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Epidemiology of *Taenia solium* Cysticercosis in western Kenya

Lian F. Thomas

A Thesis submitted for the degree of Doctor of Philosophy
University of Edinburgh

2013
To my fabulous parents Ian and Jenny and my wonderful husband Paul, without all your support this would never have happened
Abstract

*Taenia solium* is a zoonotic helminth which is thought to be one of the leading causes of acquired epilepsy in the developing world. *T. solium* cysticercosis infections in pigs and humans and human taeniasis were diagnosed using antigen-capture ELISAs. The parasite was found to be endemic in the study site, with cysticercosis being detected by HP10 Ag-ELISA in 6.6% of human samples (95% C.I. 5.6-7.8%) and 17.2% (95% C.I. 10.2-26.4%) of porcine samples. Human taeniasis was detected by Copro-Ag ELISA in 19.9% (95% C.I. 18.2-21.8%) of faecal samples. The study site was found to be co-endemic with a large selection of other neglected tropical diseases, including soil transmitted helminthiasis, schistosomiasis, strongyloidiasis and amoebiasis.

Potential control measures for this parasite have been modeled and the exclusion of infective pork from the food chain through the use of a pre-slaughter test for pig farmers, traders and slaughtermen was found to have the potential to avoid 72.6% (95% C.I. 62.1-80.9%) of infective meals consumed in the area at an incremental cost-effectiveness ratio (ICER) of $0.25 (0.2-0.35). Such a diagnostic tool is currently under development and its performance was evaluated as part of this thesis. The novel, user-friendly lateral flow assay, utilising the HP10 monoclonal antibody, was evaluated using a Bayesian framework and was estimated to perform with a Sensitivity of 82.7% (95% B.C.I. 72.5-91.9%) and Specificity of 87% (95% B.C.I. 80.2-93.4), results which demonstrate the potential utility of this test in epidemiological studies and in control strategies.

Free-ranging pig production has been previously demonstrated to be a key risk factor for porcine cysticercosis and is commonly practised in this study region. A study carried out as part of this thesis found that these pigs have a home range of 15,085m² which is almost 10 times the average area of a homestead. This work indicates that pigs can be exposed to infective eggs from any human *T. solium* carriers within that homerrange area, greatly assisting transmission of this parasite. Western Kenya is a severely deprived region where pig production is becoming hugely popular and is seen as a major tool for economic development, yet the data presented in this thesis indicates an area with endemic status for the harmful parasite *T. solium*, for which effective control strategies are desperately required.
Declaration of Authorship

I, Lian Thomas, declare that the work presented within this thesis is my own, except where clearly stated. Chapters 3 & 6 have been prepared as manuscripts and so have kept the use of “we” and Chapter 2, although written as a thesis chapter, describes the study design which was a collaborative effort between myself and the Zoonotic and Emerging Disease research group and so the use of “we” is more suitable. This work has not been submitted for any other degree or professional qualification.

Signed: .................................................

Date: .......................... 6th December 2013
Acknowledgements

I would like to thank the Biological and Biomedical Research Council who supported my PhD studies through a Doctoral Training Grant and the Wellcome Trust (085308) for their support of the People, Animals and their Zoonoses project. Collection of porcine samples from slaughter facilities was supported by a University of Edinburgh Small Projects Grant and a Strategic Award from the Wellcome Trust to the Centre for Immunity, Infection and Evolution (095831) helped support the laboratory facilities in Busia. The Association for Strengthening Agricultural Research in Eastern and Central Africa (ASARECA) funded development of the prototype lateral flow assay. I thank the Department of Veterinary Services, Republic of Kenya, for their facilitation and support for the work presented in this thesis, and permission to access slaughter facilities and the Director of the Kenya Medical Reaserch Institute, for their facilitation of the human data collection.

In order to complete this thesis I was lucky to recieve the support of many people, I am especially grateful to:

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- Sarah Gabriel and Pierre Dorny (Institute of Tropical Medicine, Antwerp) who provided the polyclonal antibodies for the copro-antigen ELISA

- Phil Toye (ILRI) who obtained funding for, and directed the development of, the lateral flow assay
Acknowledgements

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I would also like to strongly thank the entire 'PAZ' field and laboratory teams in Busia and Nairobi for their hard work and diligence and most importantly to all the participating farmers, pig traders and slaughter men for their willingness to be involved in this research, without whom none of this work could have been completed.
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<td>Ab</td>
<td>Antibody</td>
</tr>
<tr>
<td>Ab-ELISA</td>
<td>Antibody capture ELISA</td>
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<tr>
<td>Ag</td>
<td>Antigen</td>
</tr>
<tr>
<td>Ag-ELISA</td>
<td>Antigen capture ELISA</td>
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<tr>
<td>AIC</td>
<td>Akaike’s second-order information criterion</td>
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<td>AIDS</td>
<td>Acquired immunodeficiency syndrome</td>
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<tr>
<td>ASARECA</td>
<td>Association for Strengthening Agricultural Research in Eastern and Central Africa</td>
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<tr>
<td>ASF</td>
<td>African swine fever</td>
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<td>African swine fever virus</td>
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<td>B.C.I.</td>
<td>Bayesian Credibility Interval</td>
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<td>BCS</td>
<td>Body condition score</td>
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<td>BSA</td>
<td>Bovine serum albumin</td>
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<tr>
<td>C.I.</td>
<td>Confidence Interval</td>
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<tr>
<td>CDC</td>
<td>Center for Disease Control</td>
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<tr>
<td>CNS</td>
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<td>CSF</td>
<td>Cerebro-spinal fluid</td>
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<td>CWGESCA</td>
<td>Cysticercosis Working Group for East and Southern Africa</td>
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<tr>
<td>DALY</td>
<td>Disability adjusted life year</td>
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<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
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<tr>
<td>DPLO</td>
<td>Divisional livestock production office</td>
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<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>EITB</td>
<td>Enzyme linked immunoelectrotransfer blot</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
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<td>ELISA</td>
<td>Enzyme linked immunosorbant assay</td>
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<td>Immunoglobulin</td>
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<td>International Livestock Research Institute</td>
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<td>Just another Gibbs sampler</td>
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<td>LoCoH</td>
<td>Local convex hull</td>
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<td>Monoclonal Antibody</td>
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<td>Optical density</td>
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<td>Odds Ratio</td>
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</table>
PAZ  People Animals and their Zoonoses
PBS  Phosphate buffered saline
PCR  Polymerase chain reaction
PCV  Packed cell volume
PDA  Personal digital assistant
PE   Physical examination
PRRS Porcine reproductive and respiratory syndrome
rpm  Revolutions per minute
SDS-PAGE Sodium docdecyl sulfate- polyacrylamide gel electrophoresis
Se   Sensitivity
Sp   Specificity
SSA  Sub-Saharan Africa
TP   Total protein
USA  United States of America
WHO  World Health Organization
YLD  Years of life lived with a disability
YLL  Years of life lost
ZN   Ziehl-Neelson staining method
Chapter 1

Introduction
1.1 Motivation

Cysticercosis, infection with the intermediate stage of the tapeworm *Taenia solium* is a zoonotic disease of increasing public health and economic importance, being a leading cause of acquired epilepsy in humans (Commission on Tropical Diseases, 1994) as well as causing substantial losses to pig farmers due to meat condemnation (Phiri *et al.*, 2003). The increasing global popularity of pork as a protein source puts a great many people and animals at risk from contracting this disease. This is exacerbated in the developing countries where the parasite life cycle is perpetuated through incomplete coverage of formal meat inspection facilities, poor education on preparation of meat, inadequate sanitation facilities and a large number of free-roaming, scavenging pigs.

*T. solium* cysticercosis has been designated as a ‘Neglected Tropical Disease’, affecting the poorest and most marginalised members of society.

On 27th May 2013 The World Health Assembly adopted resolution WHOA66.12 through which member states are urged to work towards controlling neglected tropical diseases, including *T. solium* (World Health Organization, 2012a, 2013a). For the global community to honour this commitment, there must be an improvement in our understanding of the epidemiology of the parasite and validation of evidence-based control strategies (World Health Organization, 2012a).

