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Transmission biology of porcine cysticercosis in eastern-central Tanzania

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Cover: Free-roaming pigs in a rural village of Kongwa district
(Photo: Justine Daudi Maganira)

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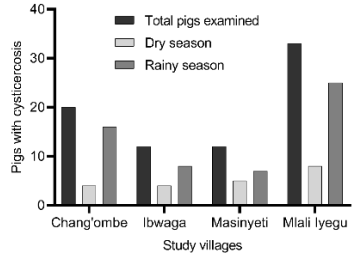

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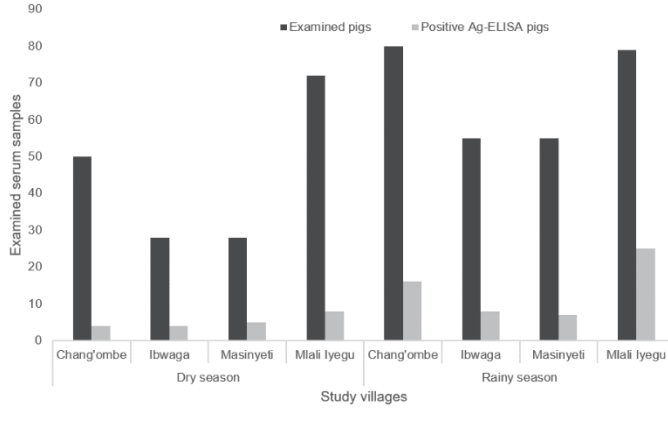

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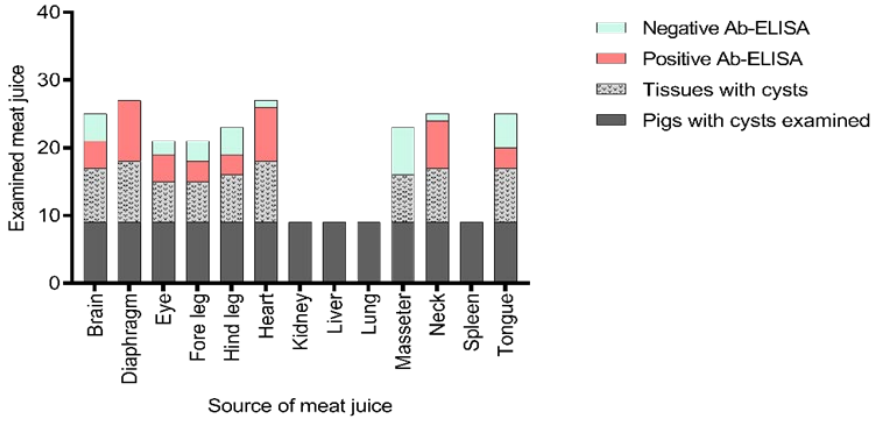

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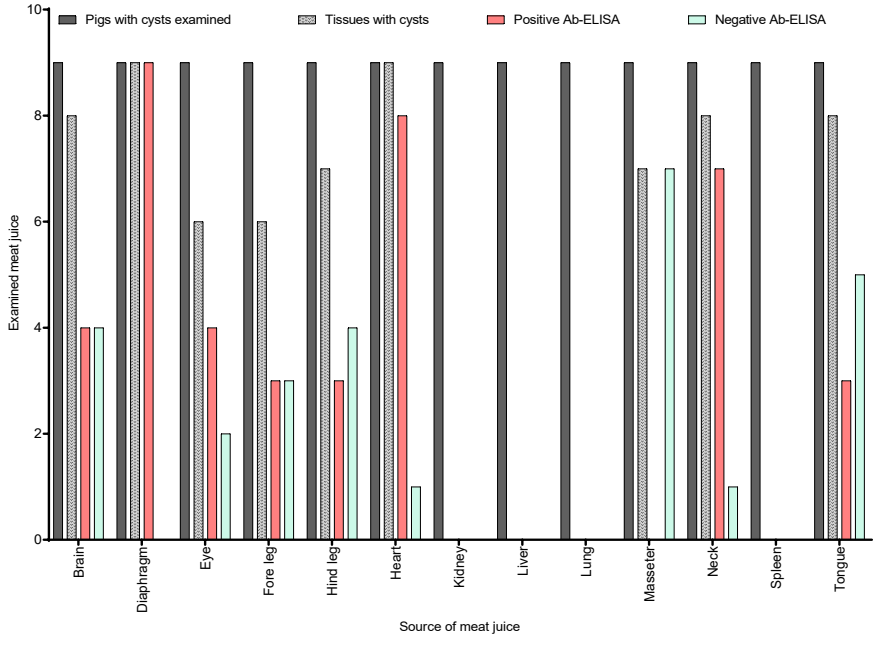

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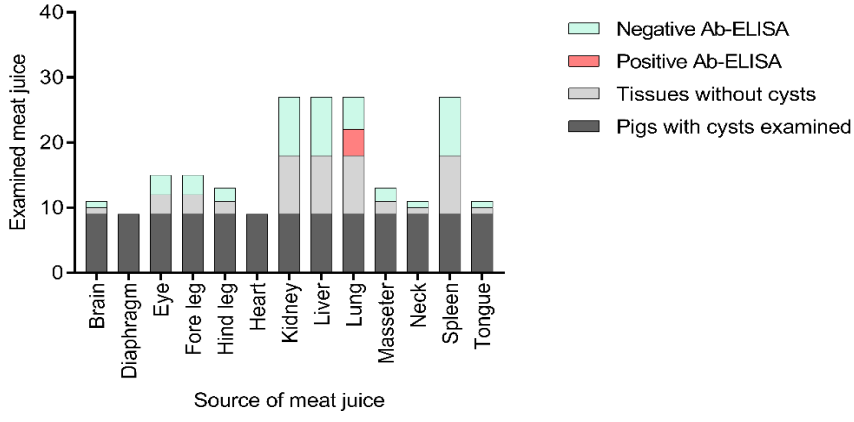

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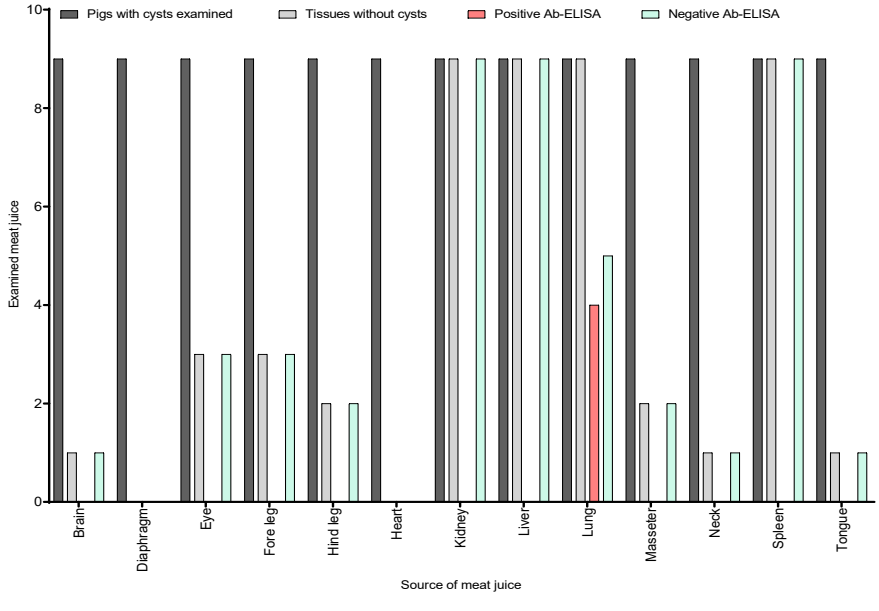

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Transmission biology of porcine cysticercosis in eastern-central Tanzania

Abstract

Porcine cysticercosis (PC) is a neglected zoonotic disease usually acquired by pigs after ingestion of *Taenia solium* eggs from food or an environment contaminated with human faeces of a pork tapeworm carrier. The disease has serious veterinary and public health implications in low-income endemic countries. The biology and molecular detection of the parasite in the intermediate host and the environment remain a serious concern. This thesis determined the seroprevalence of PC and its associated risk factors including the potential role of soil in the transmission of the eggs of the parasite in four rural villages of Kongwa district in eastern-central Tanzania; evaluated the effectiveness of a novel droplet digital Polymerase Chain Reaction (ddPCR) for detection of taeniid eggs in soil and finally; evaluated the diagnostic potential of pork meat juice for serological detection of PC. The seroprevalence of circulating taeniid antigens indicative of cysticercosis in pigs was found to be 17% using an enzyme-linked immunosorbent assay (ELISA), suggesting the presence of people harbouring adult *T. solium*. Indiscriminate defecation, free-range pig husbandry and source of reared pigs were identified using questionnaire as risk factors for PC transmission in the surveyed villages in Kongwa. The ddPCR technology was found to be effective in detecting taeniid egg DNA from soil using spiked and field soil samples. However, the rate of soil contamination by *T. solium* egg DNA detected by the ddPCR in 96 surveyed households in the surveyed villages was low (3.1%), suggesting a low risk of both pigs and humans acquiring cysticercosis through contaminated soil. The sensitivity of cysticercosis antibody ELISA using sera was 100%; whereas the overall sensitivity for meat juice was 38%. On the other hand, the sensitivity of the antibody ELISA was high in meat juice extracted from the diaphragm (100%), heart (89%) and neck (78%) muscles of infected pigs as compared to other carcass tissues. Meat juice from these tissues also gave higher mean antibody levels similar to sera suggesting that they may be used for the screening of PC after slaughter. *Taenia solium* is endemic in the study area. To safeguard veterinary and public health and curb economic losses in Kongwa, intervention measures are vital to implement.

Keywords: *Taenia solium*, porcine cysticercosis, ELISA, meat juice, taeniid eggs, soil contamination, ddPCR, Kongwa, Tanzania

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Överföringsbiologi för svincysticercos i östra centrala Tanzania

Abstract

Cysticerkos är en förbisedd zoonotisk parasitsjukdom som sprids mellan gris och människa. Sjukdomen orsakas av *Taenia solium* och förvärvas vid intag parasitens ägg via livsmedel, foder eller i en förorenad miljö. Smittan uppförökas och sprids med mänsklig avföring och påverkar den veterinära folkhälsan i utvecklingsländer. Tillgång till tillförlitlig diagnostik är viktigt för att kontrollera parasiten. Syftet med avhandlingen var att utforska prevalensen av cysticerkos hos gris och associerade riskfaktorer i fyra byar i Kongwa-distriktet i Tanzania. Förekomsten undersöktes med en serologisk metod (antigen- ELISA), och riskfaktorer kartlades med ett frågeformulär. Betydelsen av miljösmitta utvärderades med en molekylär metod (ddPCR) för detektion av *T. solium* ägg. Dessutom granskades den diagnostiska potentialen hos köttsaft från olika organ med ELISA. Prevalensen av cirkulerande antikroppar mot *Taenia* spp. hos grisarna i studien var 17% vilket visar på närvaro av personer som var infekterade med *T. solium* i de undersökta byarna. Riskfaktorer för cysticerkos hos gris var undermålig latrinhantering och frigående grisuppfödning. Taeniid-ägg-DNA detekterades med ddPCR-såväl i spikade jordprover som i fältprover. Emellertid var antalet positiva prover lågt (3,1%) i 96 undersökta hushåll. Detta tyder på låg risk för att grisar och människor i byarna drabbas av cysticercosis via jord förorenad med parasitens ägg. Vid ELISA analys av sera från 9 grisar med makroskopiskt synliga cystor var känsligheten 100%. Den var däremot endast 38% i köttsaft från 13 olika organsystem som undersöktes. Likväl var den hög i köttsaft från diafragma (100%), hjärta (89%) och halsmuskulatur (78%), med antikroppsnivåer liknande de i sera. Detta tyder på att köttsaft från dessa tre organ bör i första hand undersökas vid screening efter slakt. För att förbättra den veterinära folkhälsan och begränsa de sociala och ekonomiska förlusterna i Kongwa är fortsatta undersökningar som leder till förbättrade kontrollåtgärder viktiga att genomföra.

Nyckelord: *Taenia solium*, cysticerkos, ELISA, köttjuice, taeniid ägg, jordföroreningar, ddPCR, Kongwa, Tanzania

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Dedication

To my lovely wife, *Hyacinthe Kariba Muganda* - Thank you for your wonderful love, support and prayers and always being there all through. God bless you abundantly.

To my children (*Allan Daudi Justine*, *Allen Elias Justine* and *Allena Bertha Justine*) - You have made me stronger than I could have ever imagined. I love you so much.

To my lovely mother, *Bertha Bundala* - If not for your enduring love and support, I would not have come this far. God bless you abundantly.

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Maganira, J.D., Mwang'onde, B.J., Kidima, W., Mwita, C.J. and Höglund, J. (2019). Seroprevalence of circulating taeniid antigens in pigs and associated risk factors in Kongwa district, Tanzania. *Parasite Epidemiology and Control*, 7, e00123.

II Maganira, J.D., Mwang'onde, B.J., Kidima, W., Mwita, C.J., Nkwengulila, G. and Höglund, J. (2019). Validation of droplet digital Polymerase Chain Reaction for the detection and absolute quantification of *Taenia solium* eggs in spiked soil samples. *Acta Tropica*, 200, 105175.

III Maganira, J.D., Kidima, W., Mwita, C.J., Halvarsson, P. and Höglund, J. Soil contamination by *Taenia solium* eggs in rural villages in Kongwa district, Tanzania. *Infection Ecology & Epidemiology* (Submitted).

IV Maganira, J.D., Kidima, W., Mwita, C.J. and Höglund, J. Serological detection of *Taenia solium* cysticercosis in pigs using meat juice samples. *Journal of Parasitic Diseases* (Submitted).

The contribution of Justine Daudi Maganira to the papers included in this thesis was as follows:

I Conceived field and laboratory study designs in collaboration with co-authors and as part of the SIDA-Food Security sub-programme. Performed fieldwork with support from local assistants from Kongwa and co-authors. Mainly performed laboratory and statistical data analysis in collaboration with co-authors and a statistician. Drafted the manuscript with inputs from co-authors and handled correspondence with the journal.

II Conceived field and experiment design in collaboration with the main supervisor and as part of the SIDA-Food Security sub-programme. Performed fieldwork with support from co-authors. Performed laboratory and statistical data analysis in collaboration with the main supervisor. Performed data interpretation in collaboration with the main supervisor. Drafted the manuscript with inputs from co-authors and handled correspondence with the journal.

III Conceived field and laboratory design in collaboration with co-authors and as part of the SIDA-Food Security sub-programme. Performed fieldwork with support from local assistants from Kongwa and co-authors. Performed laboratory and statistical data analysis. Performed data interpretation in collaboration with the main supervisor. Drafted the manuscript with inputs from co-authors and handled correspondence with the journal.

IV Conceived field and laboratory design in collaboration with co-authors. Performed fieldwork, laboratory and statistical data analysis. Performed data interpretation in collaboration with the main supervisor. Drafted the manuscript with inputs from co-authors and handled correspondence with the journal.

