerator to make assessment of floor, wall and roof of shelter with following scores:

1 = strong (highly protected can't offer free inlet and outlet of pigs), 2 = moderate (protected, however minimum effort can allow pigs out or in), 3 = weak (pig can get out and in when desires)

a. What is general condition of the shelter? _____

b. What is the specific condition of the floor? _____

c. Which material used for the floor? 1 = timber off cuts, 2 = tree/bamboo poles, 3 = cemented bricks,

4 = burned bricks, 5 = others (specify) _____

d. What is the specific condition of the wall? _____

e. Which materials used for the wall? 1 = timber off cuts, 2 = tree/bamboo poles, 3 = cemented bricks,

4 = burned bricks, 5 = others (specify) _____

f. Does a shelter have the roof? 1 = Yes, 2 = No

g. If yes, which materials used for the roof

1 = thatched grass, 2 = iron sheet, 3 = bamboo trees, 4 = others (specify) _____

viii. According to condition of shelter, do the pigs or piglets ever escape from their shelters?

1 = Yes, 2 = No

ix. If yes, how frequently? 1 = always, 2 = only occasionally, 3 = during off (dry) seasons, 4 = others (specify) _____

x. According to your experience in pig keeping, which are the main limitations for erecting pig shelter?

1 _____2._____

xi. Which are the main limitations for using pig shelter?

1 _____2. _____

H. Pig productive and reproductive performance

i. What is average performance of your pig with regards to following parameters

1. In your pig herd, how many sows farrowed for the past 12 months (this year) _____
2. What is the total number of farrowings for that period (past 12 months) _____
3. What is the total number of piglets borne for that period _____
4. What was the average litter size per sow at farrowing _____ and at weaning _____
5. What was the average age of piglets at weaning _____(months)
6. What is average age of gilts at first heat _____ (months), at first mating _____ (months), and at first farrowing _____ (months)

7. What is average period between farrowing to next heat _____(days) or _____ (months)
8. What is average period between one farrowing to another _____(months)

ii. Are you satisfied with your current pig productivity? 1 = Yes, 2 = No

iii. Do you want to increase pig production? 1 = Yes, 2 = No (**if no go to question v**)

iv. If yes, how do you plan to do it?

1. _____ 2_____

v. If no, why not? 1. _____ 2_____

I. Pig disposal/ off take

i. For the past 12 months, have you disposed off any pig from your herd? 1 = Yes, 2 = No

ii. If yes, what type of disposal have you done for the past 12 months (one year)

	Month											
	Jan	Feb	Mar	Apr	May	June	Jul	Aug	Sep	Oct	No	De
No disposed												
Type of disposal[1]												
Age group[2]												
Estimated weight (kg)												
Sex (1 = male, 2 = female)												
Price per pig (TZS)												
Total price (TZS)												

Type of disposal[1]: 1 = sales, 2 = gift, 3 = slaughter for home consumption, 4 = pride price, 5 = others (specify) _____

Age group[2]: 1 = piglets after weaning, 2 = grower (4 – 8 months), 3 = adult

iii. Which locations do you often sell your pigs?

Location	Tick	Rank	Name of village & market	District	Region
1. Within the village					
2. Neighbour villages/markets					
3. Far villages/markets in the					

district					
4. Other districts within the region					
5. Other places outside the region					

iv. Whom (category of buyers) you have most sell your pigs?

Market outlets	Farmers/pig keepers	Butchers	Pig roasters	Pig retailers	Truckers	Pig collecting agent	Others (specify)
Indicate (tick)							
Rank							

v. Do you have marketing place for selling pigs? 1 = Yes, 2 = No

vi. Where do you mostly meet with buyer(s)?

1 = in your household, 2 = in the market within the village, 3 = in the market outside the village, 4 = others (specify) _____

vii. Have you encountered any difficulty to sell your pigs? 1 = Yes, 2 = No

viii. If yes, which are the serious difficulties you have experienced

1. _____ 2_____

ix. Which are the important attributes (pigs and environmental) which determine selling prices for pigs you have sold?