Knowledge gaps were identified at a recent meeting of the European *T. solium* Taeniasis/Cysticercosis Working Group, including the specific requirement for data on the prevalence of taeniasis and cysticercosis in humans, risk factors for cysticercosis in humans and a better understanding of transmission dynamics, including pig movements between and within villages and seasonality of infections (Johansen and Mejer, 2010). In this thesis I aim to address this paucity of data for western Kenya, through the accomplishment of 7 research objectives, these being:

1. Quantify the risk to humans of acquiring a *T. solium* cysticercosis infection from pork butchered and sold in western Kenya

2. Determine the prevalence of *T. solium* Taeniasis and cysticercosis infections and establish the risk factors associated with their acquisition in the human population of western Kenya

3. Determine the prevalence of, and risk factors for, *T. solium* cysticercosis infection in the porcine population in western Kenya
4. Understand the diagnostic parameters of a novel, user-friendly, rapid diagnostic test for *T. solium* cysticercosis infections produced as a collaborative venture between the University of Edinburgh and the International Livestock Research Institute

5. Understand the spatial distribution of *T. solium* infections in the porcine and human population of western Kenya

6. Determine the homorange of a free-ranging pig in western Kenya and understand the spatial ecology of free-ranging pigs in relation to disease transmission

1.2 Background

1.2.1 Cysticercosis: A Neglected Zoonotic Disease

A zoonotic disease is one which is naturally transmitted between people and vertebrate animals (World Health Organization, 1959). It is estimated that such diseases represent 58% of all human pathogens and up to 75% of all emerging diseases (Woolhouse and Gowtage-Sequeria, 2006). Amongst these diseases, there are many which have not been prioritised by national or international health services despite a growing understanding of the health and socio-economic burden they impose (World Health Organization/DFID-AHP, 2005). The so-called neglected zoonotic diseases are often closely associated with poverty, generally affecting the poorest and most marginalised members of the population (Molyneux *et al.*, 2011).

Poverty can be both a cause and consequence of zoonotic diseases, with particular aspects of poverty which can lead to an increased risk of exposure being: the close spatial association between people and their animals (Cosivi *et al.*, 1998), poor hygiene and sanitation, and lack of access to safe feed sources for animals and people. Living in poverty also results in a poorer prognosis should a disease be acquired due to a lack of access to veterinary and human health resources (Alvar *et al.*, 2006; Perry *et al.*, 2002). The burden imposed by these zoonotic diseases are multifaceted, with impacts on human and animal health as well as on the economic stability of households and the wider community (Perry *et al.*, 2002). This multidimensional quality makes the burden of disease hard to quantify and as such is one of the reasons for their current neglect.

One of the zoonotic diseases which has been recognised as being neglected by health and veterinary services as well as by donors and policy makers is the zoonotic helminth
**Taenia solium** (World Health Organization/DFID-AHP, 2005). The World Health Organization (WHO) estimates that *T. solium* affects 50 million people world-wide, resulting in approximately 50,000 deaths a year (World Health Organization/DFID-AHP, 2005). In sub-Saharan Africa alone it is estimated that between 1.90 and 6.16 million people maybe infected (Winkler, 2012). This parasite is thought to be a leading cause of acquired epilepsy (Commission on Tropical Diseases, 1994) potentially responsible for up to 1/3rd of all epilepsy cases (Ndimubanzi et al., 2010) in endemic areas.

*T. solium* cysticercosis has been identified as an important disease in Latin America (Gonzalez et al., 1990), Asia (Rajeshkhar et al., 2003) and across much of Africa (Ngowi et al., 2004; Phiri et al., 2003; Poudet et al., 2002) although the nature of global travel and migration puts all countries at risk of infection. This is highlighted by the hundreds of cases of neurocysticercosis which were diagnosed in the United States between 1990-2000, with histories indicating that infection was acquired from endemic areas outside of the USA (Sorvillo et al., 2007). It is estimated that approximately 41,400 -169,000 cases of cysticercosis occur in the USA annually, predominately in the Hispanic population, with the Mexico border states of Texas and California having the greatest burden (Hotez et al., 2008).

In recognition of the importance of this parasite, *T. solium* has been recently included in a road-map for the control of 17 previously neglected tropical diseases (NTDs) in which the global community declares that by 2015 a validated control strategy will have been devised, and control will be scaled up in selected African, Asian and Latin American countries by 2020 (World Health Organization, 2012a).

### 1.2.2 Biology of *Taenia solium*

Species of *Taenia* are members of the cestode family Taeniidae, which also includes the genera Echinococcinae (Hoberg, 2002). Those *Taenia* spp. which have human definitive hosts, utilise domesticated mammals as the intermediate hosts; bovines in the case of *T. saginata* and swine in the case of *T. solium*. Their occurrence in humans has been linked historically to the domestication of their obligatory intermediate host (Hoberg et al., 2001).

*T. solium* has a two host life cycle, which is illustrated in Figure 1.2 on page 6, with humans being the definitive host and pigs the intermediate host. Humans as the definitive host, are infected after consumption of viable cystercerci in under-cooked pork and harbor the adult tapeworm in the small intestine, an infection known as taeniasis.
The adult worm has a scolex with four rows of suckers at one end, with which they attach to the intestinal mucosa (Garcia et al., 2003). Distal to the scolex the proglottids are produced to form the long strobilia, generally up to 1 or 2m in length, although worms of up to 7m in length have been reported (Sciutto et al., 2000) An adult *Taenia* spp. specimen and proglottids can be seen in figure 1.1.

The attachment of the parasite to the gut mucosa is non-symptomatic in the majority of cases, but has been reported to cause abdominal discomfort, diarrhea and nausea (Garcia et al., 2003). Gravid proglottids, containing approximately 50,000 eggs with infective embryophores, detach from the adult tapeworm and are excreted in faeces in an intermittent fashion (Garcia et al., 2003).

Ingestion of these eggs, by either pigs or humans, results in the oncospheres penetrating the intestinal wall, moving through the lymph and blood vessels to encyst in muscle, eyes or the central nervous system (CNS) as cysticerci, the larval stage of the parasite (Flisser, 2006). The cysticerci is a fluid-filled vesicle up to 2cm in diameter with a small invaginated scolex (Sciutto et al., 2000). Porcine cysticercosis is generally asymptomatic and is characterised by cysts found in the heart, diaphragm, masseters thigh and loin musculature and was recognised as early as 380BC (Hawk et al., 2005).

Signs of cysticercosis infection in man are variable. Large numbers of larval stages encysting in muscles can cause stiffness, pain and muscular pseudohypertrophia (Garcia et al., 2003). Ocular cysticercosis can cause disturbances in vision, but the greatest morbidity is that caused by neurocysticercosis (NCC), the most common symptom of which is epileptic seizures (Carabin et al., 2011; Quet et al., 2009), although symptoms can also include nausea, headaches and dizziness, ataxia, dementia and strokes (Hawk et al., 2005).
The wide range and severity of NCC symptoms seem to stem from both the location and number of lesions and the variability of individual host inflammatory response, which can range from a ‘convivial’ relationship with little or no inflammatory cell infiltrate to a prominent infiltrate surrounding necrotic cysticerci (Sciutto et al., 2000). The importance of NCC as an etiological agent of epilepsy is discussed in section 1.2.6 on page 21.

1.2.3 Diagnosis of Taeniasis and Cysticercosis

There are a great many diagnostic techniques for the detection of T. solium cysticercosis and taeniasis and, as is often the case when many tests are available, none are perfect. The range of diagnostic tests also relates to the different clinical manifestations of infection and includes techniques which are suitable for different clinical or epidemiological settings. The most common techniques for diagnoses of this parasite are described in the following sections and the diagnostic parameters of several of these are outlined in Table 1.1 on page 15
1.2. BACKGROUND

**Serological Diagnostics for Cysticercosis**

In both the human and porcine host serological assays have been used extensively in the epidemiological setting and are based around the detection of antibodies raised towards the parasite or of specific parasite antigens. Two techniques dominate the literature, being the Enzyme linked immunosorbant assay (ELISA) and the Enzyme-linked immunoelectrotransfer blot (EITB).

The EITB assay combines sodium dodecyl sulfate- polyacrylamide gel electrophoresis (SDS-PAGE) and ELISA techniques to detect circulating antibodies in sera or cerebrospinal fluid (CSF) (Tsang *et al.*, 1983). Lentil-lectin bound glycoproteins extracted from homogenised *T. solium* cysts provide seven glycoprotein bands to which anticysticerci antibodies in sera or CSF will bind. A signal from any one of these bands being considered to indicate exposure to *T. solium* (Tsang *et al.*, 1989).

High sensitivity (se) and specificity (sp), of 98%/100%, has been reported in human samples (Tsang *et al.*, 1989) and 100%/100% in pigs (Tsang *et al.*, 1991). In NCC cases with multiple active cysts, the EITB has been shown to perform with very high sensitivity (Blocher *et al.*, 2011). It, has, however been shown to have a low sensitivity for detection of NCC in patients with a single calcified lesion (Blocher *et al.*, 2011). The assay has performed well when compared to antibody and antigen ELISAs (Diaz *et al.*, 1992) but the relative technical difficulties in protein purification and standardiseing the polyacrylamide gel system (Handali *et al.*, 2010) combined with relatively higher costs makes ELISA a favored choice in a developing nation or field setting (Dorny, 2003; Rodriguez *et al.*, 2012).