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Abbreviations

ASF	African swine fever
cox1	Cytochrome c oxidase subunit I
ddPCR	Droplet digital polymerase chain reaction
GDP	Gross Domestic Product
DNA	Deoxyribonucleic acid
EAC	East African Community
FAO	Food and Agriculture Organization of the United Nations
FAOSTAT	FAO Statistical databases
HC	Human cysticercosis
mt	Mitochondrial
NBS	National Bureau of Statistics
NIMR	National Institute for Medical Research
NTDs	Neglected Tropical Diseases
PC	Porcine cysticercosis
SIDA	Swedish International Development Cooperation Agency
SLU	Swedish University of Agricultural Sciences
SPSS	Statistical Package for Social Sciences
SUA	Sokoine University of Agriculture
UDSM	University of Dar es Salaam
WHO	World Health Organisation

1 Background

Smallholder farmers in rural areas of low-income countries depend on livestock for both nutrition and economic survival (Maziku *et al*. 2017). In the past few years, pig production has tremendously increased (Figure 1) in the East Africa Community (EAC) states (FAO 2012; Maziku *et al*. 2017). This has largely been due to low cost of investment, high demand for pork in both rural and urban areas and recognition by communities of the fast and greater earnings of the pig sector as compared to other livestock (Phiri *et al*. 2003; Ngowi *et al*. 2004; Secka *et al*. 2010). One of the problems facing the pig production sector in the EAC particularly Tanzania is the existence of parasitic zoonoses such as *Taenia solium* cysticercosis. *Taenia solium* cysticercosis in pigs (porcine cysticercosis, PC) leads to significant economic losses such as reduced market value, condemnation or omission of infected pig carcasses from the food chain (Praet *et al*. 2009). Similarly, infection with *T. solium* cysticercosis in humans (human cysticercosis, HC) results in public health and socioeconomic concerns (Trevisan *et al*. 2017). For example, neurocysticercosis presents a predominant manifestation of human cysticercosis in the central nervous system and a major cause of acquired epilepsy in endemic regions (Assana *et al*. 2013; Mwape *et al*. 2015; Mwang'onde *et al*. 2018).

The economic burden of cysticercosis in the pig sector in Tanzania has been estimated at $3 million, while the direct and indirect costs imposed by human neurocysticercosis associated epilepsy has been reported to be about $5 million (Trevisan *et al*. 2017). In 2014, in Iringa rural district in the southern highlands of Tanzania, the respective annual losses due to cysticercosis in pigs and management of epilepsy in humans were estimated at $144 thousand and $79 thousand (Nkwengulila 2014). In other African countries such as in Mozambique, the annual costs due to cysticercosis has been estimated at $90 thousand of which 72% were costs associated with HC and 28% to pig sector losses (Trevisan *et al*. 2018). The total annual losses due to *T. solium* cysticercosis were estimated at €10.3 million in Cameroon of which 4.7% were

due to PC and 95.3% to direct and indirect losses caused by HC (Praet *et al*. 2009). Therefore, *T. solium* cysticercosis not only poses significant public health problems but also prevents smallholder farmers from achieving nutritional safety and security, livelihood and economic development in endemic low-income countries, Tanzania being no exception.

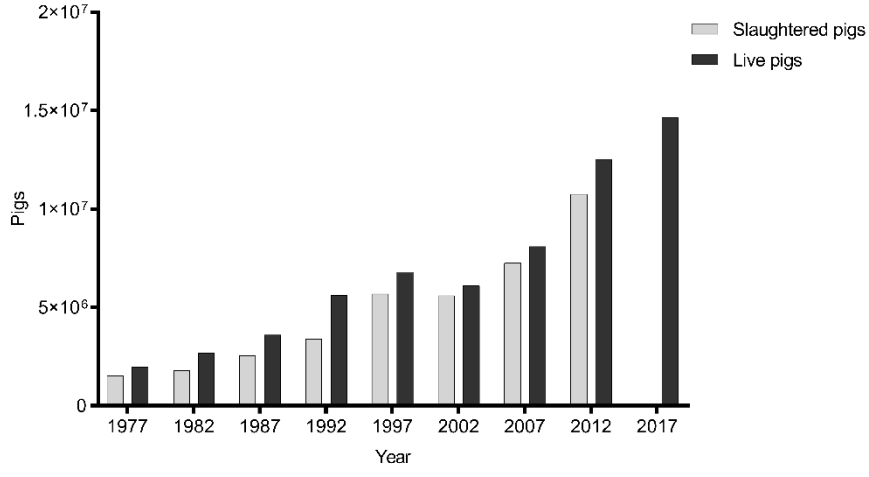

Figure 1. Trends in pig production and consumption for five East Africa Community countries (Burundi, Kenya, Rwanda, Tanzania, and Uganda) from 1977 – 2017. Pig production and consumption data for one EAC state (South Sudan) is missing whereas data for slaughtered pigs in the five EAC states is not available after 2013 (FAOSTAT 2017).

Taenia solium can be eradicated; however, meaningful control and monitoring programmes can probably be more efficient when more details in the infection cycle are understood. After years of research on this zoonotic parasite, the main focus of epidemiological studies has been on infection levels in pigs and humans. Even though a relatively high prevalence of the parasite has been reported in low-income countries almost worldwide, certain regions within these countries are still unsurveyed. The prevalence of *T. solium* cysticercosis in Tanzania and elsewhere in Sub-Saharan Africa has been reported to range between 0.12% to over 50% in humans or pigs (Phiri *et al*. 2003; Assana *et al*. 2013). It is a common practice in many rural areas of sub-Saharan Africa that pig farming is a small-scale farmer's activity in mixed agro-ecological farming system. Interestingly, the potential role of various environmental transmission routes of *T. solium* cysticercosis including soil remains inadequately researched, even though the soil is regarded as a common reservoir of geohelminths eggs (Collender *et al*. 2015). This is partly attributed to lack of sensitive standardised techniques for detection of the eggs of *T. solium* in environmental samples such

as soil. The paucity of information on the role of soil in the transmission of *T. solium* cysticercosis limits both local and international efforts to plan for control and elimination of the parasite in endemic areas.

Lack of control and monitoring programmes in Tanzania still allows infected pork to enter the food chain, thereby maintaining the persistence of the parasite. Therefore, generation of *T. solium* cysticercosis diagnostic information suitable for epidemiological studies, monitoring the health status of pig herds or parasites control programmes is necessary to provide safe pork to consumers.

Despite, the inclusion of *T. solium* cysticercosis to the list of Tanzania's health research priorities in 2013 by the National Institute for Medical Research (NIMR), there has been no control or national monitoring programmes (NIMR 2013). Nevertheless, it is globally urged that research should focus on establishing the extent and trends of zoonoses and device mechanisms for control to safeguard public health, minimize production problems and economic losses to ensure food safety and security (WHO 2010; NIMR 2013; FAO and WHO 2014). Consistent with both the country's and global health research agenda on neglected tropical diseases, the University of Dar es Salaam (UDSM) and Swedish University of Agricultural Sciences (SLU) developed a joint research and training programme within the framework of the Swedish International Development Cooperation Agency (SIDA) bilateral research programme 2015-2020. Funding from SIDA bilateral programme provided an opportunity to carry out a study on *T. solium* in the form of this thesis in Kongwa district in Dodoma region in eastern-central Tanzania. Unlike the southern and northern highland regions of the country, studies on PC in central Tanzania are scarce. Dodoma together with other regions in central Tanzania keep about 30% of the pigs in the country. These pigs are usually slaughtered in Dodoma and other heavily populated regions such as Arusha and Dar es Salaam. Thus, carrying out this study was in a view to safeguard public health following fast population increase in the region in recent years mainly due to two reasons; Firstly, the establishment of the University of Dodoma (UDOM) in 2007 has caused the population of university students to grow since its beginning with 1,200 students in 2007/2008 to 25,000. UDOM is designed to host 40,000 students at full capacity; this number will probably be reached after 2020 (www.udom.ac.tz). Secondly, the 2016 decision to relocate the government of Tanzania to the new capital of Dodoma from the commercial capital of Dar es Salaam have increased the human population. Within the region, Kongwa has the largest pig population (48%) as compared to other districts. SIDA's bilateral research programme provided an opportunity to explore the extent of PC and associated risk factors in Kongwa in order to recommend proper control strategies for food safety and security and so to safeguard public health. It also gave an opportunity to address the

methodological limitations for the detection of *T. solium* eggs in the soil as well as in pork entering the food chain. Importantly, a better understanding of the role of soil in the transmission of PC in rural households is important for planning future intervention strategies.

2 Introduction

2.1 Pig production in Tanzania

The livelihood and economy of more than 70% of the population in rural areas of Tanzania depend upon agriculture (NBS 2014). It is estimated that, out of the 5.8 million households involved in agriculture, 60% are engaged in crop production, 2% in livestock production, and 38% in both crop and livestock production (NBS 2016). Livestock production in Tanzania has been estimated to contribute 7.4% to the gross domestic product (GDP) with the majority of the livestock and products originating from smallholder farmers in rural areas (Lekule and Kyvsgaard 2003; Phiri *et al*. 2003; Michael *et al*. 2018). The major livestock contributing to the household and national economy include cattle (28.8 million), goats (16.7 million), sheep (5.0 million) and pigs (2.0 million) (Michael *et al*. 2018).

Although pigs only represent about 4% of the national population of livestock, pig production has tremendously increased in the past few decades due to high demand for pork in both rural and urban areas (Phiri *et al*. 2003; Wilson and Swai 2014; Michael *et al*. 2018). The increase in pig production has also been due to a shortage of grazing land for ruminants, low cost of investment and quick and high economic returns accrued from investing in pig production as compared to ruminants (Phiri *et al*. 2003).

Despite the widespread distribution of pigs throughout Tanzania, the majority of the pigs are reared in areas with prominent crop agriculture. About 54% of the pigs are reared in Mbeya, Iringa, Njombe, Rukwa and Ruvuma regions in the southern highlands of the country (Kimbi *et al*. 2015; NBS 2016), whereas about 30% are reared in Dodoma, Tabora, Singida and Morogoro regions in central Tanzania (Wilson and Swai 2014; NBS 2016).

In Tanzania, pig production systems can be categorized into two major systems:

1) *Smallholder pig production*: This is the main pig production system carried out by about 80% smallholder farmers in rural areas and with an average of three sows per household and a litter size of 6–8 (Wilson and Swai 2014; Maziku *et al*. 2017). The majority allow their pigs to roam around scavenging for food particularly during the post-harvest period (Phiri *et al.* 2003; Maziku *et al*. 2017). In this production system pigs are exposed to *T. solium* cysticercosis leading to low productivity, food insecurity and associated economic loss (Nkwengulila 2014; Kimbi *et al*. 2015). Thus, improvements are important for one-health reasons.

2) *Commercial pig production*: This constitutes about 20% of pigs raised mostly in urban areas or where there is a shortage of land in either commercial small-scale or large-scale systems. The commercial pig production system is characterized by pigs fed on concentrates and with reasonably good productivity (Lekule and Kyvsgaard 2003). The small-scale commercial pig production system has an average of eight sows and a litter size of 8–11 (Maziku *et al*. 2017). The large-scale system has an average of more than eight sows and is confined primarily to institutional or government farms.

The major constraint facing the pig production sector in Tanzania is diseases and lack of quality feed. About 20% of piglets suffer from infectious diseases and lack of animal health services (Maziku *et al*. 2017). In particular, African swine fever virus (ASF) and *T. solium* cysticercosis are recognised as significant constraints to pig production (Lekule and Kyvsgaard 2003; Phiri *et al*. 2003; Kamaghe *et al*. 2014). Due to shortage and lack of quality feeds, scavenging has been the main source of pig feed on many smallholder farms, whereas potato and cassava peels, maize bran and oilseed cakes act as supplements when available. Shortages and lack of proper housing for pigs critically limit the pig production sector (Phiri *et al*. 2003; Maziku *et al*. 2017).

The Tanzania Livestock Master Plan suggests that the pig industry has the potential to significantly contribute to food security and increased income generation for the involved stakeholders (Michael *et al*. 2018). To improve pig productivity there is a need to transform and modernize especially the low productive smallholder pig production system.

2.2 Life cycle of *T. solium*

The life cycle of *T. solium* (Figure 2) is indirect and involves humans as the definitive host, carrying the adult worm and with pigs as the natural intermediate host, carrying the encysted larval form, cysticerci (Leon 1995; Okamoto and Ito 2013). The cysticerci develop into adult *T. solium* and colonize the human small intestine reaching a length of up to five meters with about 1,000 proglottids, after

the definitive host ingests cysticerci from raw or insufficiently cooked pork (Leon 1995; Sathe *et al*. 2011; Flisser 2013a). The adult tapeworm attaches to the mucosa in the human intestine with a scolex equipped with four suckers and double concentric rows of between 22 and 32 hooks, and it can survive for up to more than 20 years (Spickler 2005; Flisser 2013a). Between 3-6 gravid proglottids each with about 50,000 infective eggs, are periodically released per day with the feaces into the environment (Sathe *et al*. 2011; Flisser 2013b). The eggs are spherical in shape and measure between 26 and 34 µm in diameter (Flisser 2013a).

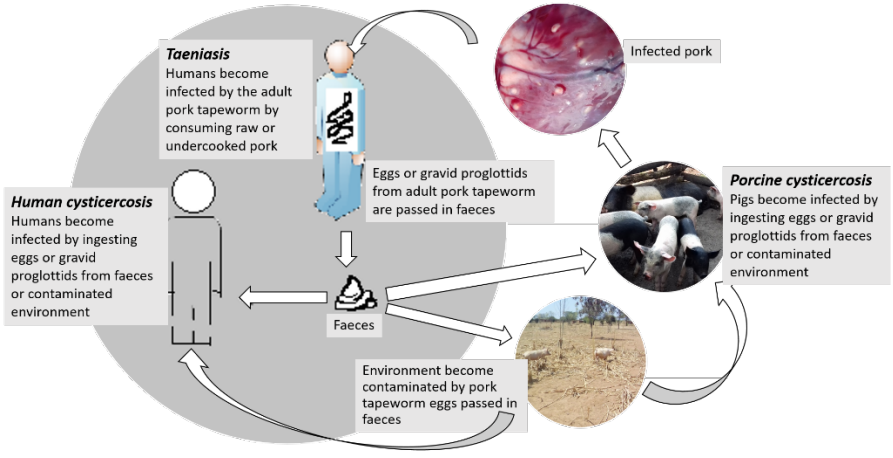

Figure 2. Taenia solium life cycle (Photo: Justine Daudi Maganira, SLU).

The natural intermediate host of *T. solium* is pigs. However, dogs and humans can be infected but then remain dead-end hosts (Leon 1995; Ito *et al*. 2002). After pigs have ingested the eggs, bile and enzymes disaggregate the embryophoric blocks and digest the oncospheral membrane. The oncosphere crosses the intestinal wall with the aid of its hooklets and it will then circulate within the body through the vascular or lymphatic system (Leon 1995; Sathe *et al*. 2011). Here the oncosphere grows and transforms into cysticercus, which relocates primarily in muscles and nervous tissues (Flisser 2013a). After ingestion by the definitive host, it takes about two months for the cysticercus to develop into mature *T. solium* tapeworm (García *et al*. 2003; Flisser 2013a). In a study from Uganda, Kisakye and Masaba (2002) found evidence of congenital transmission of *T. solium* cysticerci in fetuses recovered from a sow infected with cysticerci.