Attribute	Tick	Rank	Explain briefly how it influence the price
Breed/ecotype of pig			
Colour of the pig			
Health condition			
Sex of the pig			
Body condition status			
Fat status of the pig			
Size/weight			
Length of body			
Season of the year			
Location where pig is originated			
Others (specify)			

x. Are you satisfied with price given for your pigs? 1 = Yes, 2 = No

xi. If yes, what reasons for satisfaction? If no, what reasons for your dissatisfactions

(If Yes) Reason for satisfaction	Tick	(If No) Reason for dissatisfaction	Tick
Competition with other pig keepers is low		Competition with other pig keepers is high	
Quality of pig is good		Quality of pig is poor	
Reliable pig marketing		unreliable pig marketing	
Pig buyers prices are genuine		Cheating by buyers/buyer price not genuine	
Buyers are many/competitive		Lack of enough buyers	
Others (specify)		Others (specify)	

xii. What is the price trend for the past two years? 1 = increasing, 2 = decreasing, 3 = no change

xiii. What are the important examination do traders normally do to pigs before buying them?

Examination	Tick	Rank	Explain your preference criteria
Presence of cyst(s)			
Body condition characteristic			
Length of body			
Size of the body			
Colour			
Other health status (specify at D)			
Background history/records (i.e. reproductive & productivity (specify at D)			
Others (specify)			

xiv. Do you get information about market prices for pig and types of pigs required?.1 = Yes, 2 = No

xv. If yes, how do you get the information? 1 = hear from other pig keepers, 2 = hear from pig traders, 3 = hear from mass media, 4 = others (specify) _____

I. Awareness, knowledge and effects of porcine cysticercosis

i. What are the major pig health problems you normally experienced in your pig flock

Type of health problem experienced	Order of importance (rank)
1.	
2.	
3.	

ii. Have you ever heard or experienced about cysts in pigs? 1 = Yes , 2 = No

iii. If yes, when did you get aware of the diseases for the first time? (year) _____

iv. Briefly explain your understanding on the disease

_____

v. What is the local name for the disease _____

vi. Do you know how pigs get infected with cyst? 1 = Yes, 2 = No

vii. If yes, please indicate the causes of the infestation

1. _____ 2_____

viii. If yes, where did you get the information on the disease

1 = from my fellow pig keepers, 2 = extension officers, 3 = from researchers, 4 = from pig traders, 5 = others (specify)_____

ix. Can porcine cysticercosis cause any problem to human being? 1 = Yes, 2 = No

x. If yes, briefly explain how _____

xi. How serious is porcine cysticercosis in this village. 1 = non–existence, 2 = it is present but not serious, 3 = moderate serious, 4 = it is serious problem, 5 = I am not aware

xii. Have you ever encountered cases of cysticercosis infection in your pig herd?

1 = Yes, 2 = No, 3 = not sure

xiii. If yes, which methods do you use to understand/diagnose the infected pig

1. _____ 2_____

xiv. What do you do if you discover that your pig is infected? 1 = sell the pig, 2 = treat with _____, 3 = pierce the nodules, 4 = other (specify) _____5 = I don't know

xv. Have you experienced any losses due to cysticercosis in your pig herd? 1 = yes, 2 = No

xvi. If yes, which are the production losses have you experienced

Year	Explain production limitation/loss encountered	Monetary value of limitation/loss in TZS

xvii. If yes, which are the marketing limitations/losses have you experienced

Year	Explain market limitation/loss encountered	Monetary value of limitation/loss in TZS

xviii. What are the mitigation mechanisms do you use to avoid or reduce the mentioned limitations