ELISA techniques have been developed for the detection of circulating antibodies in sera and CSF using either a variety of antigens, crude cysticeri extracts, extracts from vesicular fluid or partially purified surface antigens. These assays have been found to have Se/Sp ranging between 80-100% in sera and CSF in NCC cases (Espinoza *et al.*, 1986), and for the detection of cysticercosis in endemic and non-endemic regions (Larralde *et al.*, 1986).

Both the EITB and Antibody-capture ELISA techniques detect circulating antibodies (Ab), which indicates exposure to the parasite, but not necessarily an active infection (Rodriguez *et al.*, 2012). This makes these assays useful for understanding the presence of the parasite in a population, but cannot identify those who are currently harboring the infection or in a clinical setting for the diagnosis of cases and is likely to lead to over-estimation of infections (Garcia *et al.*, 2001; Rodriguez *et al.*, 2012).
Active infections can be determined through detection of *T. solium* antigens (Ag) and serological detection of circulating antigen has been achieved using monoclonal antibodies (MAb), including HP10 (Harrison *et al.*, 1989) and B158/B60 (Dorny *et al.*, 2000) and various oncospheral peptides (Ferrer *et al.*, 2005). The HP10 mouse MAb is an IgM which reacts to surface and excreted/secreted glycoproteins of *T. saginata* cysticerci (Harrison *et al.*, 1989). The MAb, when used in an ELISA set up was found to detect *T. saginata* antigen in experimentally infected cattle from 4 weeks post infection, with a rapid decrease following praziquantel treatment, indicating the utility of this test for detection of only viable cysticerci (Harrison *et al.*, 1989).

The assay was found to cross-react with *T. solium* infected individuals, but was found to have low cross-reactivity with other helminth infections, including *Taenia hydatigena* (Harrison *et al.*, 1989). Cross-reactivity with *Taenia hydatigena* has been reported in the B158/B60 Ag-ELISA, which may limit the utility of the test for pigs in Asian countries where the parasite is widespread, e.g. Vietnam (Dorny *et al.*, 2004).

The HP10 and B158/B60 Ag-ELISAs have been used routinely for detection of *T. solium* infections in humans and pigs, with varying sensitivity and specificity reported, as shown in Table 1.1 on page 15. In human NCC the sensitivity of both HP10 and B158/B60 Antigen ELISAs depends on the location of lesions, with extra-parenchymal lesions more easily detected than intra-parenchymal lesions (Fleury *et al.*, 2007; Rodriguez *et al.*, 2009). The B158/B60 Antigen ELISA has very recently been commercialised (ApDia n.v., Belgium) and the manufacturers report a sensitivity of 94% in human NCC cases (n=100), of 100% in experimentally infected pigs (n=31) and specificity of 99.3% in humans (n=300) and 99.6% in pigs (n=300).

The requirement for obtaining blood samples raises ethical issues in both animals and humans. Genuine fears about having blood samples taken have been encountered by medical researchers in several countries in sub-Saharan Africa, seemingly stemming from colonial times and rumors of “Kachinja” (blood stealer) (Geissler and Pool, 2006; Geissler, 2005). Other biological fluids have been utilised for the detection of *T. solium* infections and may warrant further investigation. The use of urine in an ELISA showed a sensitivity of 90.9% (0.820.99) and specificity of 100%. in active NCC cases (Castillo *et al.*, 2009). Tears have been used for detection of ocular cysticercosis and the ELISA was found to have a sensitivity of 100% and specificity of 92% when tested on a very limited number of cases (Sahu *et al.*, 2008).

Despite the benefits of immunological diagnostics, in terms of relative sensitivity and specificity, analysis of samples requires basic consumables for sample collection, equip-
1.2. BACKGROUND

ment such as refrigerators, centrifuges, access to utilities such as water and electricity and technical expertise, all of which may be lacking in the developing country settings in which \textit{T. solium} is prevalent (Petti \textit{et al.}, 2005). These limitations have lead to the call for a more user-friendly, cheap and rapid diagnostic modality for use in field conditions. The good performance of the HP10 Antigen ELISA for the detection of neurocysticercosis cases has lead to the suggestion that this MAb be incorporated into a lateral flow format assay which could fulfill this role (Fleury \textit{et al.}, 2007). In Chapter 5 on page 121 of this thesis, the prototype of such a lateral flow assay (LFA) is evaluated.

Field Diagnosis of Cysticercosis in Pigs

Cheaper and more widely accessible than other diagnostics available, is the diagnosis of cysticercosis in pigs by lingual palpation. This is a well established technique that is routinely used by farmers in Peru to screen their own pigs before being sent to slaughter (Gonzalez \textit{et al.}, 1990). The pigs are restrained and their mouths opened with a stick and the tongue grasped and pulled out of the mouth, the ventral surface of the tongue is then felt for the presence of cysticerci as described by Ngowi \textit{et al.}, (Ngowi \textit{et al.}, 2004). The technique, although requiring no special equipment, does require technical skill to identify the parasite and has a very low sensitivity and is only likely to detect heavily infected animals (Dorny \textit{et al.}, 2004). There are some who suggest that this technique is useful for the rapid assessment of areas for the presence of the parasite (Gonzalez \textit{et al.}, 1990), but I would hesitate to promote lingual palpation as a reliable diagnostic tool.

Meat inspection is used the world over, to a greater or lesser extent, for the condemnation of meat which is unfit for human consumption for various reasons, such as for;

- the detection of zoonotic parasitic infections including; trichinosis, echinococcosis and cysticercosis
- the detection of signs indicative of systemic disease such as septicemia and jaundice
- the detection of notifiable diseases
- to ensure the correct and hygienic handling of meat for the prevention of food borne bacterial contamination
Provision is made within official meat hygiene regulations laid down by governments for specific procedures to be undertaken for the detection of cysticercosis. These include parallel incisions into the external and internal masseter muscles, a longitudinal incision along the length of the tongue, incision into the heart septum and three incisions into the triceps muscle (Boa et al., 2002).

The specificity of meat inspection is high, but the sensitivity has been reported to be low, 38.7% (95% C.I. 0.22-0.58) as practiced in Zambia (Dorny et al., 2004) especially when there are few cysts present in the pigs (Boa et al., 2002; Dorny et al., 2004; Gonzalez et al., 1990). In many of the countries in which T. solium is endemic there is often an informal trade in pigs, which circumvent the official meat inspectors and thereby allow un-inspected pigs into the food chain (Boa et al., 2006), as is the case in western Kenya (Kagira et al., 2010) and Uganda (Nsadha et al., 2010). It has been noted also that fewer meat inspectors are available than required to achieve full coverage of inspection and that pork may sometimes be consumed by abattoir workers before inspection (Kagira et al., 2010).

There is therefore a lack of sensitive and specific diagnostic tests available for the detection of T. solium which are suitable for use in the field setting. Again, development of a lateral flow format assay utilising the HP10 MAb as previously suggested (Fleury et al., 2007) could also have utility in porcine diagnostics to fill this current requirement.

*Imaging Modalities for Neurocysticercosis*

Clinical signs of NCC are equivocal and serological diagnostics can indicate the presence of (Ag-ELISA) or exposure to (EITB/Ab-ELISA) a parasite but will not definitively indicate this as the etiological agent (Hawk et al., 2005).

Diagnostic criteria for NCC have been proposed, to increase the accuracy with which NCC is diagnosed. The diagnostic criteria fall into four categories as follows:

- **Absolute:** Histological demonstration of the parasite, neuroimaging of a cystic lesion with scolex, direct visualisation of the parasite by fundoscopy
- **Major:** Evidence of highly suggestive lesions on neuroimaging, positive serum on EITB, resolution of lesions after albendazole or praziquantel therapy, spontaneous resolution of small single enhancing lesion.
- **Minor:** Evidence of NCC compatible lesions on neuroimaging, suggestive clinical
manifestations, positive CSF Ab or Ag-ELISA, evidence of extra-CNS cysticercosis

- Epidemiological: history of living in or traveling to an endemic area, evidence of contact with a *T. solium* infected person

From these criteria one may make a definitive (one absolute or 2 major and a minor or epidemiological criteria) or probable (one major and 2 minor criteria, one major, one minor and one epidemiological criteria or three minor and one epidemiological criteria) diagnosis (Del Brutto, 2012; Del Brutto *et al.*, 1996). These criteria have been used extensively over the last 10 years in both field and hospital settings to avoid over and under-diagnoses of NCC (Del Brutto, 2012).

Neuroimaging technologies such as Computed Tomography (CT) and magnetic resonance imaging (MRI) are as such invaluable for the accurate diagnoses of NCC, but the availability and cost of such modalities are, however, prohibitive to their use in many of the endemic areas and in the majority of epidemiological studies (Foyaca-Sibat *et al.*, 2009; Gilman *et al.*, 2012). This issue has lead to the under-diagnoses of NCC in patients with false-negative serological results and the possible misdiagnoses of serologically positive people with neurological signs or history as NCC, when they may have been suffering from idiopathic epilepsy (Del Brutto *et al.*, 1996). It has been suggested however, that in areas where neuro-imaging is lacking that the addition of a positive B158/B60 Ag-ELISA as a major diagnostic criteria could increase the number of true NCC cases identified as definitive cases as opposed to probable cases (Gabril *et al.*, 2012).