Humans may also be accidentally infected by *T. solium* eggs from contaminated hand or after ingesting the eggs on contaminated food by for

example soil or water when there are poor hygiene and sanitation conditions (Phiri *et al*. 2003; Mwang'onde *et al*. 2014). Occasionally, internal autoinfection might occur when proglottids reach the stomach by reverse peristalsis causing massive infections, but it is not clear that it actually occurs (Gilman *et al*. 2000). In the human body, the eggs develop into cysticerci, which primarily relocate into muscle tissue and the brain.

2.3 Zoonotic potential of *T. solium*

As a zoonotic parasite, the adult *T. solium* tapeworm causes taeniasis in humans, while its larval form causes cysticercosis in both humans and pigs. *Taenia solium* cysticercosis has been increasingly reported as a neglected parasitic zoonosis with major veterinary and public health concerns in endemic regions (Phiri *et al*. 2003; Assana *et al*. 2013; Coral-Almeida *et al*. 2014; Okello *et al*. 2015).

Taeniasis is usually asymptomatic but may be characterized by mild broad-spectrum symptoms such as abdominal or epigastric pain, nausea, hunger, diarrhoea, constipation, and chronic indigestion (Sathe *et al*. 2011; Okamoto and Ito 2013). The prevalence of taeniasis ranges between 0.1 – 8.7% in African countries (Assana *et al*. 2013), 0.2 – 2.8% in Latin America (Flisser *et al*. 2003) and 0.06 – 26.1% in Southeast Asia (Wu *et al*. 2017), with the highest prevalence in hospital patients.

As stated earlier pigs contract cysticercosis by ingestion of *T. solium* eggs in human faeces or contaminated feed-stuff, water and/or soil (Okamoto and Ito 2013; Johansen *et al*. 2014). The cysticerci have a predilection to the cardiac and skeletal muscles or brain of pigs and remain viable at least for one year (Flisser 2013a). The prevalence of cysticercosis in pigs has been reported to be as high as 35% in Mexico and southeast Asia (Flisser *et al*. 2003; Wu *et al*. 2017) and up to 57% in Africa (Assana *et al*. 2013). Some studies reported the absence of noticeable signs associated with PC infection (García *et al*. 2003). However, researches in India noticed clinical signs, such as excessive salivation, blinking and tearing, suggestive of neurocysticercosis (Prasad *et al*. 2006). Another similar study conducted in Tanzania, reported dullness, sluggishness, somnolence, apathy, and loss of consciousness in pigs naturally infected with cysticerci (Mkupasi *et al*. 2014). Pigs with cysticercosis have also been reported to suffer from seizures like humans with symptomatic neurocysticercosis (Trevisan *et al*. 2016).

When humans become accidental intermediate dead-end hosts, the eggs release the oncospheres that develop into cysticerci that primarily relocate into striated muscles, eyes and the brain. The relocation into the brain causes neurocysticercosis with symptoms such as severe headache and epilepsy, and in

the eyes may result into impaired visual acuity (Coral-Almeida *et al*. 2014; Mwape *et al*. 2015; Mwang'onde *et al*. 2018). A prevalence of HC of 11%, 22% and 35% has been reported in Mexico, Africa, and Asia, respectively (Flisser *et al*. 2003; Assana et al. 2013; Wu *et al*. 2017).

2.4 Distribution of *T. solium*

Taenia solium has attained a worldwide distribution (Figure 3) but it is most prevalent in low-income countries of Asia, Latin America and Africa (Schantz *et al*. 1992; Flisser *et al*. 2003; Phiri *et al*. 2003). The prevalence of *T. solium* PC has been reported in almost all sub-Saharan African countries with a range of up to over 50% (Phiri *et al*. 2003; Assana *et al*. 2013).

The worldwide geographical distribution of *T. solium* is based on data obtained from pigs and humans. In contrast, data from studies on the contamination of the environment including soil in endemic areas are scarce or non-existent. This thesis partly explored the prevalence of *T. solium* in soil from endemic rural villages of Kongwa district in eastern-central Tanzania.

As indicated earlier the prevalence of *T. solium* in Tanzania is favoured by traditional pig husbandry under poor sanitation, which is motivated by lifestyle, ignorance, and poverty. Due to improved sanitation, commercial pig-production and education, *T. solium* infection is currently uncommon in countries of Europe (Zammarchi *et al*. 2013) and in the United States of America (Sorvillo *et al*. 2011). Thus, in developed countries, cysticercosis in humans is predominantly an imported disease due to increased globalisation (Sorvillo *et al*. 2011; Zammarchi *et al*. 2013). The parasite is also uncommon in countries where pork is not consumed under religious grounds. However, it is worth noting that HC can be acquired by anyone, regardless of either ethnicity or religious beliefs (Schantz *et al*. 1992), as far as eggs from a human harbouring the adult *T. solium* are ingested through faecally contaminated water, food or in soil. A well-known example is the diagnosis of cysticercosis in four families of Orthodox Jews in New York, who had neither eaten pork nor associated with pigs (Schantz *et al*. 1992).

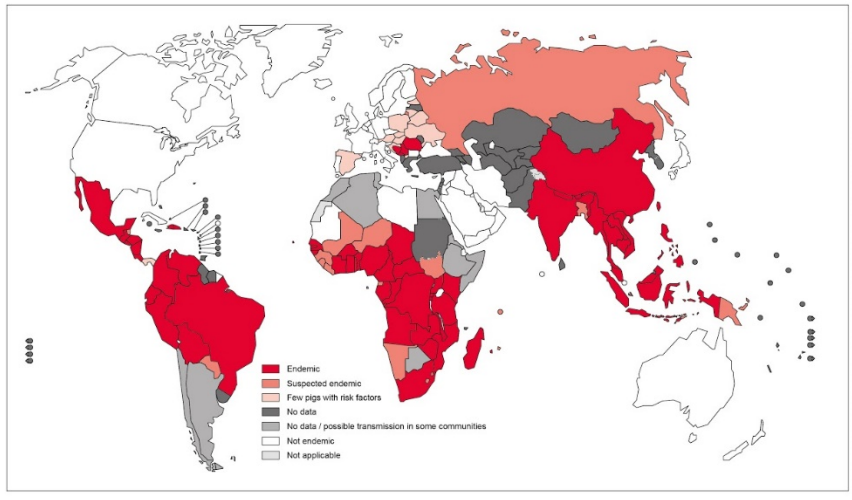

Figure 3. Global distribution of *Taenia solium* (Modified from Donadeu *et al*., 2016).

2.5 Prevalence of *T. solium* in Tanzania

In Tanzania, PC has been reported as an emerging problem since 1980s. In these years, pigs originating from the Mbulu district in the northern highlands of the country were exported to Kenya, and they were found to be heavily infected with the parasite at slaughter (Phiri *et al*. 2003). This finding was followed by a retrospective survey of slaughter slab records from Mbulu district where the prevalence of PC was reported to increase from 0.4% to 5% between 1985 and 1989 (Phiri *et al*. 2003). This was followed by another study in 1993, which reported between 5% to 38% PC prevalence based on *postmortem* examination of pigs slaughtered in the northern highland regions of Tanzania (Boa *et al*. 1995). Since then, survey studies mostly conducted in rural areas of northern and southern highlands of the country (Figure 4) have reported prevalence of PC as high as 33% (Ngowi *et al*. 2004; Boa *et al*. 2006; Ngowi *et al*. 2010; Komba *et al*. 2013; Yohana *et al*. 2013; Braae *et al*. 2014; Ngowi *et al*. 2014; Kabululu *et al*. 2015; Kavishe *et al*. 2017; Shonyela *et al*. 2017; Maganira *et al*. 2018). Furthermore, human taeniasis (Mwanjali *et al*. 2013; Braae *et al*. 2017) and cysticercosis (Winkler *et al*. 2009; Mwang'onde *et al*. 2012; Mwanjali *et al*. 2013; Ngugi *et al*. 2013; Hunter *et al*. 2015) have been reported in both the southern and northern highlands of Tanzania. On the contrary, despite keeping about 30% of the pigs in the country and the presence of possible risk factors for

transmission, only two reports have reported on *T. solium* in central Tanzania. The first study based on a microscopic examination of *T. solium* eggs from faecal samples among school children in Kongwa district in Dodoma region, reported a prevalence of 0.4% (Eom *et al*. 2011). The second study determined the co-endemicity of cysticercosis and gastrointestinal parasites in rural pigs in Kongwa. This study estimated the overall prevalence of PC of 15% but used a lingual examination technique (Ngowi *et al*. 2014). Furthermore, the prevalence of PC based on studies at slaughter slabs in various areas in Tanzania has been reported as high as 18% (Mkupasi 2008; Kavishe *et al*. 2017; Shonyela *et al*. 2017).

Previous studies reporting on the rate of contamination of soil by *T. solium* eggs are non-existent not only in central Tanzania regions but also countrywide. The paucity of information on the rate of soil contamination by *T. solium* eggs limits the understanding of PC and HC infection risk at a village and household level not only in Tanzania but also elsewhere in endemic areas.

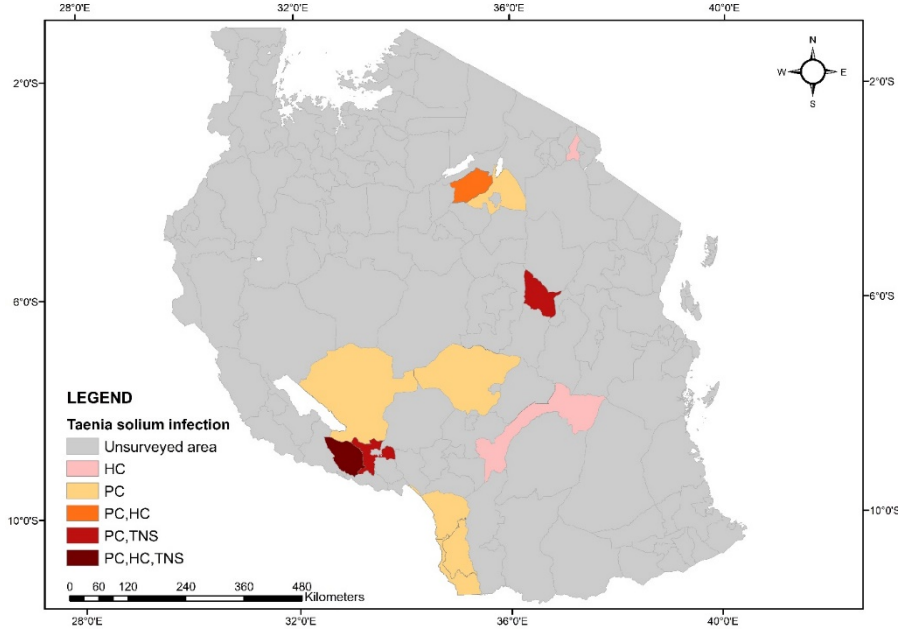

Figure 4. Locations of previous epidemiological studies for *Taenia solium* prevalence in Tanzania (Ngowi *et al*. 2004; Boa *et al*. 2006; Winkler *et al*. 2009; Ngowi *et al*. 2010; Mwang'onde *et al*. 2012; Komba *et al*. 2013; Mwanjali *et al*. 2013; Ngugi *et al*. 2013; Yohana *et al*. 2013; Braae *et al*. 2014; Ngowi *et al*. 2014; Hunter *et al*. 2015; Kabululu *et al*. 2015; Braae *et al*. 2017; Kavishe *et al*. 2017; Shonyela *et al*. 2017; Maganira *et al*. 2018).

2.6 Taeniid cestodes

The family *Taeniidae* is composed of several genera, but *Taenia* and *Echinococcus* are the most medically important (Taylor *et al*. 2007; Okamoto and Ito 2013). Usually, two mammalian hosts are required to maintain the life cycle of tapeworms in the two genera. Although the eggs are identical, adult tapeworms in the genus *Taenia* are much longer than the dwarf tapeworms within the genus *Echinococcus* (Sathe *et al*. 2011; Flisser 2013a; Okamoto and Ito 2013).

The genus *Taenia* currently has 43 species in a range of mammalian host including the recently described species *Taenia lynciscapreoli* from the Eurasian lynx (Okamoto and Ito 2013; Haukisalmi *et al*. 2016). However, the most medically important zoonotic species are *T. solium* (pork tapeworm), *T. saginata* (beef tapeworm) and *T. asiatica*, which all have humans as their definitive hosts. Because all taeniids are morphologically similar such as in the shape and size of eggs (26-36 µm) (Taylor *et al*. 2007; Okamoto and Ito 2013), it is important to differentiate them especially as they can occur in the same area or mammalian host. Given morphological similarities, taeniid eggs are impossible to distinguish through conventional methods employing microscopy (Eom *et al*. 2011; Okamoto and Ito 2013). On the other hand, the identification of the adults with conventional diagnostic methods can be increased by examining morphological characteristics in the scolex and proglottids after staining with Indian ink (Okamoto and Ito 2013). The scolex of *T. solium* has a double row of small hooks while that of other taeniid cestodes such as *T. saginata or T. asiatica* lack hooks (Flisser 2013a; Okamoto and Ito 2013). Depending on the number of uterine branches, taeniid cestodes can be identified as *T. solium* (7-11 branches), *T. asiatica* (12-26 branches) or *T. saginata* (14-32 branches) (Flisser 2013a).

From a research perspective, morphological similarities of taeniid eggs can offer an opportunity of using one species as a proxy for understanding some aspects of another species. For example, eggs of either taeniid species can be utilized in the validation of detection methods such as for eggs in the soil.

2.7 Risk factors for *T. solium* transmission in pigs

The primary risk factor for cysticercosis acquisition in both pigs and humans is the presence of adult *T. solium* human carrier (Flisser *et al*. 2003). Nevertheless, the inclusion of infected pork in the food chain is the main risk factor of acquiring the adult *T. solium* in humans. The role of other risk factors can only ensure the accessibility of pigs or humans to *T. solium* eggs from human faeces.

Free-range pig husbandry and absence or poor usage of latrines are identified as the main risk factors exposing pigs to *T. solium* cysticercosis infection in

many low-income countries in Latin America (Alcobedes *et al*. 2010), Asia (Khaing *et al*. 2015; Okello *et al*. 2015) and Sub-Saharan Africa (Pondja *et al*. 2010; Braae *et al*. 2015; Shonyela *et al*. 2017). Other risk factors for PC identified include increased age and feedstuff (Pondja *et al*. 2010; Khaing *et al*. 2015) or on a dirt floor (Braae *et al*. 2015). Thus, PC is mainly contracted when roaming pigs have free access to human faeces contaminated with *T. solium* eggs in endemic areas, where there is lack or improper use of latrines resulting in open defecation. In recent years, the government of Tanzania has been emphasizing the establishment of sanitary facilities in rural settings in a bid to fight infectious diseases. However, reports of PC and the presence of open defecation from such areas calls for health education regarding the proper use of such establishment.

Generally, *T. solium* infection persists in low-income endemic areas where pigs are free-roaming, meat inspection is insufficient or non-existent, indiscriminate defecation is practiced, personal and meat hygiene is poor. These risk factors largely are associated with ignorance, lifestyles and poverty.