1. _____ 2_____

xix. Do you know how to prevent your pig from get infected with cyst? 1 = Yes, 2 = No

xx. If "Yes" which are the techniques involved in prevention

1. _____

2_____

xxi. Do you know how to treat pigs which are infested with cyst? 1 = Yes, 2 = No

xxii. If yes, briefly explain how_____

K. Pork slaughter, inspection, and eating behaviour

i. Do you or any member in the household use pork? 1 = Yes, 2 = No

ii. If no, what reasons made you not to use pork_____

iii. If yes, how often do you eat pork in a month and year? _____times a month, _____times a year

iv. If yes, which places do you buy pork for home consumption

1. _____ 2. _____

v. Did you ever slaughter pig at home? 1 = Yes, 2 = No

vi. If yes, how often do you slaughter pigs at home? _____ times a month, _____ times a year

vii. If "ever" how did you know weather or not it was fit for human consumption.

1 = by using our traditional inspection methods, 2 = by observing the background of slaughtered pig, 3 = by using official meat inspector, 4 = no any consideration made, 5 = others (specify) _____

viii. Within your household, which is the pork preparation method mostly preferred

1 = boiling, 2 = frying, 3 = raw, 4 = barbecue, 5 = others (specify) _____

ix. In this village, do you have a place(s) where someone can get prepared/cooked pork?1 = Yes, 2 = No

x. If 'Yes' which are place(s) located? 1. _____ 2. _____

xi. In these places, which are commonly pork preparation method used

1 = boiling, 2 = frying, 3 = raw, 4 = barbecue, 5 = others (specify) _____

xii. How often, do you or member of household use pork from these places? 1 = _____ times a week, _____ times a month, _____times a year, 2 = never

L. Hygiene: extent of latrine use, water assess and use

i. Presence and use of latrine (enumerator should request permission to assess the latrine)

1 = present and being used, 2 = present but not used, 3 = the construction started, 4 = absent

ii. Type of latrine 1 = pit latrine, 2 = others (specify) _____

iii. For household using latrine, the interviewer should assess the following

a) The status of walls 1= completed/strong with enough protection, 2 = incomplete/weak

b) The status of roof 1 = reasonable strong, 2 = present but week, 3 = latrine has no roof

c) Is the latrine having a closing door? 1 = Yes, 2 = No

d) Latrine base floor 1 = earthed, 2 = cemented, 2 = timber floor,

e) Presence of human faeces on the floor surface or elsewhere around the latrine: 1 = Yes, 2 = No

f) Who are the household members allowed to use latrine; 1 = every body, 2 = parents only,

3 = male only, 4 = females only, 5 = every body except children, others (specify) _____

g) Who constructed latrine for this household 1 = father, 2 = mother, 3 = causal labourer,

4 = others (specify)_____

iv. Which are the sources of water for your household? 1 = tap water, 2 = shallow borehole,

1 = deep borehole, 4 = springs, 5 = river, 6 = others (specify) _____

v. Location of water source: 1 = within the household, 2 = within the village, 3 = outside the village

vi. If outside the household, what is the distance to the most used water source for your household _____(Km),

vii. Do you boil your drinking water? 1 = always, 2 = sometimes, 3 = never

viii. Under following situations how often do you wash your hand

Practice for hand washing	**Most often**	**often**	**sometimes**	**never**
1. Before eating some food				
2. before eating some food using spoon				
3. After eating some food				
4. After using latrine				

L. Institutional elements, services and accessibility

i. Do you get extensions services for your pig production activities? 1 = Yes, 2 = No

ii. If “Yes” who provides you the services

1 = government extension services, 2 = private extension services, 3 = research,

4 = my own experience, 5 = neighboring farmers, 5 = relatives

iii. How often do you get extension service?

1 = most often (at least once per two months), 2 = often (at least once per three months),

3 = less often (at least once per six months), 4 = sometimes (at least once per year)

iv. How often are the following extension services provided to your pig enterprise

Extension cervices	Most often	often	sometimes	never
1. Treatment of sick pigs				
2. Construction of pig shelter				
3. Management of piglets				
4. Management of adults				
5. Pig feeding				
6. General control of diseases				

v. Which are main constraints limiting your pig production

Constraints	Ranking
1.	
2.	