**Diagnosis of Taeniasis**

Traditional diagnoses of adult *Taenia* carrier relies on direct microscopy of expelled eggs in faeces. The sensitivity of microscopy is, however, estimated to be approximately 39% (Allan *et al.*, 1996) to 52.5% (Praet *et al.*, 2013), due to the intermittent nature of egg shedding. The specificity of microscopy is high at the species level, but as the as the eggs of *Taenia* spp. appear identical under the light microscope there is a requirement for observation of expelled proglottids for speciation (Allan and Craig, 2006; Wilkins *et al.*, 1999). In order to improve the detection of taeniasis cases; immunodiagnostic assays, on faecal or sera samples, have been developed with a great improvement in sensitivity and specificity.
Copro-antigen diagnostics, based upon the detection of parasite specific secretory antigens, was first reported in the 1960’s although did not gain widespread scientific attention until the 1980’s (Allan et al., 2003). Specific secretory antigens are produced independently from reproductive material and are therefore not reliant on active shedding of eggs or proglottids. Coproantigen ELISA has now been used in a variety of situations to detect Taenia carriers. A field trial in Mexico, achieved a Se/Sp of 98%/99.2% with copro-antigen ELISA in comparison to a 38% sensitivity achieved with microscopy (Allan et al., 1996). The copro-Ag ELISA currently available are not species specific, detecting both T. solium and T. saginata (Allan et al., 1990) and cross-reactions have been reported with a variety of other gastro-intestinal parasites, including; Ascaris lumbricoides, Trichuris trichiura, Hymenolepis nana and parasitic protozoa (Rodríguez-Hidalgo, 2003).

To obtain a species specific diagnosis of T. solium work has been done on DNA based diagnostics. A rapid nested Polymerase chain reaction (PCR) assay using primers based on the published gene sequence of the oncospheral protein Tso31 achieved a 100% specificity, even under field conditions, whilst achieving a sensitivity of 97%-100% (Mayta et al., 2008).

The problems associated with diagnostic assays on faecal material regarding biohazards and cultural acceptability, have indicated a place for serological diagnoses of adult Taenia carriers. This has been achieved with an immunoblot assay for the detection of antibodies towards T. solium excretory secretory (TSES) antigens. The assay achieved a Se/Sp of 95%/100% when used to analyse sera of known infection status, including sera from T. saginata carriers and Echinococcosis infections (Wilkins et al., 1999). The use of native proteins, however, was a limitation on the utility of this test in the field, and recombinant proteins have now been expressed in a baculovirus system for use in diagnostic assays (Levine et al., 2004). These protein antigens (rES33 & rES38) are currently being used in an EITB format in the Peruvian cysticercosis elimination programs, both having shown high sensitivity (97%/98%) and specificity (100% /91% respectively) in field trials (Levine et al., 2007).

The use of immunodiagnostic techniques for the detection of Taenia carriers is a vast improvement over microscopic detection and their use in epidemiological surveys and in the clinical setting has an important role in the control and possible elimination of T. solium.
1.2.4 Understanding Diagnostic Test Parameters

The ideal or ‘gold standard’ diagnostic test would pick up all truly infected individuals as positive, i.e. 100% sensitivity (Se), whilst returning a result of negative for all truly un-infected individuals, even when other pathogens are present, i.e. 100% specificity (Sp). Not every diagnostic test, however will achieve such results and the true Se/Sp will be dependent upon characteristics both of the population under study and the test itself. Population variables include disease prevalence and individual variables such as clinical stage of disease, concurrent diseases and host immunity. Although the sensitivity should be independent of the prevalence of disease in the population, the specificity will increase with decreased prevalence (Greiner and Gardner, 2000b).

Characteristics of the test itself, such as the nature of the result, e.g. being categorical or continuous data, will also impact upon the test parameters. When dealing with a normal distribution of continuous variables, such as optical density returned from an ELISA, there is a trade-off between sensitivity and specificity and a cut-off value needs to be set to obtain the best balance between false negatives and false positives depending on the use of the test. Sensitivity and specificity for several regularly used diagnostic tests for *T. solium* are described in Table 1.1 on page 15.

For epidemiological studies and in the clinical setting, knowledge of test parameters is important in order to adjust for misclassification error (false positives/ false negatives) for accurate estimation of prevalence and for identification of risk factors. Techniques for this adjustment have been described for both individual and herd-level analysis (Greiner et al., 2000).

Determination of the diagnostic test parameters is generally done with reference to a ‘Gold Standard’ diagnostic test (Bronsvoort et al., 2010). In the case of many diseases, *T. solium* infection included, there are no established or appropriate gold-standard tests. In these situations a Bayesian approach can be utilised to allow comparison of tests without reference to a gold standard, to model the estimation of prevalence and test parameters (Dorny et al., 2004).

Bayes theorem uses the prior probability distribution (e.g. the probability of an individual being diseased independently of a test result) updated by data gathered (e.g. test result from that individual) to inform a posterior probability distribution (e.g. true disease status in that individual). A Bayesian approach can be extended for use in the epidemiological setting, with the prior probability distributions incorporating known information on disease prevalence and diagnostic test parameters, including the levels of
uncertainty about these values, and using these to update the information gained from
the epidemiological study to obtain estimates for true prevalence and test parameters
(Dunson, 2001).

The use of priors, and the strong influence they have on the posterior distribution is
one of the more controversial aspects of the Bayesian frameworks (Dunson, 2001). It
is for this reason that multiple-test, multiple population models have become popular
to reduce the strong influence of the prior distribution in the model (Branscum et al.,
2005). Several studies have been undertaken to estimate Se/Sp for diagnoses of T.
solium and the true prevalence estimate for that population, using single-population,
multiple-test Bayesian models (Assana et al., 2010; Dorny et al., 2004; Krecek et al.,
2008; Poudret et al., 2002) some of these prevalence estimates can be found in Table 1.2
on page 20. In Chapter 5 on page 121 of this thesis, Baysean methods have been used to
evaluate the performance of a prototype LFA for the diagnosis of porcine cysticercosis
in East Africa.
## Table 1.1: Sensitivity and Specificity for Some Commonly Used Diagnostic Tests for *T. solium*