2.8 Detection of *T. solium* in soil

Soil is the main reservoir of common geo-helminths in many developing countries (Collender *et al*. 2015; Steinbaum *et al*. 2017). However, there is no standard technique for detecting eggs of these parasites in the soil, which hinders rigorous research and inclusion of the environment in control programmes.

Even though there is a lack of a standard method, previous studies mainly screening soil for nematode eggs have depended on sieving, flotation, and microscopy (Collender *et al*. 2015; Steinbaum *et al*. 2017). Studies screening soil for *T. solium* eggs are scarce. Molecular methods have been used in previous studies and are known to reduce human errors in detecting parasite eggs compared to microscopy, which cannot distinguish, taeniid eggs. However, most of these methods require standard curves and are prone to restrictions from inhibitors (Demeke and Dobnik 2018). A standard molecular technique for detecting and quantifying parasite eggs such as those of *T. solium* in the soil would be essential for rigorous assessment of environmental contamination and sound estimation of infection risk. Such a method would be useful for epidemiological studies assessing soil contamination in order to better understand the environmental transmission pathway for effective planning for control strategies.

Even though conventional molecular techniques have not been applied for environmental screening of *T. solium* eggs, in recent years, these techniques (e.g. Multiplex, PCR-Restriction Fragment Length Polymorphism) have often been used as a confirmatory test for taeniid DNA in faecal samples (copro-DNA)

(Yamasaki *et al*. 2004), cysticerci (Abuseir *et al*. 2006; Chembensofu *et al*. 2017) or eggs and proglottids in human stool (Yamasaki *et al*. 2004; Eom *et al*. 2011). This thesis evaluates a novel molecular technique (droplet digital Polymerase Chain Reaction, ddPCR) for detecting and quantifying taeniid eggs from the soil. DdPCR is a third generation PCR technology for absolute quantification of target DNA (Pinheiro *et al*. 2012). This PCR technology quantifies the total number of individual target DNA in a digital format (Pinheiro *et al*. 2012). The advantages of the ddPCR technique as compared to other PCR methods include improved sensitivity and the ability for precise quantification without the need of standard curves (Pinheiro *et al*. 2012; Demeke and Dobnik 2018). In addition, the ddPCR is not prone to restrictions from inhibitors (Demeke and Dobnik 2018). The ddPCR technology has been applied in the detection of geo-helminths eggs such as those of *Ascaris lumbricoides* in reclaimed water and screening of bacteria in soil (Kim *et al*. 2014; Soto *et al*. 2017). This technology has also been applied in the detection of genetically modified organisms (Dobnik *et al*. 2015), human malaria and schistosomiasis (Koepfli *et al*. 2016; Weerakoon *et al*. 2017) and gastrointestinal nematodes of sheep (Elmahalawy *et al*. 2018). However, the use of ddPCR technology for screening *T. solium* eggs from soil has not been reported before.

2.9 Diagnosis of *T. solium* in pigs

The diagnosis of PC is critical to prevent taeniasis in humans and subsequent cysticercosis in pigs and humans. The routine diagnosis of *T. solium* cysticercosis in pigs depends on *antemortem* through lingual and conjunctival inspection, *postmortem* examination or serological testing for circulating antibodies or antigens (Dorny *et al*. 2003; Chembensofu *et al*. 2017).

2.9.1 Tongue and carcass inspection

The most common ways for the detection of cysticercosis in pigs in endemic areas have been tongue or carcass inspection, where cysticerci are identified in the tongue by palpation or in the carcass by visual inspection (Gonzalez *et al*. 2001; Chembensofu *et al*. 2017). In Africa, most of the epidemiological studies on PC have used tongue or carcass inspection (Phiri *et al*. 2006; Thomas *et al*. 2016; Chembensofu *et al*. 2017). Though not commonly used, conjunctival examination demonstrates the presence of cysticerci in the eyes of pigs. Both tongue and conjunctival examination require a certain level of technical skill and are only effective in heavily infected pigs (Chembensofu *et al*. 2017).

Pig carcass inspection has commonly been used for accurate diagnosis for the presence of cysticerci in pigs. Different countries have different regulations concerning the *postmortem* examination of carcasses, but invariably the masseter, ham, lingual and cardiac muscles are incised and examined (Taylor *et al*. 2007). The occurrence of cysticerci in the brain has also been reported (Chembensofu *et al*. 2017).

Tongue and carcass examination have most often been used to check the quality of pork entering the food chain and are reported to be specific, but lack sensitivity for low cysticerci infection levels (Dorny *et al*. 2004; Phiri *et al*. 2006; Taylor *et al*. 2007). In a study conducted in Zambia, tongue palpation and meat inspection failed to detect the infection in 84% and 61% of infected pigs, respectively (Phiri *et al*. 2006). Low sensitivity of detecting cysticercosis in pigs using tongue palpation (21%) or routine meat inspection (22%) has also been estimated using a Bayesian approach (Dorny *et al*. 2004). Failure to detect a low level of cysticerci infections by these diagnostic tests allows pork with cysticerci to enter the food chain unnoticed and hinders epidemiological studies to accurately estimate the true prevalence of PC in endemic areas. Thus, meat inspection seems to be an ineffective means of detecting infected pigs and removing the parasite from the food chain.

2.9.2 Serodiagnosis in pigs

Serological detection of cysticercosis in pigs can be based on the detection of either circulating parasite antigens or specific antibodies in serum using enzyme-linked immunosorbent assays (ELISA) or by detection of antibodies by enzyme-linked immunoelectrotransfer blot (EITB) (Dorny *et al*. 2003; Lightowlers *et al*. 2016). Unlike carcass inspection, serological tests can be used on live pigs, thus widely useful for epidemiological and disease monitoring as well as in studies evaluating control programmes (Dorny *et al*. 2003). Even though serological tests are reported to be more sensitive than meat inspection (Dorny *et al*. 2000), they are in general limited by the lack of species specificity when performed in pigs (Akoko *et al*. 2019). For example, currently, most assays cannot differentiate *T. solium* from other taeniid species such as *T. hydatigena* and *T. asiatica* (Dorny *et al*. 2003; Dermauw *et al*. 2016; Akoko *et al*. 2019). Therefore, the aspect of cross-reactivity should be considered especially in areas where *T. asiatica* and other taeniids such as *T. hydatigena* are common. The cysticerci of *T. hydatigena* are reported to be common in small ruminants in Africa, but uncommon in pigs (Dorny *et al*. 2004). In contrast to the Ab-ELISA, which measures exposure to the parasite and not necessarily current infection, the Ag-ELISA detects only viable cysticerci (Nguekam *et al*. 2003). In this respect, Ag-

ELISA can be a useful tool for monitoring the performance of an anthelminthic intervention in pigs (Nguekam *et al*. 2003; Deckers and Dorny 2010). Measurement of exposure to the infection may also overestimate the true prevalence because antibodies may last longer even after the elimination of the parasite by the immune system or drug treatment (García *et al*. 1997). Thus, unlike antibody detection, the measurement of circulating antigens permits the differentiation of cysticercosis cases with viable cysticerci from degenerated or calcified cysticerci. Moreover, the sensitivity of the Ag- ELISA has been estimated to be higher than that of the Ab-ELISA (Nguekam *et al*. 2003; Dorny *et al*. 2004). It has been shown using the cysticercosis Ag- ELISA in experimentally infected pigs that circulating antigens can be detected between two to six weeks post-infection, with the minimum number of detectable living cysts being one (Nguekam *et al*. 2003; Dorny *et al*. 2004). These diagnoses challenges spell an urgent need for the development of a specific diagnostic test for parasite epidemiological studies as well as monitoring and evaluation of control programmes.

2.10 Control of *T. solium* in pigs

The control of cysticercosis in pigs in most low-income countries is important to safeguard veterinary and public health and curtail economic losses in the pig sector. Although the WHO considers that *T. solium* infection can be prevented and eradicated, sustainable interruption of the transmission cycle has not yet been reported. The absence of a large-scale control programme in sub-Saharan African has maintained the prevalence of *T. solium* transmission in pigs and humans. The available control approaches and their relevance for *T. solium* control in endemic areas are considered below.

2.10.1 Treatment of humans with taeniasis

Humans represent the only definitive host of *T. solium*, thus carry the adult worm, which is the source of eggs causing cysticercosis in both pigs and humans. Therefore, chemotherapy treatment of humans represents a viable option to prevent the pig intermediate host from acquiring cysticercosis. Praziquantel and niclosamide have proven to be safe and effective in the treatment of taeniasis. It has been shown in a study in Guatemala that the prevalence of taeniasis declined from 35% before treatment to 1% ten months after treatment of a human population with niclosamide and consequently, cysticercosis in pigs declined from 55% to 7% (Allan *et al*. 1997). The potential of chemotherapy against taeniasis to control cysticercosis was also demonstrated in a study carried out in

a rural community in Mexico using praziquantel (Sarti *et al*. 2000). Treatment of humans with praziquantel at doses higher than 10 mg/kg has shown to be highly effective but there is a risk of inducing seizures in humans post treatment (Flisser *et al*. 1993; Sarti *et al*. 2000). Unlike praziquantel, niclosamide does not cross the blood-brain barrier to induce seizures (Sarti and Rajshekhar 2003). Furthermore, niclosamide does not require cold storage and has a long shelf life (Sarti and Rajshekhar 2003). Thus, this drug is the best choice for many low-income countries. However, it is advised that mass therapy should be administered twice a year over five or more years to have a major impact on taeniasis load in the target population.

Chemotherapy is one of the feasible strategies in the control *T. solium* parasite, however, its effectiveness can be maximized when combined with prophylactic strategies such as educating people regarding safe disposal of human faeces to prevent contact of pigs with contaminated faeces. Seeking community compliance during mass chemotherapy can also demonstrate positive impacts and sustainability of the intervention in reducing taeniasis and cysticercosis. For example, PC increased after chemotherapy intervention for taeniasis in a Mexican village due to poor community compliance with treatment recommendations and poor disposal of human faeces (Keilbach *et al*. 1989).

2.10.2 Treatment of pigs with cysticercosis

The treatment of pigs infected with cysticercosis is important in buffering pig farmers from economic losses due to the loss of infected pigs as well as health problems. However, there is currently no registered drug for the treatment of PC. Still, various drugs such as albendazole, fenbendazole, flubendazole, oxfendazole and praziquantel, have been tested with varying success (Gonzalez *et al*. 1997; Gonzalez *et al*. 2012; Pondja *et al*. 2012; Mkupasi *et al*. 2013). Treatment of PC with oxfendazole has been reported to be highly effective when administered as a single dose at 30 mg/kg bodyweight (Gonzalez *et al*. 1997; Garcia *et al*. 2016). Oxfendazole has also been reported by Gonzalez *et al*. (2001) to protect pigs from new cysticercosis infections for at least three months after treatment. Alternatively, treating pigs with cysticercosis using albendazole sulphoxide has been shown in Mexico to be effective in controlling muscle cysts when administered subcutaneously for eight consecutive days at 15 mg/kg bodyweight (Peniche-cardeña *et al*. 2002). More work is clearly needed to validate the usefulness of anthelminthics against PC. Meanwhile, efforts to prevent pigs from acquiring cysticercosis in endemic areas are important in order to safeguard public health.

2.10.3 Meat inspection and processing

As stated before pigs intended for human consumption can be inspected for cysticercosis infection by tongue examination or meat inspection at slaughter (Thomas *et al*. 2016). Although these techniques have low sensitivity, they are cheap, easy to perform and have the potential to exclude heavily infected pork from the food chain if correctly and strictly performed (Phiri *et al*. 2003; Dorny *et al*. 2004; Thomas *et al*. 2016). However, in many endemic rural areas, meat inspection service is lacking or where available it is not strictly performed and this likely allows infected pork to enter the food chain undetected. Furthermore, following meat inspection, farmers may incur huge economic losses due to reduced market value or condemnation of infected pork carcasses. Consequently, some smallholder pig farmers and traders in Tanzania occasionally perform tongue examination themselves for cysticerci before selling or buying the pigs to avoid condemnation. Pigs found infected are then slaughtered in clandestine places where they are informally marketed and sold at a cheap price. Farmers also may sell their infected pigs to remote areas where there is no meat inspection service, as more often there is no sufficient veterinarians plus the ignorance of the communities on the disease. Removing infected pork from the food chain is critical for control of *T. solium* but to achieve complete removal pig farmers should be motivated to avoid clandestine trade by compensating them for their infected pigs or pork that are subject to condemnation.

Hygiene and proper meat processing at the household or community level is also important to remove the parasite from the food chain and avoid taeniasis. It has been shown that viable cysticerci can be killed at a temperature of >65 °C for 3 h or a cold treatment at -10°C for 10 days or at -24°C for one day (Sotelo, Rosas and Palencia 1986; Rodriguez-Canul *et al*. 2002). The availability of freezers in many endemic areas is a challenge, thus cooking seems to be the most feasible strategy to kill any viable cysticerci in pork. This method should be brought to the attention of people preparing and consuming pork in contrast to the loved grilling or frying methods, which may not kill the cysticerci (Assana *et al*. 2013). Therefore, health education on meat hygiene and preparation is vital for effective and sustainable removal of the parasite from the infection cycle.

2.10.4 Improvement in hygiene, sanitation and pig husbandry

There is evidence from some developed countries of *T. solium* parasite eradication due to compulsory meat inspection as well as improvements in sanitation and pig husbandry practices (Del Brutto 2012). High economic development is attributed to the eradication of this parasite in these countries due

to the establishment of the necessary infrastructure (Sarti and Rajshekhar 2003). Unlike developed countries, many low-income developing countries have high levels of poverty especially among smallholder farmers in rural areas, which make improvements in sanitation and pig husbandry infrastructure difficult.

The availability and proper use of latrines in *T. solium* endemic areas are important to avoid indiscriminate defecation. A considerably higher prevalence of cysticercosis in pigs reared in households lacking latrines than in those reared in households using latrines was reported in a study in the Mbulu district in Tanzania (Ngowi *et al*. 2004). In contrast, the prevalence of PC did not differ between households with or without latrines in the Angónia district in Mozambique (Pondja *et al*. 2010). Finding from these studies indicate that safe disposal of human waste through effective use of latrine should reduce the risk of cysticercosis infection in pigs and therefore in the human population. The presence of open defecation and high rates of PC in rural areas with latrines suggest poor utilization of latrines giving free-roaming pigs access to potentially contaminated human faeces and hence a risk of contracting cysticercosis. Even though improving sanitation is costly and therefore deterrent to the adoption of such measures, some developing countries such as Tanzania have been emphasizing the construction of latrines in every rural home in a bid to fight various infectious diseases. Education on the proper use of sanitary facilities is thus important to stop open defecation and thereby reduce the risk of infection for both pigs and humans. The enforcement of local bylaws by the local authorities in respective areas may also play part in the utility of the sanitary facilities.

Personal and general household hygiene is important to prevent the dissemination of *T. solium* eggs from human carriers and therefore lowering the risk of cysticercosis acquisition through contamination in both pigs and humans. Personal hygiene including washing hands after visiting a sanitary facility, adequately washing vegetables and fruits and drinking clean and safe water are all vital aspects in the fight against cysticercosis infection. The availability of clean water is therefore important in the improvement of hygiene in the household. It has been shown in Nigeria that the risk of contracting a *T. solium* cysticercosis infection is six times higher in people who do not wash their hands after defecating than those who wash their hands with soap and water *(Weka et al*. 2013). In many low-income countries including Tanzania, the provision of sufficiently clean water, especially in rural communities, is still a change and this is likely to hinder efforts towards personal and household hygiene improvements as a control strategy.

The prevalence of PC in the EAC and other sub-Saharan African countries is maintained by the traditional free-range pig production systems (Phiri *et al*.

2003; Ngowi *et al*. 2004; Weka *et al*. 2013). Investing in pig production under a free-range pig management system is cheaper than a pig confinement system. Pig confinement leads to additional investment costs to the already poor smallholder farmers due to proper pig housing and continuous food supply requirement (Lekule and Kyvsgaard 2003; Willingham and Engels 2006). Most pig farmers in rural areas are yet to adopt total pig confinement due to the high level of poverty and therefore not suitable as a long-term control strategy for *T. solium*. Placing high price tags on pigs free from cysticercosis may be one way to buffer increased production cost (Willingham and Engels 2006) but this is likely to affect markets and therefore household economy and nutrition. Farmers need to be motivated by the fact that confinement has the potential to prevent pigs from acquiring other parasitic diseases, and ASF (Lekule and Kyvsgaard 2003). Local bylaws and regulations if locally emphasized by local authorities and adhered to by the public can achieve some level of pig confinement but these can effectively work when poverty among rural communities is reduced or eliminated.

Although some low-income countries are on the rise economically, a high level of poverty in many endemic countries is still a challenge. Tackling this challenge would pave a way for the adoption of improved hygiene, sanitation, pig husbandry practices by the general community, and possibly produce results similar to developed countries in interrupting the transmission cycle of the parasite.

2.10.5 Public health education

Taenia solium infection has persisted in low-income countries where pigs are free-roaming, hygiene and sanitation are poor and health education is lacking (Phiri *et al*. 2003; Lightowlers 2010). Public health education is thus critical in changing these practices to interrupt the transmission of the parasite.

A few studies have assessed the effectiveness of public health education for the control of *T. solium* (Sarti *et al*. 1997; Ngowi *et al*. 2009). One study in Mexico found that public health education, developed along with community involvement, improved knowledge on the parasite transmission and reduced access of pigs to contaminated human faeces (Sarti *et al*. 1997). Another study in northern Tanzania evaluated the effectiveness of public health education as an intervention for control of *T. solium* and reported a significant reduction of consumption of pork infected with cysticercosis by 20% as well as a reduction of the incidence rate of PC by 43% (Ngowi *et al*. 2009). Thus, educating communities in endemic countries regarding the diseases and life cycle of *T. solium* parasite has great potential in cultivating behaviours necessary to

interrupt disease transmission. However, studies evaluating the sustainability of such public health education promotions are scarce.

Even though public health education can be an effective control strategy, it can be expensive as its success requires multidisciplinary input and compliance of the community (Sarti and Rajshekhar 2003). Improvements in infrastructure such as the construction of latrines and pigpens are important to enable changes in the attitudes and practices of the involved community but the implementation of these measures may be difficult due to high levels of poverty prevailing in many endemic low-income countries.

2.10.6 Pig vaccination

Vaccination has been widely used in the control of many infectious diseases worldwide. Given the fact that pigs are the main intermediate host of *T. solium*, vaccination seems an appropriate control measure. Although there is currently no registered vaccine (Flisser *et al*. 2004; Assana *et al*. 2010; Lightowlers 2010), the experimental work of vaccines is promising. For example, two recombinant *T. solium* oncosphere antigens, designated TSOL18 and S3Pvac, have been evaluated with encouraging results (Huerta *et al*. 2002; Flisser *et al*. 2004; Sciutto *et al.* 2007). Both antigens were effective in inducing high levels of protection in three independent vaccine trials against experimental challenge infection with *T. solium* eggs in pigs in Mexico and Cameroon. Vaccination with the TSOL18 recombinant antigen alone induced 99.5% and 100% protection against the development of cysticerci following experimental challenge infection (Flisser *et al*. 2004). Another study by Assana *et al*. (2010) conducted in Cameroon proved that the TSOL18 vaccine was 100% effective in protecting pigs against naturally acquired *T. solium* infection. The prevalence of cysticercosis was reduced by 53% and, the intensity of infection with viable cysts by 98% among pigs vaccinated with the S3Pvac vaccine in a field trial in rural Mexico (Huerta *et al*. 2002).

With an effective commercial vaccine against *T. solium*, consideration of several aspects is important before including it as a control strategy. The vaccine must be cheap or subsidized to be affordable to smallholder pig-farmers in low-income countries. The vaccine must also be easy to administer in a mass intervention campaign and offer long-term protection (Sarti and Rajshekhar 2003). Nevertheless, the cost of vaccination is likely to be high because of the short life span of pigs which means the interval between the vaccination campaigns must be adjusted accordingly as well as the availability of the vaccine must be secured (Sarti and Rajshekhar 2003). There is some evidence that pigs acquire cysticercosis mainly early in life, this suggests that vaccination must be

done in piglets (Gonzalez *et al*. 2003). However, the immune system of piglets is immature and therefore vaccination may not induce an effective protective immune response (Gonzalez *et al*. 2003). Before vaccines against *T. solium* become commercially available, other means of preventing pigs from acquiring the disease as discussed in this thesis should be adopted.

3 Aims of the thesis

The overall aim of this thesis was to investigate the transmission biology of cysticercosis in pigs including the potential role of soil in the transmission cycle of the parasite in rural areas of Kongwa district in eastern-central Tanzania. The diagnostic potential of meat juice for serological detection of PC was also evaluated.

The specific objectives of the study (papers I – IV) were to:

I Investigate the exposure and potential risk factors associated with PC transmission in rural villages of Kongwa district, Tanzania.

II Validate the effectiveness of a novel ddPCR technology for detection, identification and absolute quantification of taeniid eggs from experimental soil samples using *T. solium* and *T. lynciscapreoli* eggs.

III Explore the prevalence of *T. solium* eggs in household soil from rural villages of Kongwa district using the validated ddPCR technology.

IV Evaluate the diagnostic potential of pork meat juice in comparison to serum samples in the detection of PC using cysticercosis antibody ELISA.

4 Materials and Methods

4.1 Study area (paper I – IV)

Choices for the study area and sample collection centred on objectives outlined in the "Sustainable agricultural productivity, processing, and value chain for enhancing food security in Tanzania" project and the available *T. solium* zoonoses general literature. Some adjustments were made based on *T. solium* infection information available in Tanzania. Samples for this thesis were collected from Dodoma and Morogoro regions in eastern-central Tanzania. For detailed information on materials and methods, refer to papers I and IV.

Ethical clearance for the present study was obtained from the UDSM on behalf of the Tanzania Commission for Science and Technology (COSTECH) as well as from authorities in Kongwa district. The Research Ethics Committee of the Sokoine University of Agriculture (SUA) approved ethical statement (RPGS/R/ETHICS/32) for slaughtered pigs involved in this study, in compliance with Tanzania's Animal welfare act of 2008. Furthermore, informed oral consent was sought from heads of pig keeping households surveyed to participate in this study. Permit to transport biological and soil samples to SLU in Sweden was granted by Tanzania's Ministry of Agriculture, Livestock and Fisheries and the Ministry of Energy and Minerals.

4.2 Sample collection

4.2.1 Blood sampling (paper I, IV)

To determine the seroprevalence of PC, blood samples were collected from the auricular vein of pigs aged three months and above belonging to smallholder farmers in rural villages of Kongwa district. Strict venepuncture procedure was followed and a 23-gauge needle was used in the blood collection. The blood was stored in vacutainer tubes before serum was extracted. During blood sampling, pigs were restrained using a hog catcher and the ear was sterilized with 70% alcohol. For detailed information, refer to papers I and IV.

4.2.2 Household questionnaire (paper I)

Information related to the pig production system, awareness of PC, socio-cultural settings, hygiene, and sanitation were collected through a structured questionnaire administered to either the head or one of the family members of the household from which blood samples were collected from pigs.

4.2.3 *Taenia solium* cysticerci sampling (paper I – IV)

Taenia solium cysticerci for molecular confirmation in paper I and IV and as control samples in paper II and III were collected from the neck, hind legs, forelegs, diaphragm, heart muscles and the brain of naturally infected slaughtered pigs found during meat inspection in Kongwa district.

4.2.4 Source of soil samples (paper II, III)

Clay, silt and sand soil used in the experimental study in paper II were collected from domestic livestock free areas in the Morogoro region, Tanzania. Loam soil was made available for use in the experiment by mixing clay, silt, and sand according to Foth (1990). Household soil in paper III was collected from inside the houses, areas around the houses, nearby the toilet, backyard and the waste disposal areas in four rural villages of Kongwa district. Household soil was collected both during the dry and rainy seasons approximately at the same locations from pig-keeping households in all surveyed villages and then stored at room temperature before analysis.

4.2.5 Source of taeniid eggs (paper II, III)

Taeniid eggs in paper II were recovered from adult *T. solium* and *T. lynciscapreoli* tapeworm proglottids obtained from the SUA, Tanzania and the National Veterinary Institute, Sweden, respectively. Prior to egg recovery, the proglottids were inactivated for biosecurity reasons at −80 °C for seven days and then stored at −20 °C before the spiking experiment. In paper III, *T. solium* eggs were recovered from contaminated household soil obtained in rural villages of Kongwa district.

4.2.6 Meat sample collection (paper IV)

Tissue samples for meat juice recovery were obtained from slaughtered pigs in rural villages in Kongwa district in Dodoma region and from Nyandira in Mvomero district in Morogoro region, Tanzania. Pigs infected with *T. solium* were identified via tongue palpation and then confirmed to be infected following visual inspection for cysts at slaughter. All pigs harbouring cysts were free-ranged and obtained from villages in Kongwa. Similarly, pigs reared under more strict hygienic conditions with no cysts were obtained from Mvomero. After seeking oral consent from pig owners and careful visual inspection of the carcass tissue samples were collected from the pigs naturally infected with cysticercosis, whereas control tissue samples were collected from pigs without visible cysts.

4.3 Laboratory analysis

Samples were processed and analysed in Sweden at the SLU and in Tanzania at the UDSM, SUA and Kongwa district hospital laboratories.

4.3.1 Extraction of serum and meat juice samples (paper I, IV)

In paper I and IV, serum samples were recovered in the laboratory at Kongwa district hospital and collected in labelled cryogenic vials within 6 to 8 hours after blood collection by centrifuging at 2000xg for 10 minutes. In paper IV, meat juice was extracted at SUA from frozen meat samples cut into small pieces and left to thaw for 5 to 6 hours at room temperature using special containers (CC Plast A/S, Hillerød, Denmark) for meat juice collection (Figure 5). All sera and meat juice samples were stored at -20 °C until further analysis at SUA.

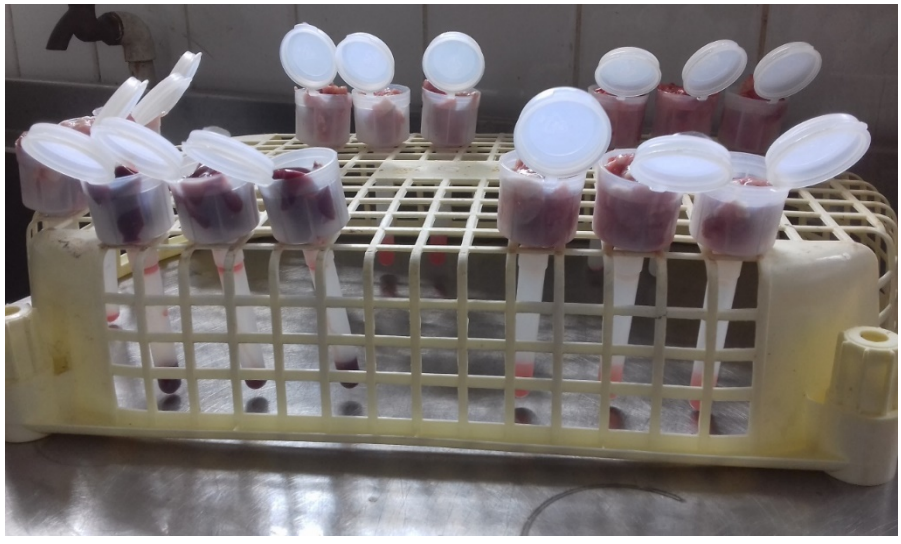

Figure 5. Extraction of meat juice from pig carcass tissues (Photo: Justine Daudi Maganira, SLU).

4.3.2 Cysticercosis antigen and antibody ELISA test (paper I, IV)

In paper I, the seropositivity of *Taenia* spp. antigens in serum were measured using a commercially available cysticercosis antigen ELISA (apDia, Belgium). The assay can determine down to one viable cysticercus but it is *Taenia* genus-specific in porcine serum samples (Deckers and Dorny 2010; Akoko *et al*. 2019). In paper IV, a commercial cysticercosis antibody ELISA (NovaTec Immundiagnostica, Germany) was used for the qualitative immunoenzymatic determination of specific antibodies against PC infection in serum and meat juice samples. Both antigen and antibody ELISA tests were done following test protocol provided by the manufacturer with minor modifications.

4.3.3 Soil-egg spiking experiment (paper II)

In paper II, all soil types were sterilized at 130 °C for 60 min in a hot oven, before spiking soil with taeniid eggs at SLU. Then, about 5 g of each soil was artificially spiked in triplicates with about 10, 50, 100 and 500 eggs of either *T. solium* or *T. lynciscapreoli*. In addition, positive control was prepared by spiking approximately 10, 50, 100 and 500 eggs in triplicates in 100 µl of distilled water without soil. Similarly, unspiked soil served as negative controls.

4.3.4 Soil-egg isolation (paper II, III)

Taeniid eggs in soil were recovered at SLU according to a protocol originally developed for the detection of eggs of *Echinococcus multilocularis* in fox faeces (Mathis *et al*. 1996; Miller *et al*. 2016). The protocol combines flotation using zinc chloride solution (density 1.45 g/ml) and sequential sieving of the supernatant using modified Falcon tubes with nylon sieves of 41/100 µm and 20/100 µm mesh size (Figure 6).