<table>
<thead>
<tr>
<th>Diagnostic Test</th>
<th>Sample &amp; Species</th>
<th>Estimated Sensitivity% (95% C.I)</th>
<th>Estimated Specificity% (95% C.I)</th>
<th>citation</th>
</tr>
</thead>
<tbody>
<tr>
<td>HP10 Ag-ELISA (Harrison <em>et al.</em>, 1989)</td>
<td>Pig sera</td>
<td>70.4 (52.7-84.7)</td>
<td>66.1 (44.6-85.1)</td>
<td>(Krecek <em>et al.</em>, 2011)</td>
</tr>
<tr>
<td></td>
<td>Pig sera</td>
<td>44.4-84</td>
<td>45-100</td>
<td>(Sciutto <em>et al.</em>, 1998)</td>
</tr>
<tr>
<td></td>
<td>Human sera</td>
<td>84.8 (74.4-95.2)</td>
<td>94 (90.2-97.8)</td>
<td>(Fleury <em>et al.</em>, 2007)</td>
</tr>
<tr>
<td></td>
<td>Human csf</td>
<td>91.3 (83.2-99.4)</td>
<td>97.7 (93.2-100)</td>
<td>(Fleury <em>et al.</em>, 2003)</td>
</tr>
<tr>
<td></td>
<td>Human sera</td>
<td>75 (66.5-83.5)</td>
<td>96.5 (93.4-99.5)</td>
<td>(Ferrer <em>et al.</em>, 2005)</td>
</tr>
<tr>
<td>B158/B60 Ag-ELISA (Dorny <em>et al.</em>, 2000)</td>
<td>Pig sera</td>
<td>89.5 (80.4-99.4)</td>
<td>94.7 (90.2-99.7)</td>
<td>(Assana <em>et al.</em>, 2010)</td>
</tr>
<tr>
<td></td>
<td>Pig sera</td>
<td>63.3 (46.8-81.6)</td>
<td>87.0 (78.294.9)</td>
<td>(Krecek <em>et al.</em>, 2011)</td>
</tr>
<tr>
<td></td>
<td>Pig sera</td>
<td>64.5 (45-81)</td>
<td>91.2 (76-98)</td>
<td>(Dorny <em>et al.</em>, 2004)</td>
</tr>
<tr>
<td></td>
<td>Human Sera</td>
<td>60-100</td>
<td></td>
<td>(Rodriguez <em>et al.</em>, 2009)</td>
</tr>
<tr>
<td></td>
<td>Human CSF</td>
<td>73.3-100</td>
<td></td>
<td>(Rodriguez <em>et al.</em>, 2009)</td>
</tr>
<tr>
<td>apDia Cysticercosis Ag-ELISA</td>
<td>Pig sera</td>
<td>100 (83.8-100)</td>
<td>99.7 (98.2-99.9)</td>
<td>Kit information</td>
</tr>
<tr>
<td>apDia n.v. Ref. 650501</td>
<td>Human Sera</td>
<td>94 (87.4-97.8)</td>
<td>99.3 (97.6-99.9)</td>
<td></td>
</tr>
<tr>
<td>Ab-ELISA (Dorny <em>et al.</em>, 2004)</td>
<td>Pig sera</td>
<td>45.2 (27-64)</td>
<td>88.2 (73-97)</td>
<td>(Dorny <em>et al.</em>, 2004)</td>
</tr>
<tr>
<td>EITB (Tsang <em>et al.</em>, 1989)</td>
<td>Pig sera</td>
<td>49.0 (36.462.8)</td>
<td>84.0 (75.091.8)</td>
<td>(Krecek <em>et al.</em>, 2011)</td>
</tr>
<tr>
<td></td>
<td>Pig sera</td>
<td>100 (90.4-100)</td>
<td>100 (90.4-100)</td>
<td>(Tsang <em>et al.</em>, 1991)</td>
</tr>
<tr>
<td></td>
<td>Pigs pre-mortem</td>
<td>7.3 (0.815.1)</td>
<td>80.8 (71.090.4)</td>
<td>(Krecek <em>et al.</em>, 2011)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16.1 (5-34)</td>
<td>100 (90-100)</td>
<td>(Dorny <em>et al.</em>, 2004)</td>
</tr>
<tr>
<td>Meat Inspection</td>
<td>Pig Carcass</td>
<td>38.7 (22 58),</td>
<td>100 (90100)</td>
<td>(Dorny <em>et al.</em>, 2004)</td>
</tr>
<tr>
<td>Microscopy</td>
<td>Human Faeces</td>
<td>28.6 (17.8-42.4)</td>
<td>85.7 (60.1-96)</td>
<td>(Allan <em>et al.</em>, 1996)</td>
</tr>
<tr>
<td></td>
<td>Human Faeces</td>
<td>52.5 (11.1-96.5)</td>
<td>99.9 (99.5-100)</td>
<td>(Pract <em>et al.</em>, 2013)</td>
</tr>
<tr>
<td>Copro-Antigen ELISA (Allan <em>et al.</em>, 1990)</td>
<td>Human Faeces</td>
<td>98 (89.3-99.6)</td>
<td>99.1 (98.4-99.4)</td>
<td>(Allan <em>et al.</em>, 1996)</td>
</tr>
<tr>
<td>rES33 EITB (Levine <em>et al.</em>, 2007)</td>
<td>Human sera</td>
<td>97.6 (93.9-99.3)</td>
<td>99 (97.2-99.7)</td>
<td>(Levine <em>et al.</em>, 2007)</td>
</tr>
</tbody>
</table>
1.2.5 Prevalence & Risk Factors for Cysticercosis in sub-Saharan Africa

Worldwide *T. solium* cysticercosis in humans is under-reported due to a combination of: the poor sanitary conditions and poor husbandry practices found in marginalised communities in developing countries which allow the parasite to thrive (Carabin *et al.*, 2009), poor access to health care and lack of reporting or surveillance systems in these communities (Coulibaly and Yameogo, 2000; Singer and Ryff, 2007; Singh *et al.*, 2006), the wide range of non-specific clinical signs (Sciutto *et al.*, 2000) and absence of affordable, reliable diagnostic techniques (Murrell *et al.*, 2005).

In 2005 the WHO convened the first of a series of international meetings on the “Neglected Zoonotic Diseases” (World Health Organization, 2007, 2010; World Health Organization/DFID-AHP, 2005). During these meetings it was recognised that *T. solium* has been under-reported, and therefore neglected by the global health community, but that it is likely to be causing a large health and economic burden upon the communities where it is present. There is now increasing research activity surrounding this parasite, in response to initiatives such as the regional action plan drawn up by the Cysticercosis Working Group for East and Southern Africa (CWGESA) (Boa *et al.*, 2003).

The epidemiological picture in sub-Saharan Africa is now becoming clearer, especially in the porcine population, and it appears that apart from in Muslim areas, *T. solium* is present in practically all countries in the region (Zoli *et al.*, 2003). Estimates of *T. solium* prevalence vary greatly within the region as indicated in Table 1.2 on page 20, the prevalence estimate depends in part on the parameters of the diagnostic technique used as discussed in section 1.2.3 on page 6, as well as the area of the country studied, due to the focal nature of the parasite. In many of the countries studied there appear to be hyper-endemic foci of porcine infections, with prevalence rates between 30 and 50% not uncommon.

Table 1.2 on page 20 shows the results of some of the most recent papers on prevalence of *T. solium* in the porcine and human host from sub-Saharan Africa. Data were obtained through a search on both PubMed and Google Scholar and where no published literature was available through these searches I have stated ND. No data were available on either human or porcine infections in: Botswana, Congo (Brazzaville), Djibouti, Eritrea, Ethiopia, Guinea, Guinea-Bissau, Lesotho, Liberia, Malawi, Mali, Namibia, Niger, Sierra Leone, Somalia, Sudan or Swaziland. I have endeavored to provide the most recent data from each country and have given priority to data obtained by Antigen
1.2. BACKGROUND

ELISA or multi-test Bayesian estimates. The small island nations of Cape Verde, Comoros, Mauritius, Reunion, Seychelles and Sao Tome & Principle were omitted from this literature search.

Although in many countries data is becoming available for both human and porcine infections, there have been few studies, especially in Africa, which have attempted to collect this data simultaneously and to investigate the relationship between infections in the two species. The best example of simultaneous study of the porcine and human hosts in Africa was conducted in Burkino Faso, where Antigen ELISA was used to identify infection in both humans and pigs (Carabin et al., 2009; Ganaba et al., 2011). This is in contrast to 2 other multi-species studies in Africa in which differing (and less sensitive) diagnostics were used, such as interview based questionnaires for detection of NCC in Zambia (Phiri et al., 2002) and lingual palpation for porcine cysticercosis, with microscopy for the identification of taeniasis in Kenya (Githgia et al., 2006). In Chapter 4 on page 87 of this thesis prevalence and risk factors for 

In Burkino Faso the prevalence data from pigs and humans indicates a strong relationship between prevalence of porcine and human cysticercosis in the three villages sampled (Nyonyogo; human prevalence = 0% & porcine = 0%, Batondo; human = 1.4% & porcine = 32.7%, Pabre; human = 10.3% & porcine = 48.2%) with the strongest risk factor for either porcine or human infection being the village (Carabin et al., 2009; Ganaba et al., 2011).

The identification of high risk villages gives an indication of the pattern of spatial distribution of this parasite, which appears to occur around particular foci. A study in Tanzania explored the clustered nature of porcine infections formally, using the concept of random labeling and Ripley’s K function to determine consistency of cases with the null hypothesis of spatial randomness. Localised clustering was analysed with Bernoulli probability and Poisson models in SatScan\textsuperscript{TM} \url{http://www.satscan.org/} and significant clustering was found in the Ag-ELISA incidence data between 600m to 5km, approximating to clustering on a village level (Ngowi et al., 2010).

Clustering of 

Clustering of 

Another Peruvian study has also demonstrated clustering of 

This study demonstrated
increased likelihood of being copro-Antigen positive (OR = 9.4, 95% C.I. 1.2-71.8), rES33-ELISA positive (OR = 3.4, 95% C.I. 1.1-10.9) and EITB positive (OR = 2.2, 95% C.I. 1.2-4.1) if living within 100m of a lingual-positive pig (O’Neal et al., 2012).

The clustered nature of this parasite is one of the factors which may have led to its neglect by the global health community. Country or region-wide estimates of prevalence may dilute the burden experienced by localised communities, for example, a nationwide porcine prevalence of 0.57% reported by official meat inspection records in Burkino Faso (Coulibaly and Yameogo, 2000), compared to the up to 48.2% prevalence found by Ag-ELISA in one village (Ganaba et al., 2011). The national figure may also be artificially low due to a high degree of rurally raised pigs which are informally slaughtered without inspection in endemic areas (Phiri et al., 2003), an activity that not only allows the parasite to propagate, but also contributes to under-reporting of the disease.

The geospatial pattern for \textit{T. solium} infections discussed here indicate that certain areas have a higher risk of infection than others, relating to the presence or absence of risk factors in those areas. Looking at the biology of the parasite, we can identify that risk factors must relate to the ability of pigs to access infected faecal material and the consumption by humans of undercooked infected pork for the propagation of the parasites life-cycle and faecal-oral infection of people with infective \textit{T. solium} eggs.