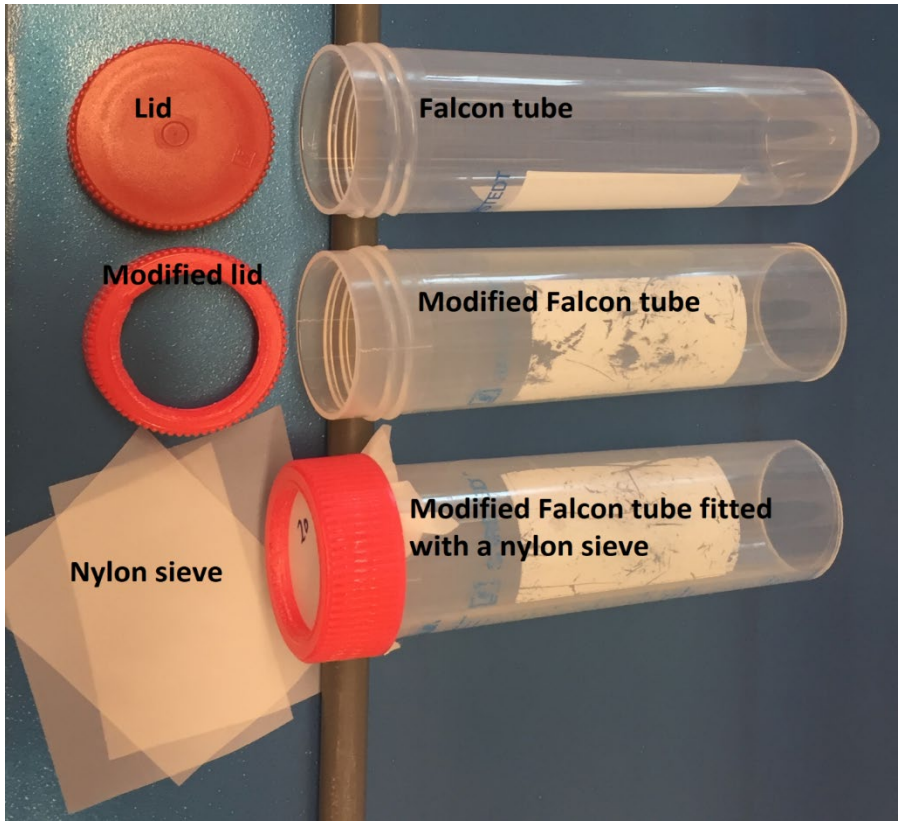

Figure 6. Nylon sieves and the modified Falcon tubes used in sieving the supernatant (Photo: Justine Daudi Maganira, SLU).

4.3.5 Molecular techniques (paper II, III)

In paper I and IV, total deoxyribonucleic acid (DNA) was extracted from *T. solium* cysticerci from pigs (Figure 7) with QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) and the quality of the DNA assessed by running extracted DNA on 1% Agarose gel. Parasite species were then identified after amplification and sequencing (Macrogen, Amsterdam, Netherlands) using forward (JB3) and

reverse (JB4.5) universal primers targeting the mitochondrial (mt) cytochrome c oxidase subunit I (*cox1*) gene (Bio-Rad Laboratories) (Bowles *et al.* 1992; Bowles and McManus 1994; Okamoto and Ito 2013). The sequences were edited and trimmed using CodonCode Aligner version 7.1.2 and then blasted in GenBank to determine the species identity.

In paper II and III, DNA was extracted from the sample pellets recovered from the soil-egg spiking experiment or household soil using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol with minor modifications. Prior to DNA extraction, alkaline lysis and neutralization procedures were performed on the sample pellets (Stefanic *et al*. 2004; Miller *et al*. 2016). The extracted DNA was stored in labelled Eppendorf tubes at −20 °C. Finally, DNA from soil sample pellets was screened for the presence of taeniid DNA using ddPCR using universal primers (JB3 & JB4.5) targeting the mt *cox1* gene in the EvaGreen supermix as described in paper II. In paper III a second ddPCR assay employing a *T. solium* specific primer and probe set, in ddPCR supermix for probe was used as a confirmatory test. In addition, this second assay is targeting the mt *cox1* gene for *T. solium*. For details, refer to papers II and III.

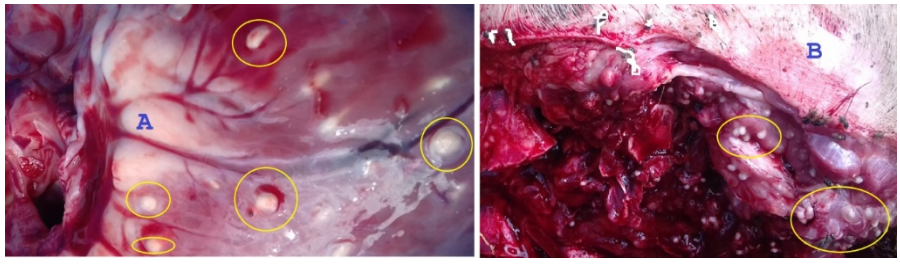

Figure 7. Cysts in the pig heart (A) and neck muscle (B) (Photo: Justine Daudi Maganira, SLU).

4.4 Statistical analysis

In paper I, the Chi-Square test of independence was used to compare differences between seasons, villages and pig age groups and forward step-wise binary logistic regression model was used to compute predictors of PC transmission. In papers II and III, the analysis of data generated by the QX200 Droplet Reader was performed using QuantaSoft version 1.7.4 software (Bio-Rad Laboratories). Analysis of variance (ANOVA) was performed to compare mt *cox*1 detection results between species, soil types or samples, and egg concentrations. When ANOVA results were statistically significant ($p < 0.05$), Tukey's or Bonferroni's multiple comparisons *post hoc* test was used to compare the groups as appropriate. Kruskal-Wallis non-parametric test was used to compare antibody

concentration in meat juice from different organs and muscles in paper IV. Statistical data analyses were performed in either R version 3.4.3 for Windows (R Foundation, Vienna, Austria), Statistical Package for Social Sciences (SPSS) version 24 software, GraphPad Prism version 8.0.0 for Windows (GraphPad Software, La Jolla California, USA) or Microsoft Excel 2016 for Windows (Microsoft Corporation, Redmond, Washington, USA). Detailed procedures for the statistical analyses can be found in the respective papers.

5 Results and discussion

Below is a brief description of the general outcomes of papers I-IV. For a detailed description of the results, refer to the individual paper.

5.1 Prevalence of PC and risk factors (paper I)

The seroprevalence of *Taenia* spp. in rural villages of Kongwa district was for the first time established in paper I before the assessment of household soil contamination by *T. solium* eggs in paper III and evaluation of the PC diagnostic potential of meat juice using the cysticercosis antibody ELISA test in paper IV.

The results of paper I showed that the overall prevalence rate of *Taenia* spp. in pigs from the surveyed villages was 17%. The presence of *T. solium* cysts from pigs was confirmed by sequencing of the mt *cox1* gene. This proves that PC is endemic in rural villages in Kongwa district. The seropositivity was significantly higher in the rainy than the dry season ($\chi^2 = 5.50$, $p = 0.019$) (Figure 8). The level of PC seropositivity reported herein from Kongwa is similar to what has been previously reported in the southern (16.7%) and northern (17.4%) highlands of Tanzania (Ngowi *et al*. 2004; Boa *et al*. 2006). However, it was higher than that recently reported in the southern highland district of Ludewa (Maganira *et al*. 2018). These local differences suggest that pigs in different districts in Tanzania have different rates of exposure to *T. solium* eggs from human carriers.

The seasonal differences in the observed seroprevalence may be attributed to the different seasonal pig production systems. This is because pigs are mostly confined in the rainy season during which they are fed on crops from their farms and other foodstuffs locally available, while in the dry season after crops have been harvested pigs are in general left to freely roam and scavenge. Interestingly, a significantly higher seroprevalence was recorded in the rainy than in the dry season. It was noted that during pig confinement in the rainy season, banana,

cassava and potato peels and other plant materials including legumes formed the greatest portion of the pig feed. It is likely that the eggs of the parasite contaminated some feed. It has been shown in a previous study that confinement of pigs as a sole intervention for control does not necessarily prevent PC (Braae *et al*. 2014). Furthermore, the number of pigs sampled during the dry season was significantly lower than in the rainy season due to an outbreak of the ASF, which decimated the pig population before the commencement of the present study. Thus, the difference in the number of sampled pigs may also explain the seasonal differences in the reported seroprevalence of cysticercosis.

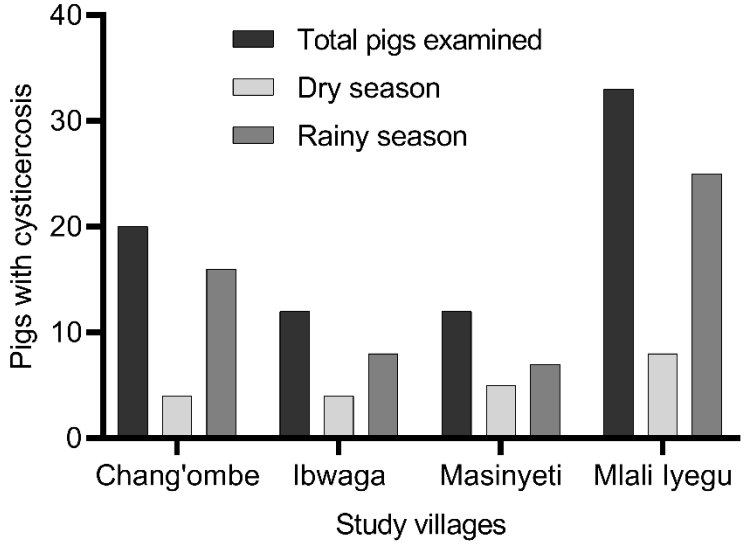

Figure 8. Seroprevalence of taeniid spp. antigens measured for each season from the corresponding villages in the Kongwa district, central Tanzania.

Nevertheless, the level of PC recorded in this study indicates the presence of people in the community infected with the adult tapeworm, *T. solium*. These results also suggest a need for *T. solium* control programmes in the study area such as improved sanitation and pig husbandry and health education that have shown to eliminate the parasite from most developed countries (Del Brutto 2012). There is a need for further studies on taeniasis and cysticercosis in the human population for informed control strategies of the parasite in Kongwa.

The risk factors for PC transmission identified by paper I in the study areas included open defecation, free-range pig husbandry and origin or source of reared pigs. Free-range pig husbandry and open defecation give pigs unrestricted access to faeces contaminated with *T. solium* eggs from human carriers

(Mwang'onde *et al*. 2014; Khaing *et al*. 2015; Shonyela *et al*. 2017). The government of Tanzania has been, in recent years, emphasizing the construction of sanitary facilities in every rural home for safe disposal of human faeces in a bid to fight various infectious diseases. However, a high level of PC reported from these areas and the occurrence of open defecation suggests that such established sanitary facilities are not properly utilized. It was observed that 98% of the households in the surveyed villages have pit latrines, though unimproved. Human faeces containing the eggs of *T. solium* when not properly disposed of can be a source of contamination to soil, water or food (Braae *et al*. 2014). Thus, pigs produced and reared in the same household in Kongwa were likely to contract cysticercosis. More work is needed to investigate when and where PC infection is contracted. For example, does it mainly occurs when the pigs are free-roaming or when they are in the pens?

5.2 Soil-egg spiking experiment (paper II)

In paper II, we experimentally tested for the first time a novel ddPCR technology for the detection of taeniid eggs spiked in four different types of soil after egg recovery according to a protocol previously developed for detection of *Echinococcus multilocularis* eggs in fox faeces (Mathis *et al*. 1996; Miller *et al*. 2016).

5.2.1 Assay optimization

In paper II, the ddPCR assay was first optimized to identify the optimal annealing temperature for the detection of the mt *cox1* gene. The mt *cox1* detection sensitivity of the assay was also tested along a serial dilution gradient. The DNA used in the optimization reactions originated from *T. solium* cysticerci and proglottids of *T. lynciscapreoli*.

Figure 9 presents an example of a 1-D amplitude data generated by the QuantaSoft software for mt *cox1* gene detected in the ddPCR. During ddPCR assay thermal gradient optimization, the average number of accepted droplets was 16,000 with ddPCR detection ranging between 7,180 – 61,400 mt *cox1* copies per reaction mixture. It was found that 54°C was the optimal annealing temperature when tested in duplicates with a set of universal primers, JB3 & JB4.5 suggested by (Okamoto and Ito 2013).

The detection range of mt *cox1* by ddPCR along a two-fold dilution gradient varied from 344 to 67,000 copies per ddPCR reaction mixture. The concentration of mt *cox1* copies in both species declined gradually relative to serial dilution.

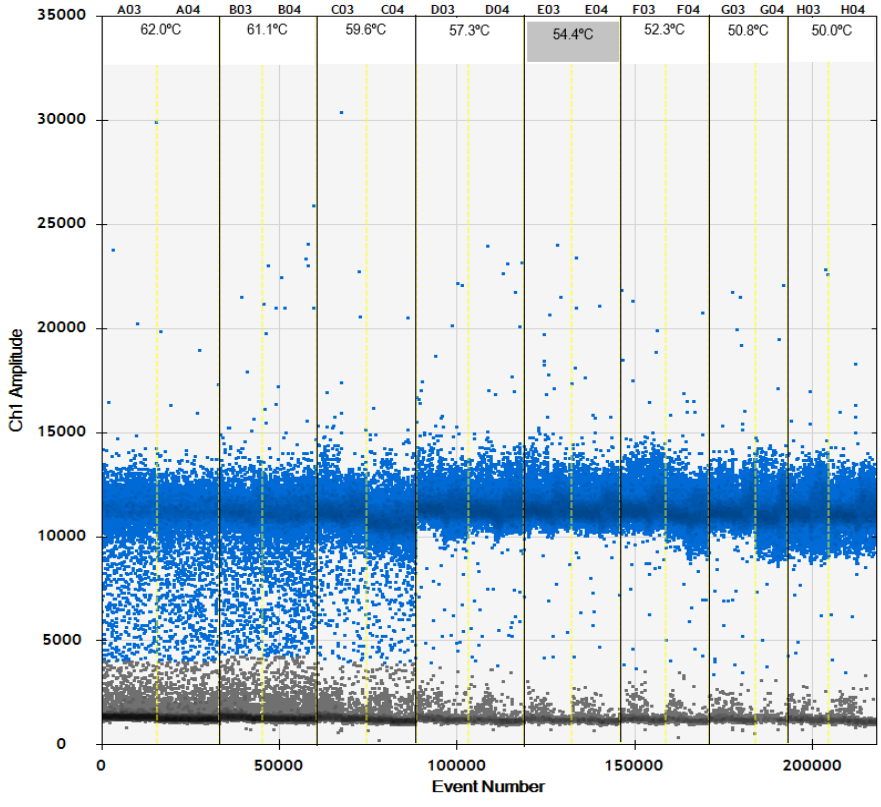

Figure 9. Example of a 1-D scatter plot showing thermal gradient optimizing for primer annealing temperature in ddPCR uniplex assay for mitochondrial *cox1* detection using *Taenia solium* cysticerci DNA as a template for method validation. Black dots are negative droplets representing the baseline, whereas blue dots droplets contain DNA that reacted.

5.2.2 Detection of mt *cox1* among spiked soil types

In paper II, egg DNA from both *T. solium* and *T. lynciscapreoli* was detected in all control samples without soil (positive controls), as well as in most of the different soil types spiked with eggs. The highest mt *cox1* copies were detected in the positive controls in comparison to spiked soil samples with the detection ranging between 28 – 55,400 mt *cox1* copies per reaction mixture. The detection range of mt *cox1* by ddPCR in the spiked soil samples was between 10 and 14,220 copies per ddPCR reaction mixture. Generally, the number of mt *cox1* copies increased with the number of taenia eggs per gram in the spiked soil samples (Figure 10).

The average number of mt *cox1* copies detected by ddPCR in the spiked soil were significantly different for both *T. solium* ($F_{3,32} = 3.091$, $p < 0.05$) and *T. lynciscapreoli* ($F_{3,32} = 37.61$, $p < 0.05$). The highest mt *cox1* DNA copies recovery

was detected in sand for all four egg concentrations in both *T. solium* and *T. lynciscapreoli*; whereas the lowest mt *cox1* copy number was detected in silt and clay (Figure 10).

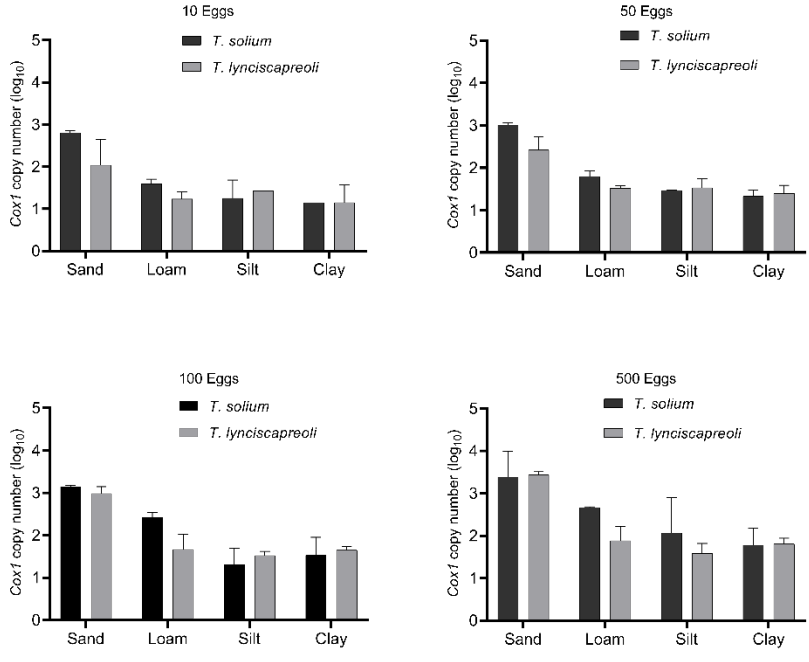

Figure 10. Copies of mt *cox1* detected by ddPCR per eggs spiked in soil samples for both *Taenia solium* and *T. lynciscapreoli*. Error bars represent the standard deviation (SD).

Furthermore, there were significant differences in the mean number of mt *cox1* copies between the different soil types. The mean number of mt *cox1* copies detected in soil spiked with either *T. solium* or *T. lynciscapreoli* eggs differed significantly between sand and all other soil types (loam, silt and clay). Pairwise comparisons between loam, silt and clay showed no significant difference in mean mt *cox1* copies for neither *T. solium* nor *T. lynciscapreoli* (Table 1).

In paper II, a novel ddPCR technology was for the first time tested for the detection of taeniid eggs in four different types of soil after egg recovery according to a protocol originally developed for detection of eggs of the closely related species *E. multilocularis* in fox faeces (Mathis *et al*. 1996; Miller *et al*. 2016). The results of the present study show that ddPCR is a promising technology for the detection of mt *cox1* gene copies of different taeniids eggs from different types of soil.

Table 1. *Statistical analysis of mean cox1 copies of Taenia solium and T. lynciscapreoli detected by ddPCR in different soil types and egg concentrations*

	T. solium		*T. lynciscapreoli*	
	MD	*p* value	MD	*p* value
Sand vs. Loam	1739	<0.05	1017	<0.05
Sand vs. Silt	1866	<0.05	1041	<0.05
Sand vs. Clay	1913	<0.05	1031	<0.05
Loam vs. Silt	127	>0.05	24	>0.05
Loam vs. Clay	174	>0.05	14	>0.05
Silt vs. Clay	47	>0.05	10	>0.05

*Multiple comparisons using Tukey's post hoc test; MD = Mean Difference

The increasing number of taeniid eggs recovered for both species may explain the observed gradual increase in mt *cox1* copy numbers in proportional to the number of eggs spiked in soil samples and subsequently increasing the amount of taeniid DNA recovered from the eggs. These results suggest an acceptable egg recovery efficiency and DNA detection efficiency by ddPCR regardless of the concentration of eggs. Similar findings using ddPCR technology were reported in the detection of other helminths such as nematode eggs of *Ascaris lumbricoides* in water samples (Soto *et al*. 2017). The copy number of target DNA detected using ddPCR technology in a study involving *Schistosoma japonicum* in an experimental mouse model also increased with microscopically assessed infection levels (Weerakoon *et al*. 2017).

Sand soil gave a significantly higher mean copy number of mt *cox1* gene than all other types of soil with varying egg concentrations for both *T. solium* and *T. lynciscapreoli* suggesting that soil type is an important factor in the recovery of taeniid eggs and detection of the target gene. In addition, there was less variation in the mean number of *cox1* copies detected in loam, silt and clay soils. Higher recovery rates were also recorded in sand compared to other soil types during recovery of *Toxocara canis* eggs using the flotation method (Nunes *et al*. 1994). Similarly, the recovery efficiency of other soil-transmitted helminths eggs including those of the parasitic nematodes *Ascaris lumbricoides* and *Trichuris trichiura* was higher in the sand than loam soils in studies carried out in Kenya and Bangladesh (Steinbaum *et al*. 2017). The difference in soil texture may explain the variation in the number of eggs recovered from soil and hence *cox1* copies detected by ddPCR. Soil texture accounts for variation in the distribution pattern of helminth eggs in different soil types (Nunes *et al*. 1994; Oge and Oge 2000). Large sand particle size offers a homogeneous egg distribution pattern

because of holding eggs loosely compared to small clay or silt particle size that holds the eggs tightly resulting in a more irregular distribution. This suggests that spiked sand soil had a homogeneous distribution of eggs, which resulted into the highest recovery of the eggs and then *cox1* copies. Therefore, it is important to consider the effect of soil type in the dispersion pattern of helminth eggs in environmental soil from endemic areas.

Even though *cox1* detection was significantly higher in sand soil, possibly due to its large particle size, compared to clay or silt soils (Nunes *et al*. 1994), care must be taken when screening field samples. Differences in egg recovery not only may suggest variation in soil texture but also variation in soil properties such as moisture and chemical composition or other factors such as rainfall and wind. With this in account, there is a need for further exploration of how other factors than soil type influence the distribution of taeniid eggs in environmental soil from endemic areas to determine infection dynamics in the pig and human population. It should be noted also that the detection of taeniid egg DNA in soil *per s*e does not necessarily indicate the presence of viable, infectious eggs. Thus, it is important to consider the viability of the eggs in the environment when the risk of transmission is determined.

Homogenization of the soil samples through thoroughly mixing by blending may increase the effectiveness of recovering parasite eggs from soil with a heterogeneous distribution pattern or with a low concentration of the eggs. Although the results of this study based on spiked soil samples weighing 5 g each, it is natural that the probability of recovering taeniid eggs or DNA will increase with the amount of soil as suggested by Oge and Oge (2000). To avoid the difficulties associated with recovering DNA from taeniid eggs using the DNA extraction kit, as described in this study, alkaline lysis and neutralization seem to be important procedures to be performed prior to carrying out DNA extraction kit procedures. This is crucial because the successful recovery of DNA from taeniid eggs is a key factor for effective detection and quantification by ddPCR. Still, alternative options for DNA extraction based on bead beating in combination with other commercially available DNA extraction protocols needs to be further evaluated and validated.

5.3 Soil contamination by *T. solium* DNA (paper III)

For the first time, soil contamination by *T. solium* eggs was investigated in the field by the use of ddPCR technology (Paper III). The samples were first processed by a flotation-double sieving technique, followed by screening for the presence of worm DNA using the universal ddPCR assay and confirmation of *T. solium* DNA by the specific ddPCR assay (Paper II).

The overall prevalence of *T. solium* DNA contamination in soil in the surveyed households in four rural villages in Kongwa district during the dry and rainy seasons was found to be 3.1% (n=3/96). Contamination by *T. solium* was only confirmed in two villages and only during the dry season. These results are consistent with those in a European study, that found both *Taenia* and *Echinococcus* eggs on vegetables and fruits (Rojas *et al*. 2018), but differ from previous studies using microscopy, which did not detect *T. solium* eggs (Diaz *et al*. 1992; Mwita *et al*. 2013; Adekeye *et al*. 2016). On the other hand, the level of soil contamination by *T. solium* egg DNA in the resent study is lower than reported for *E. multilocularis* in soil from northeast Poland (Szostakowska *et al*. 2014), that employed other methods for egg recovery and DNA detection. Furthermore, they are lower than those reported for other soil-transmitted helminths such as *Ascaris*, *Trichuris* and *Ancylostoma* (Steinbaum *et al*. 2017). However, it is difficult to compare these results as they are based on various methods for recovery and detection of parasite eggs from various sources and in different environments.

The relatively low prevalence of *T. solium* DNA recorded in the soils in this study might be due to the heterogeneous distribution of the cestode eggs. Gravid proglottids of *T. solium* are non-motile, unlike those of *T. saginata* and *T. asiatica*, which account for a clumped heterogeneous distribution of eggs once in the environment (Okamoto and Ito 2013). In addition, the amount of soil analysed per household was relatively low and may have contributed to the recorded low level of contamination. Soil samples were collected from five sampling points per household but these were pooled (5 x 100 g), from which a single sample of about 10 g was analysed in each season. Thus, our sampling design might have underestimated the presence of *T. solium* eggs in the soil. Nevertheless, the likelihood of detecting contamination of *T. solium* eggs in the household environment in endemic areas may be improved for example by sampling hotspots such as around remnants of deposited human faeces. However, this requires further investigation.

The contamination of soil by *T. solium* DNA was exclusively detected in the dry season. Similarly, a lower rate of other soil-transmitted helminths' eggs has been recorded in the rainy as compared to the dry season (Nwoke *et al*. 2013). The absence of soil contamination by *T. solium* eggs in the rainy season might be because rainwater washes off the eggs into surface water, where they may contaminate rivers and streams (Oyebamiji *et al*. 2018). In both seasons, the weight of each sample collected was equivalent (≈200 g), and thus the volume of soil collected during the rainy season was in theory lower compared to the dry season. The difference in the volume of soil investigated in both seasons might also partially explain the difference in the detection of *T. solium* DNA.

Still, an important outcome of paper III was the direct applicability of the ddPCR technology for screening *T. solium* eggs in soil samples without the need for confirmatory sequencing. The ddPCR is obviously a promising technology for the assessment of *T. solium* eggs contamination in environmental samples due to its robustness against inhibitors and the high sensitivity (Demeke and Dobnik 2018). Although more studies need to be done, it is promising that ddPCR technology can be used for screening the eggs of *T. solium* or other parasites from a variety of environmental samples such as water. A limitation with ddPCR for low-income countries is that the costs for equipment and consumables are high (Koepfli *et al*. 2016).

Generally, given the low rate of contamination recorded in this study in rural villages of Kongwa district, the risk of acquiring a *T. solium* infection through the soil to both pigs and humans seems to be relatively low.

5.4 Detection of PC using meat juice samples (paper IV)

Previous studies have examined the use of meat juice from pigs for the detection of the zoonotic protozoan parasite *Toxoplasma gondii* (Wallander *et al*. 2015). However, meat juice as a diagnostic specimen for the detection of helminth infections such as PC has never been evaluated before. Thus, paper IV assessed the diagnostic potential of meat juice for serological detection of PC using a cysticercosis antibody ELISA with serum samples as reference standard specimens.

The results of paper IV indicate that all (n=9) serum samples from *T. solium* cysts positive pigs from Kongwa gave a positive response in the ELISA test, whereas all serum samples collected from pigs (n=6) with no visible cysts from Mvomero gave a negative reaction. Of all 117 meat juice tissue samples from pigs with cysts, 45 (38%) tested positive while 72 (62%) tested negative for PC antigens. Out of 68 meat juice samples from tissues with visible cysts, 41 (60%) tested positive for PC (Figure 11). The carcass tissues that most often tested positive were diaphragm, heart and neck muscles. Of 49 meat juice samples extracted from tissues of pigs with cysticercosis but from samples without visible cysts (kidney, liver, lung, and spleen), only 4 (8%) samples from the lung were positive (Figure 12). Similarly, all 78 meat juice samples from the six pigs with no cysts tested negative in the cysticercosis antibody ELISA.

Both the sensitivity and specificity of cysticercosis antibody ELISA using serum samples was 100%. In contrast, the average sensitivity and specificity of the ELISA using meat juice samples collected from different tissue were 38% and 100%, respectively. However, the sensitivity of the ELISA was high in meat juice extracted from the diaphragm (100%), heart (89%) and neck muscle (78%) of

PC infected pigs. Higher mean OD values were recorded in serum than in all of the meat juice samples. However, meat juice from the heart and diaphragm gave the highest mean OD values as compared to meat juice from other tissue from the carcasses.

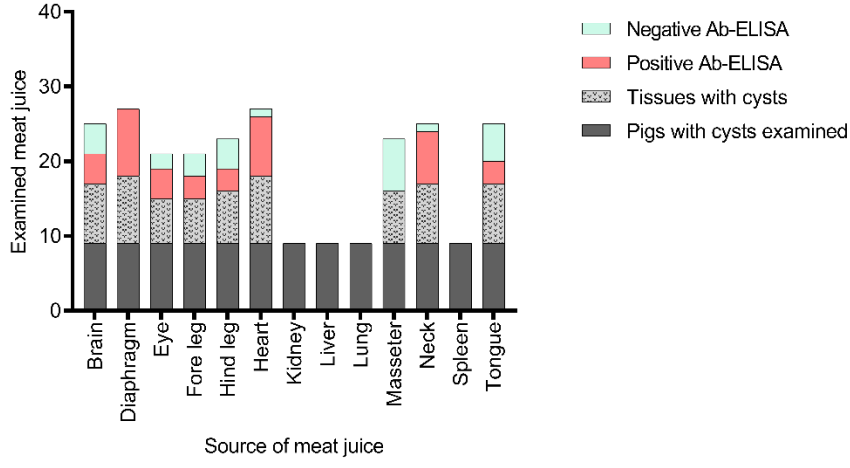

Figure 11. Detection of antibodies against porcine cysticercosis in meat juice tissue samples from organs or muscles of nine infected pigs with visible cysts.

The overall sensitivity and specificity of meat juice in detecting PC using cysticercosis antibody ELISA were lower than that in serum (Paper IV). These results are consistent with the results of a study involving cattle experimentally and naturally infected with *T. saginata* cysticercosis, which also reported a lower sensitivity for meat juice than serum samples (Abuseir *et al*. 2007). However, a higher sensitivity (83%) of an antibody ELISA employing HP6-2 peptide in the detection of bovine cysticercosis using meat juice from undefined tissues with *T. saginata* cysts, than what is reported in the present study was reported (Abuseir *et al*. 2007). Still, the overall performance of meat juice in detecting PC using antibody ELISA in the present study showed relative higher sensitivity (38%) than the sensitivity of detecting porcine cysticercosis using tongue inspection (21%) or routine meat inspection (22%) estimated using a Bayesian approach (Dorny *et al*. 2004). Although the average sensitivity for meat juice from different tissues was low (38%) in the present study, the sensitivity of the ELISA test was high in specific tissues ranging between 78% and 100% for meat juice from the neck muscle, heart, and diaphragm.