Many papers have looked for association between putative risk factors and cysticercosis infections in pigs or humans. Risk factors which have been found to be significantly associated with porcine \textit{T. solium} infection in sub-Saharan Africa are: Lack of a latrine in the compound, (OR=2.03 (95% C.I. 1.25-3.5) (Ngowi et al., 2004), OR=3.2 (95% C.I. 1.2-8.55) (Mutua et al., 2007), OR= 3.8 (95% C.I. 1-14.8) (Kagira et al., 2010) and OR=1.9 (95% C.I. 1.32-37) (Eshitera et al., 2012)), being kept free range (OR=3.81 (95% C.I. 2.08-7.06) (Pondja et al., 2010) and OR=1.68 (95% C.I. 1.362.07) (Sikasunge et al., 2007)), and the pig being over 12mths of age (OR=3.56, 95% C.I. 2.04-6.19) (Pondja et al., 2010)).

Significant risk factors associated with human \textit{T. solium} infection in sub-Saharan Africa are: consumption of pork (OR = 8.75 (95% C.I. 1.11-68.88) (Carabin et al., 2009), 1.7 (95% C.I. 1.1-2.5) (Prado-Jean et al., 2007)), being male (OR=2.5, 1.8-3.4) (Prado-Jean et al., 2007) and being over 70 years of age (OR=2.8, 95% C.I. 1.14-3.81) (Kanobana et al., 2011).

Some putative risk factors, however, are not consistently significant on data analysis,
with some being shown, somewhat obscurely, to be protective factors, such as the non-availability of a pig pen (OR=0.446, 95% C.I. 0.231-0.860, \( p =0.016 \)) (Assana \textit{et al.}, 2010), which one would instinctively consider to increase the risk of infection.

In other studies potential risk factors have been shown to have no association with infection, such as the presence or absence of a latrine in the house (Pouedet \textit{et al.}, 2002). A community based study in Benin found a 3.5% (95% C.I. 1.3-8) sero-prevalence for \textit{T. solium} in humans, although failed to find any linkage with consumption of pork or owning free-range pigs (Adjidé \textit{et al.}, 1996). It may be that within the majority of the communities studied there is significant homogeneity of risk factors throughout the community to preclude strong identification of risk factors on an individual level. There may also be individual factors predisposing to infection, such as co-infections and immune system disorders, un-related to other, more general risk factors. Cases of NCC/HIV co-infections have also been reported (Chianura \textit{et al.}, 2006; Delobel \textit{et al.}, 2004) although little is still known about either the influence of HIV/AIDs on the epidemiology of the parasite or on the natural history of the clinical disease.

A common factor across the key risk factors investigated, are their frequent association with the poorest homesteads in a community. These are also the same households where access to health care is often prohibitively expensive (Abel-smith and Rawal, 1992; Xu \textit{et al.}, 2003) or inaccessible due to distance, or cost of transport (Gele \textit{et al.}, 2009; Odiit \textit{et al.}, 2004). Cases of taeniasis in these households are likely to go undetected and untreated, increasing the risk of cysticercosis in all those living within or near to the homestead. Therefore, when identifying people at risk of this disease, as will need to be done for the implementation of control measures, I am of the opinion that we would do better to identify high risk communities, as opposed to individuals at risk.
<table>
<thead>
<tr>
<th>Country</th>
<th>Porcine Prevalence% (95% C.I)</th>
<th>Human Prevalence% (95% C.I)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angola</td>
<td>0-6.8 (Zoli et al., 2003)</td>
<td>ND</td>
</tr>
<tr>
<td>Benin</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Burkina Faso</td>
<td>0-48.2 (Ganaba et al., 2011)</td>
<td>ND</td>
</tr>
<tr>
<td>Burundi</td>
<td>ND</td>
<td>26.1(23.2-28.9)*** (Prado-Jean et al., 2007)</td>
</tr>
<tr>
<td>Cameroon</td>
<td>26.6(15.6-31.0) (Assana et al., 2010)</td>
<td>0.4-3* (Nguekam et al., 2003)</td>
</tr>
<tr>
<td>Central African Republic</td>
<td>ND</td>
<td>2.9(0.5-5.31)*** (Druet-Cabanac et al., 1999)</td>
</tr>
<tr>
<td>Chad</td>
<td>40.8(32.2-49.4) (Assana et al., 2001)</td>
<td>ND</td>
</tr>
<tr>
<td>D.R. Congo</td>
<td>38.4-41.2% (Praet et al., 2010)</td>
<td>21.6(18.3-25.0) (Kanobana et al., 2011)</td>
</tr>
<tr>
<td>Cote d’Ivoire</td>
<td>2.5 Meat inspection* (Zoli et al., 2003)</td>
<td>reported (Heroin et al., 1972)</td>
</tr>
<tr>
<td>Equatorial Guinea</td>
<td>ND</td>
<td>reported (Díaz-Menéndez et al., 2012)</td>
</tr>
<tr>
<td>Gabon</td>
<td>ND</td>
<td>Reported (Okome-Nkoumou et al., 2010)</td>
</tr>
<tr>
<td>Gambia</td>
<td>4.8(3.46-5.5) (Secka et al., 2010)</td>
<td>1.74(0.72-2.8)*** (Secka et al., 2010)</td>
</tr>
<tr>
<td>Ghana</td>
<td>11.7(5.7-19.83) (Permin et al., 1999)</td>
<td>Reported* (Zoli et al., 2003)</td>
</tr>
<tr>
<td>Kenya</td>
<td>32.8(26.8-39.2) (Eshitera et al., 2012)</td>
<td>Reported* (Phiri et al., 2003)</td>
</tr>
<tr>
<td>Madagascar</td>
<td>Reported (Vega et al., 2003)</td>
<td>18* (Geerts et al., 2002)</td>
</tr>
<tr>
<td>Mozambique</td>
<td>34.90(22.166.7) (Pondja et al., 2010)</td>
<td>12.1(9.2-15)***** (Vilhena et al., 1999)</td>
</tr>
<tr>
<td>Nigeria</td>
<td>14.4(8.1-20.7) (Gweba et al., 2010)</td>
<td>ND</td>
</tr>
<tr>
<td>Rwanda</td>
<td>10-30%* (Zoli et al., 2003)</td>
<td>7* (Zoli et al., 2003)</td>
</tr>
<tr>
<td>Senegal</td>
<td>6.4-13.2% (Secka et al., 2010)</td>
<td>11.9(8.9-15.4) (Secka et al., 2011)</td>
</tr>
<tr>
<td>South Africa</td>
<td>56.7(40.6-76.3) (Krecek et al., 2011)</td>
<td>5.5(4.3-6.7) (Shasha et al., 1991)</td>
</tr>
<tr>
<td>Tanzania</td>
<td>17.40(12.5-22.3) (Ngowi et al., 2004)</td>
<td>16.3(13.2-19.4) (Mwangonde et al., 2012)</td>
</tr>
<tr>
<td>Togo</td>
<td>17* (Zoli et al., 2003)</td>
<td>3.8(2.8-4.8) (Balogou et al., 2000)</td>
</tr>
<tr>
<td>Uganda</td>
<td>8.5 (6-11) (Waiswa et al., 2009)</td>
<td>ND</td>
</tr>
<tr>
<td>Zambia</td>
<td>16.9-30.0% (Sikasunge et al., 2008)</td>
<td>5.8(4.17.5) (Mwape et al., 2012)</td>
</tr>
<tr>
<td>Zimbabwe</td>
<td>28.6% (Phiri et al., 2003)</td>
<td>Reported* (Phiri et al., 2003)</td>
</tr>
</tbody>
</table>

Table 1.2: Prevalence of Cysticercosis in sub-Saharan Africa

*aAg-ELISA, bBayesian estimate, cMeat inspection, dLingual palpation, eEITB&Ag-ELISA, fWestern Blot(Ab), gAb-ELISA &EITB
hAutopsy iundisclosed, *Review **case-control study (combined prevalence in cases and non-neurological controls) ***Epileptics
****hospital study
1.2.6 *Taenia solium* & Epilepsy in sub-Saharan Africa

Epileptic seizures appear to be the most common manifestation of *T. solium* NCC (Carabin *et al.*, 2011; Quet *et al.*, 2009). A meta-analysis of 21 papers on the clinical manifestations of NCC, found that 78.8% (95% C.I. 65.1-89.7) of patients of all ages with NCC, confirmed by neuroimaging or autopsy, presented with seizures (Carabin *et al.*, 2011). In order, however, to determine the true burden of NCC in the population in terms of epilepsy, it is necessary to understand what proportion of epileptic cases are likely to be attributable to NCC. A Meta-analysis of 12 papers from across the world gave a pooled estimate for the percentage of NCC among people with epilepsy of all ages of 29.0% (95% C.I. 22.93-35.5) using a random effects model (Ndimubanzi *et al.*, 2010). The estimate appeared to be consistent across age, gender and type of epilepsy, with no statistical difference between epileptics in a clinical or community setting (Ndimubanzi *et al.*, 2010).