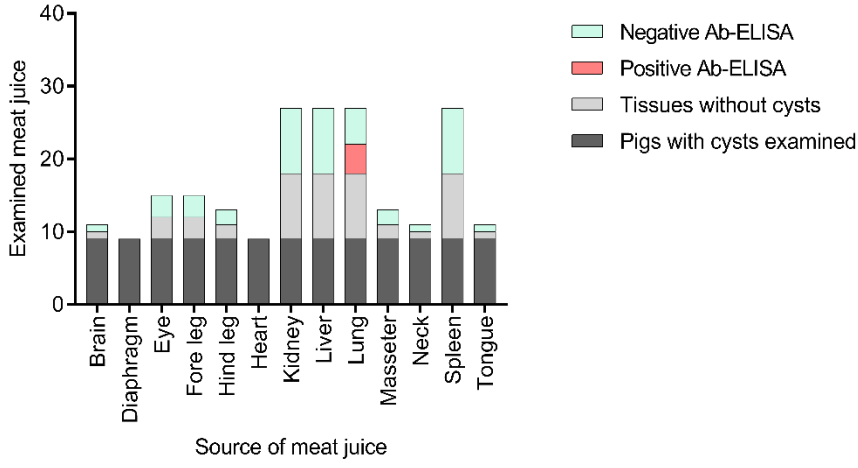

Figure 12. Detection of antibodies against porcine cysticercosis in meat juice samples from organs or muscles of nine infected pigs but without visible cysts.

The results of paper IV indicate that the serological test using ELISA is a sensitive and reliable technique for the detection of PC when pig serum is used as a diagnostic sample. However, the performance of meat juice samples from the heart and diaphragm in the diagnosis of PC using ELISA was acceptable and comparable to that of serum samples. As demonstrated in this study, higher OD values were recorded in the serum than in the corresponding meat juice samples suggesting a higher level of antibody concentrations in serum than in meat juice samples. The lower antibody levels in meat juice samples suggest a lower chance of detecting PC, likely leading to an underestimation of the true prevalence when the cysticercosis antibody ELISA test is performed on meat juice as compared to serum samples. Similar findings have been previously reported in a study involving the detection of antibodies from serum and meat juice of pigs experimentally infected with the protozoan, *T. gondii* (Forbes *et al*. 2012; Wallander *et al*. 2015). Similarly, underestimation of bovine cysticercosis using ELISA performed on meat juice has been reported in cattle experimentally infected with bovine cysticercosis (Abuseir *et al*. 2007).

The findings of paper IV indicated that PC was mainly detected in meat juice from tissues with several visible cysts (60%), whereas samples from pigs with few or no cysts were mostly not detected (Figure 10 and Figure 11). This indicates that antibody levels are related to the number of cysts present in the carcass. The most interesting finding in paper IV is that the sensitivity and mean OD values in meat juice from the heart and diaphragm were relatively similar to those in serum samples suggesting the suitability of meat juice from these organs

in the detection of PC as compared to the other tissues investigated herein. In addition, meat juice from the neck muscles showed to have both reasonably high sensitivity (78%) and antibody levels almost similar to meat juice from the heart, which also suggests the suitability of this tissue in detecting PC. The promising suitability of meat juice extracted from the diaphragm or heart was also demonstrated by the ability to detect PC by using meat juice from both heavily and lightly infected pigs. Thus, pig carcass meat juice samples, especially from the diaphragm and heart, can be used as an alternative to serum for the screening of PC. Again, the possible impact of the presence of other taeniids that may cross-react in the ELISA needs further evaluation. It would also be worthwhile to expand the investigation in pigs with variable levels of PC.

6 General conclusions

The results of this thesis determined the baseline information on the seroprevalence of cysticercosis in pigs in rural villages of Kongwa district in eastern-central Tanzania. The potential role of soil in the transmission of the eggs of the parasite was examined for the first time using ELISA and the ddPCR technology. In addition, the diagnostic potential of meat juice for the detection of PC was evaluated. The findings of this study call for further studies on taeniasis and cysticercosis in the human population as well as exploration of other environmental and ecological factors than soil that pose risks of cysticercosis transmission. The results of this study are important for the planning of intervention strategies against PC in the study area.

Specific conclusions drawn from this thesis are outlined below:

- For the first time, a high seroprevalence of taeniid antigens was found in pigs from rural villages of Kongwa district suggesting *T. solium* endemicity. Sequencing of cysticerci DNA from infected pig carcasses confirmed *T. solium* as a causative agent of infection.
- Free-range pig husbandry, practice of open defecation and the origin of the reared pigs were identified as risk factors for PC transmission.
- The ddPCR technology is an effective screening tool for taeniid DNA from both spiked soil and household soil, suggesting its potential applicability in large-scale screening of environmental contamination from endemic areas.
- In the spiking experiment, the ddPCR recovered the highest DNA copies from sand and the lowest from clay soil, suggesting that soil type may be an important factor requiring consideration when screening soil from endemic areas.
- Even though soil contamination in household soil was found to be low, soil contaminated by *T. solium* eggs can still be a source of cysticercosis infection for both pigs and humans in the studied villages.

- Even though the low rate of *T. solium* detection in soil was likely affected by the uneven distribution of eggs and the amount of soil sample analysed, screening for the presence of *T. solium* using serum samples from pigs yielded opposite results.
- Meat juice from the diaphragm or heart can be used as an alternative diagnostic specimen to serum for ELISA screening of PC given the high sensitivity in these tissues and for the detected antibody concentration levels.

7 Future perspectives

To date, there is no shortage of knowledge about *T. solium* and its impact on pigs and humans health. Although the epidemiology of *T. solium* is well known in pigs and humans, studies assessing environmental contamination by *T. solium* eggs are scarce. This thesis sets the basis for future studies related to the assessment of environmental contamination by *T. solium* eggs in endemic areas using the validated ddPCR technology. It also sets the basis for exploring and utilizing meat juice in the monitoring of cysticercosis infection in pigs from endemic areas. Below are some important questions worth addressing in future research regarding *T. solium* epidemiology and detection methods.

- Paper I raised a question as to whether the prevalence of cysticercosis in pigs reflected cysticercosis or taeniasis in the general human population. It would be interesting to investigate the magnitude of taeniasis and cysticercosis in the human population from the same region in order to combine control efforts from the veterinary and public health perspectives.
- As shown in papers II and III, ddPCR technology is a promising technology in screening *T. solium* eggs from environmental samples such as soil. However, the question of cost-effectiveness is born. The cost for ddPCR equipment and consumable is high, thus, efforts to make the ddPCR technology affordable by low-income countries for future epidemiological studies are encouraged.
- In paper II, taeniid eggs were successfully recovered from different soil types in a laboratory-based artificial experiment. More research using different soil types from endemic areas is necessary to confirm parasite egg screening methods across soil types. In addition, alternative options for DNA extraction to the method used in the present study would be worthwhile to explore.
- Even though soil contamination by *T. solium* was vividly reported in paper III, it seems the distribution of *T. solium* eggs in household soil

is uneven leading to the low rate of parasite detection. The most important question, therefore is, where in the household environment is the *T. solium* eggs most prevalent? Further investigation on hotspots of soil contamination by *T. solium* eggs at a household level in endemic rural communities is important to direct proper control strategies. The amount of soil collected and analysed per household was relatively low and may also have contributed to the recorded low level of contamination, thus the sampling design needs to be refined and standardized.

- Studies regarding the role of other environmental and ecological factors that pose risks of cysticercosis infection would be meaningful. Such studies may include a detailed exploration of pig feed, vegetables and water sources in endemic areas. The role of wind in the distribution of parasite eggs may also be interesting to explore.
- Detection of *T. solium* egg DNA in household soil in paper III was important, but was the DNA originating from viable or non-viable eggs? It is important to understand whether the eggs in the environmental soil are viable or not, to determine their infectiousness. If viable, for how long do they remain viable in the soil? What soil or environmental conditions influence their viability?
- In paper IV, meat juice collected from the heart and diaphragm showed a promising utility as a diagnostic specimen in the detection of PC, more studies on a relatively large sample size of pigs with varying degrees of cysticercosis are needed to further prove the concept. It would also be interesting to explore the diagnostic potential of other specimens such as saliva or mucus in addition to meat juice samples in the detection of PC.

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Popular science summary

Pig production in Tanzania has tremendously increased in the past few decades. This increase has been due to low cost of investment and high demand for pork in both rural and urban areas. However, one problem for animal husbandry is that pigs can contract parasitic zoonoses such as cysticercosis, caused by the pork tapeworm, *Taenia solium*. The parasite makes the meat unsafe for consumption because insufficiently prepared meat can also cause infection with the parasite in humans. In addition, the parasite leads to financial loss for pig producers. One of Tanzania's research priorities is to address neglected tropical diseases including zoonoses. Cysticercosis was added to the list of priority research areas in Tanzania in 2013 to ensure food safety and minimize production problems. Devising meaningful strategies for cysticercosis control cannot be achieved unless all the details in the transmission cycle of the pork tapeworm are understood.

The aim of this thesis was to: I) Determine the prevalence of cysticercosis in pigs in rural villages of Kongwa district. II) Investigate the risk factors for the transmission of *T. solium* eggs and potential role of soil in the transmission of the eggs of the parasite. III) Explore whether meat juices from different organs can be used to detect cysticercosis in slaughtered pigs.

To determine the prevalence of cysticercosis in pigs and the risk factors for infection, blood samples were collected from pigs. Then, antigens against the pork tapeworm were measured in sera using antigen ELISA (enzyme-linked immunosorbent assay). Farmers were also asked to fill out a questionnaire. A total of 447 pigs from four villages in Kongwa were surveyed, of which 17% were tested positive with ELISA. This indicates that there are people nearby who are infected by the pork tapeworm. During the rainy season more pigs with cysticercosis were found than during the dry season. The results of the survey show that pigs were more likely to get cysticercosis when they were allowed to roam freely and where there were no proper use of latrine. Improved sanitary

conditions and a modern view of pig production are needed to prevent the continued transmission of pork tapeworms in the study area.

The role of soil in the transmission of cysticercosis was investigated in 96 households in Kongwa using a molecular method (droplet digital Polymerase Chain Reaction) developed and validated with nailed soil samples. The method was effective in detecting even low amounts of taeniid egg DNA from soil. In contrast, DNA from the pork tapeworm's eggs was found in only 3.1% of the soil samples from households. This indicates that there is a low risk of both pigs and humans acquiring cysticercosis through soil contaminated with the parasite's eggs.

Meat juices from 13 different organs were analyzed by ELISA for the diagnosis of cysticercosis in 9 slaughtered pigs with visible cysts and the result was compared with diagnostics from blood serum. Sensitivity was found to be high when meat from the diaphragm, heart and neck muscles is used from infected pigs. Accordingly, meat juices from these organs can be used in routine post-slaughter cysticercosis studies in pigs. Especially tissue from the diaphragm and heart is recommended because they have the highest antibody levels.

In conclusion, porcine cysticercosis is common in rural areas in Kongwa district. To limit the social and economic impact, improved veterinary public health is recommended with a control program against pork tapeworm.

Populärvetenskaplig sammanfattning

Grisproduktionen i Tanzania har ökat enormt under de senaste decennierna. De två främsta anledningarna är låga investeringskostnader samt hög efterfrågan på fläskkött både på landsbygden och i städerna. Ett problem för djurhållningen är att grisar kan drabbas av parasitiska zoonoser såsom cysticerkos, orsakad av svinbandmasken, *Taenia solium*. Parasiten gör köttet osäkert för konsumtion eftersom otillräckligt tillrett kött kan leda till att människor också drabbas av parasiten. Osäkerheten kring köttets parasitstatus leder till ekonomiska förluster för grisproducenterna. En av Tanzanias forskningsprioriteringar är att ta upp försummade tropiska sjukdomar inkludernade zoonoser. Cysticerkos lades 2013 till listan över prioriterade forskningsområden i Tanzania för att säkerställa livsmedelssäkerhet och minimera produktionsproblem. Först när det finns kunskap om alla detaljer i svinbandmaskens livscykel kan meningsfulla kontrollstrategier vidtas.

Målet med denna avhandling var att I) studera förekomsten av cysticerkos hos grisar på landsbygden i Kongwa-distriktet. II) undersöka riskfaktorer som finns vid överföring av parasitägg och vilken roll som miljösmitta spelar vid överföringen. III) utforska om köttsaft från olika organ kan användas för att upptäcka cysticerkos hos slaktade grisar.

För att bestämma hur vanligt cysticerkos är hos grisar och vilka riskfaktorer som finns för smitta togs blodprov från grisar. Därefter undersöktes antikroppar mot svinbandmasken i sera med antigen-ELISA (enzymbunden immunosorbentanalys). Lantbrukarna ombads även att fylla i ett frågeformulär. Totalt undersöktes 447 grisar från fyra byar i Kongwa varav 17 % testades positivt. Detta tyder på att det finns människor i närheten som är infekterade av svinbandmasken. Under regnperioden hittades fler grisar med cysticerkos än under torrperioden. Resultatet från enkätundersökningen visar att grisar hade högre sannolikhet att få cysticerkos när de fick gå fritt och där det saknades välordnade latrinsystem. För att förhindra fortsatt smitta av svinbandmask i

studieområdet, krävs förbättrade sanitära förhållanden och en modern syn på grisproduktion.

Betydelsen av miljösmitta för smittspridning av cysticerkos undersöktes hos 96 hushåll i Kongwa med en molekylär metod (droplet digital Polymerase Chain Reaction) som utvecklades och validerades med spikade jordprover. Metoden var känslig och kunde hitta prover med små mängder DNA från parasitägg i jorden. Däremot hittades DNA från svinbandmaskens ägg endast i 3,1% av jordproverna från hushållen. Detta tyder på att det är låg risk för både grisar och människor att smittas via jord förorenad med parasitens ägg.

Köttsaft från 13 olika organ analyserades med ELISA för diagnos av cysticerkos hos 9 slaktade grisar med synliga cystor och resultatet jämfördes med diagnostik från blodserum. Det visade sig att känsligheten är hög när köttsaft från mellangärdet (diafragma), hjärtat och nackmuskulatur används från infekterade grisar. Köttsaft från dessa organ kan följaktligen användas vid rutinundersökningar av cysticerkos hos gris efter slakt. Speciellt rekommenderas vävnad från mellangärdet och hjärtat eftersom de har de högsta antikroppsnivåerna.

Sammanfattningsvis är cysticerkos hos grisar vanligt förekommande på landsbygden i Kongwa-distriktet. För begränsa se sociala och ekonomiska konsekvenser rekommenderas förbättrad veterinär folkhälsa med ett kontrollprogram mot svinbandmask.

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The aim of this thesis was to investigate the transmission of *Taenia solium* porcine cysticercosis in eastern-central Tanzania. Results show that porcine cysticercosis is endemic and may pose economic and public health concerns in rural villages of Kongwa district. A Low level of contamination by *Taenia solium* eggs detected by ddPCR in household soil in Kongwa suggests a low risk of pigs and humans acquiring cysticercosis via soil. These results provide research evidence justifying control strategies against the parasite.

Justine Daudi Maganira received his doctoral education at the Department of Biomedical Sciences and Veterinary Public Health, Swedish University of Agricultural Sciences. He obtained his Master of Science degree from the University of Dar es Salaam, Tanzania.

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