Although the estimate of approximately 1/3rd of epilepsy being attributable to NCC appears consistent across endemic regions, it is worthwhile to note, however, that in specific communities this estimate may be an over or under estimation due to the clustered nature of the parasite. For example three villages studied in Burkino Faso showed highly divergent prevalence of cysticercosis (detected by Ag-ELISA) of 0%, 1.3% and 4.5%, thought to be due to the differing nature of pig keeping and pork consumption in the villages (Carabin *et al.*, 2009). It would therefore be highly unlikely that the same proportion of people with epilepsy could be attributed to NCC in each of these three villages, although on a national scale the estimate may stand.

A study in The Gambia also refuted the claim that 1/3rd of epilepsia cases are due to NCC, finding a non-significant Odds Ratio of 0.75 (95% C.I. 0.13-3.15) between epilepsy and cysticercosis infection as detected by Ag-ELISA (Secka *et al.*, 2010). I believe though, that the results of this study, in which only 3/210 cases and 6/420 controls underwent a CT scan, are not sufficient to warrant the blanket conclusion of the paper that “epilepsy is not caused by *T. solium* cysticercosis in The Gambia”, although it certainly seems not to be an important factor in this study population.

Understanding the burden of NCC-related epilepsy in individual countries requires knowledge of the prevalence of epilepsy in that country (Ndimubanzi *et al.*, 2010). Valid epidemiological data on epilepsy in sub-Saharan Africa is scare, but epilepsy is thought to be two to three times more common than in industrialised countries (Preux and Druet-Cabanac, 2005). The prevalence of epilepsy in sub-Saharan countries, as
ascertained by door-to-door surveys has been estimated as an average of 15 cases per 1000 people (Preux and Druet-Cabanac, 2005). Between countries there are variations in this estimate, with 5.2 (4658) cases per 1000 people in Ethiopia to 74.4 (4301049) per 1000 in Ivory Coast, although studies returning higher prevalence figures in general consisted of smaller number of participants, as reviewed by Preux et al. 2005. A recent review utilising this data, estimated that across the population of sub-Saharan Africa (total population approximately 850 million people) 0.76-2.46 million people maybe suffering from epilepsy attributable to NCC (Winkler, 2012).

1.2.7 The Burden of *Taenia solium* Cysticercosis

In order to guide health policy in a world where resources are finite, some aspect of prioritisation, of diseases and of patients, must occur. To allow this to happen, in 1990 a new metric, the disability adjusted life year (DALY) was introduced by the Global Burden of Disease (GBD) study. This study was commissioned by the World Bank to standardise the calculation of burden that any disease imposes upon the global community in terms of both mortality and morbidity (Murray and Acharya, 1997).

The DALY incorporates years of life lost (YLL) and the years of life lived with a disability (YLD). YYLs are calculated as the number of deaths at each age multiplied by a standard life expectancy at that age (Murray et al., 2013). The YLDs utilise the number of cases at a given age of onset, the duration of disease at that age of onset and incorporates a disability weight (DW) that ranges from 0 (full health) to 1 (dead) reflecting the severity of the disability caused (Mathers et al., 2007).

The 1990 GBD study was critcsed on several issues, some of which have been modified for the most recent study (Murray et al., 2013). Firstly, the age-weighting system, which valued lives lived by young-middle aged adults over and above those of the very young or very old (Barker and Green, 1996), was criticised for failing to take into account societal differences in the value of lives of differing ages and has now been omitted (Murray et al., 2013).

The disability weightings were also criticised in the 1990 study, in which they were determined by a panel of health care providers, for their inability to fully account for the burden of chronic debilitating diseases, especially within a developing country context (King and Bertino, 2008). It was thought that, for those neglected tropical diseases which were included in the study, that these issues, along with poor epidemiological data on which to base the calculations, led to an underestimation of the DALYs (Engels and
1.2. BACKGROUND

Savioli, 2006). The disability weightings have now been determined through surveying 31000 members of the public in 5 countries (USA, Peru, Tanzania, Bangladesh, and Indonesia) and an open internet survey. It is hoped that this may have alleviated some of the criticisms of the previous system (Murray et al., 2013).

The GBD 2010 has recently published its results, with, for the first time, a DALY estimation for cysticercosis. The total DALYs lost globally for all causes were calculated to be 2,490 million (95% C.I. 2,349-2,638 million) for all ages, or 36,145 DALYs/100,000 people. The global burden of cysticercosis in terms of the DALY was calculated to be 503,000 (95% C.I. 379,000-663,000) globally or 7/100,000 people, a 2.1% reduction from the (newly estimated) DALYs lost in 1990 (Murray et al., 2013).

Putting this figure into context, the global combined burden of those diseases categorised as the ‘neglected tropical diseases’ was calculated to be 108,739,000 (95% C.I. 87.8 – 137.6 million) DALYs and the annual global burden of cysticercosis is similar to that of Human African Trypanosomiasis (95% C.I. 560,000 (95% C.I. 76,000-1.8 million) DALYs) and Onchocerciasis (494,000 (95% C.I. 360,000-656,000) DALYs). The ‘big three’ communicable diseases of HIV/AIDS, Malaria and TB were estimated to account for 81,547,000 (95% C.I. 75-88 million), 28,685,000 (95% C.I. 63.4-109.8 million) and 49,396,000 (95% C.I. 40.156.1 million) DALYs lost globally respectively.

Regional breakdowns for the DALY estimates indicated the focal nature of the neglected tropical diseases, with rates of this group of diseases varying across regions by 961 fold (Murray et al., 2013). The regional figures for DALYs lost/100,000 due to cysticercosis in sub-Saharan Africa are not yet published, but will no doubt be higher than the global estimate per 100,000 people.

The global burden of diseases as calculated by the DALY is a useful tool, but there is still much discussion over the correct calculation of this metric. One indicator of the likely under-estimation of the DALYs lost through cysticercosis, is to look at the symptomatic category of epilepsy. Epilepsy, attributable to all causes, is estimated as losing 253 (95% C.I. 205308) DALYs/100,000 people globally and was ranked as the 14th, 19th & 24th leading cause of DALYs lost in western, southern & eastern and northern sub-Saharan Africa respectively, in comparison to a ranking of 55th and 52nd in Australia and high-income North America (Murray et al., 2013).

If the estimation of 1/3rd of epilepsy in cysticercosis endemic countries is correct (Ndimubanzi et al., 2010) this could indicate that cysticercosis may be responsible for 84 DALYs lost/100,000 people, and potentially more due to the higher importance
of epilepsy in sub-Saharan counties.

It is interesting to note that this estimate is still tenfold lower than one of the first DALY calculations performed for cysticercosis on a single country basis. The DALYs lost due to *T. solium* in Cameroon were found to be 9.0 per 1000 persons, utilising the 1990 GBD disability weightings (Praet *et al.*, 2009). The economic cost was estimated as 10,255,202 euro (95% C.I. 6,889,048-14,754,044) per year (Praet *et al.*, 2009), illustrating the importance of this disease in Cameroon.

This single-country DALY estimate indicates the potential risk that global DALY scores for diseases with focal distributions may continue to result in their neglect and under-allocation of resources for control.

1.2.8 Pig Production in western Kenya

Demand for pork is rising dramatically, with global pork production increasing 350% between the 1960’s and 2000, with much of this increase occurring in the less developed countries (Delgado *et al.*, 2000). In Kenya, pig production has also been steadily rising with a 9.3% annual increase in the number of pigs kept in the country in the years 1990-2000 (FAO, 2005). Pigs play a valuable role in poverty alleviation, important in a highly deprived region such as western Kenya, where up to 64% of the population live under the rural poverty line (Kristjanson *et al.*, 2004). Pigs are low on the “livestock ladder” (Kristjanson *et al.*, 2004) being cheap to purchase and therefore accessible to all but the poorest households. Pigs provide not only an income from the sales of meat or piglets, but act as a secure financial instrument, with the ability to sell and release the capital at times of unforeseen financial need (Lekule and Kyvsgaard, 2003).

Pig keeping in western Kenya generally occurs as a small-holder industry relying on family labor, with between 2 and 10 pigs per farm (Kagira *et al.*, 2010) the majority of which are kept under a free-range system (Githgia *et al.*, 2006; Mutua *et al.*, 2007), see Figure 1.3 on the next page. These are generally low-input systems, relying on cheap, locally available foodstuffs, such as ground maize (Ugali) and household waste to feed the pigs (Mutua *et al.*, 2012). The rations fed rarely fulfill the nutritional requirements of growing pigs, the pigs achieving average weights of only 30kg and 42kg at 6-10 and 11-24 months (Mutua *et al.*, 2011) with the majority of pigs being slaughtered at more than 9 months of age (Kagira *et al.*, 2010).

One of the key constraints cited by pig farmers in Busia district was the high cost
Figure 1.3: Free-range Pigs are Regularly Observed in Western Kenya
and unavailability of feed for their pigs (Mutua et al., 2012). Farmers therefore utilise
the scavenging nature of pigs to supplement any rations they do provide (Lekule and
Kvysggaard, 2003). This free-roaming behavior does of course come with the risk of
infection with *T. solium* as discussed in Section 1.2.5 on page 16 (Assana et al., 2010;
Pouedet et al., 2002; Sikasunge et al., 2007; Widdowson et al., 2000), as well as a variety
of other production limiting or zoonotic diseases such as: African Swine Fever (ASF)
(Jori and Bastos, 2009), Trichinellosis (Schuppers et al., 2010), Toxoplasmosis (Kijlstra
et al., 2004) and Non-zoonotic helminths (Kagira et al., 2012). Chapter 6 on page 141
discusses the spatial ecology of free-ranging pigs within our study area of western Kenya.

The pigs raised in this way are sold into the local marketing channels for pork, either
directly to butchers or via traders who travel from farm to farm purchasing pigs (Kagira
et al., 2010). Official inspection of these pigs is required by law at the slaughter facility
(Government of Kenya, 2012), however, the ad-hoc arrangement of the marketing chain,
in conjunction with an understaffed meat inspectorate, means that some pork enters the
foodchain without inspection or with a sub-standard inspection (Kagira et al., 2010).
This puts the population at risk from food-borne diseases, including *T. solium*.

Although pig production is relatively new in western Kenya, with the majority of
farmers in Busia district having kept pigs only for an average of 6 years (Mutua et al.,
2011), it is increasingly popular, with pigs accounting for 41.4% of all livestock in
Busia district at the time of a study by Kagira (Kagira et al., 2010). The number
of livestock kept, excluding sheep, declined dramatically directly after the 2008 post
election violence experienced in the region (Dewey et al., 2011). This decline was
experienced strongly across all types of pig (sows, boars, weaners and piglets) with
the 41% decline in sow numbers from 2007 (Dewey et al., 2011) especially likely to
have a long term impact on the supply of replacement pigs to these farmers, as 95% of
farmers buy their replacement stock from other local farmers (Kagira et al., 2010). Low
numbers of pigs being produced has lead to demand outstripping supply, encouraging
farmers to sell pigs which have not yet reached an ideal slaughterweight, leading to
poor profits for both farmers and butchers (Dewey et al., 2011).

The pig industry in western Kenya has been further damaged by ASF outbreaks, most
recently in 2011 (OIE, 2011). Outbreaks such as this are associated with high mortality
of pigs, leading again to scarcity of good quality slaughter pigs, and at such time farmers
may try to sell pigs which they fear to be diseased, with 19% of butchers in Busia district
having been approached to buy pigs during an outbreak (Kagira et al., 2010). Assisting
the pig industry in western Kenya to become viable and to produce a constant supply of
safe pork to consumers, will require the help and support of the veterinary authorities and non-governmental organisations through the provision of suitable extension services (Kagira et al., 2010; Mutua et al., 2011, 2012, 2011).

1.2.9 Control Options for Taeniasis and Cysticercosis

From the biology and epidemiology of this parasite as currently understood several control options have been proposed, centering around either the prevention or treatment of cysticercosis in the porcine host, prevention of infected intermediate hosts entering the food chain, or prevention or treatment of cysticercosis and taeniasis infection in the human host.

Confinement of pigs

Confinement of pigs prevents the pig from accessing human faecal material (as long as defecation is not practiced within the confinement area) and therefore disrupts the transmission of \( T. solium \). Confinement has the additional benefit of providing protection to the pigs against environmental stress (Lekule and Kyvsgaard, 2003), injury and theft, reduces the burden of other, production limiting, parasites (Kagira et al., 2012) and reduces the transmission of infectious diseases, such as African Swine Fever (Jori and Bastos, 2009). The major hindrance to widespread adoption of confined pig rearing is the requirement from the farmer to provide a shelter, the lack of which may be a manifestation of poverty (Kagira et al., 2010) and to provide all feed for the pigs, as opposed to allowing the pig to scavenge for the majority of it’s nutritional intake (Lekule and Kyvsgaard, 2003; Lightowlers, 2010a). Confinement of pigs is discussed in more detail in Chapter 6 on page 141.

Improved Sanitation

Improving sanitation would reduce the environmental contamination with \( T. solium \) eggs and would also reduces the burden of diarrheal diseases, which cause substantial mortality and morbidity in children every day (Brooks et al., 2006), and the eye infection Trachoma, which is strongly associated with poor sanitation (Ngondi et al., 2010). Sanitation improvement, however, requires solutions which are appropriate to the situation, ideally constructed with locally available materials and require a whole community to participate and therefore can be a difficult process.
CHAPTER 1. INTRODUCTION

An example of successful latrine promotion occurred in Ethiopia, where extensive training in latrine construction was provided to communities, but no cash or materials were provided. This campaign achieved a 32.3% (95% C.I. 27.938.0) overall increase in the proportion of houses with a pit latrine 3 years after the intervention (Ngondi et al., 2010). Poverty is however, still a barrier to latrine adoption, with those households building latrines tending to be the ‘richest of the poor’, although education is key to encourage latrine use, with households receiving education being 60% more likely to have built a latrine, even when household size and wealth is controlled for (Ngondi et al., 2010).

The effect on such sanitation programs has not as yet though been evaluated on T. solium control. Though in the model produced by Kyvsgaard et al., 2007 interventions which interrupt the human-pig transmission cycle, such as use of latrines or pig confinement were the most efficacious of the three control packages modeled, with large reduction in human and pig incidence which occur slowly, but were sustained over the 120 months for which the model was run.

Pig Vaccination & Treatment

Inducing immunity to infection through vaccination has been attempted with a variety of antigens including; total antigen from Taenia crassiceps metacestodes (Huerta et al., 2000; Sciutto et al., 1995), synthetic peptides GK1 and KETc12 (Huerta et al., 2002) and recombinant proteins, including the recombinant peptide-phage vaccine S3Pvac-Phage (Morales et al., 2008). The most successful vaccine produced to date was the recombinant protein TSOL18 which has achieved complete protection of vaccinated pigs in a field trial in Cameroon (Assana et al., 2010).

The complete protection achieved by TSOL18 in this study was achieved with a protocol of initial vaccination at 2-3 months, repeat vaccination 4 weeks later with concurrent treatment with oxfenbendazole (30mg/kg) to kill any cysticerci developed prior to the onset of vaccination, and a third vaccination at approximately 6 months, 3 months after the second (Assana et al., 2010). A dose of 30mg/kg oxfenbendazole has been found to be efficacious in treating porcine cysticercosis, with no viable cysts found on necropsy 8-10 weeks after treatment (Gonzalez et al., 1997). The treatment also leaves pigs refractory to re-infection for up to 4 months (Gonzalez et al., 2003), potentially due to the immunity induced by the parasite (Lightowlers, 2010a).

Using oxfenbendazole alone in a mass treatment campaign however, seems not to be an
effective route of controlling *T. solium* as the majority of pigs treated will not be infected with *T. solium* and will not therefore have immunity to the parasite and be susceptible to new infections (Lightowlers, 2010a). The ability to provide oxfenbendazole treatment close to the time of slaughter is limited by the requirement for a withholding time (as yet to be determined) (Lightowlers, 2010a) and the necrotic lesions left by the killed cysticerci, which take approximately 3 months to resolve (Gonzalez *et al.*, 1998).

The protocol combining the TSOL18 vaccination with oxfenbendazole treatment seems to offer outstanding protection and overcomes many of the difficulties associated with the each of the interventions alone (Lightowlers, 2010a). The necessity for three vaccinations and an anthelmentic treatment, however, increases costs to the farmer and may affect feasibility (Lightowlers, 2010a). The cost effectiveness of this intervention therefore needs careful consideration.

*Improvements in Meat Inspection*

If the cost of control strategies, be that construction of pig pens, or provision of a vaccine, are to be borne by the farmer, there needs to be a financial incentive for the farmer to present 'clean' pigs to slaughter. Currently there is evidence of pigs entering the food chain without inspection, for example in Kenya (Kagira *et al.*, 2010) and Uganda (Nsadha *et al.*, 2010), allowing infected pigs to be sold, possibly with little financial loss to the farmer. The system of official meat inspection and rigorous condemnation of meat needs to be fully functioning in order that farmers are disincentivised from presenting infected pigs for slaughter. If infected pigs are condemned, it has been indicated that a health education intervention encouraging the use of pig confinement, would be financially beneficial to farmers (Ngowi *et al.*, 2007).

*Chemotherapeutic Interventions in Humans*

Interventions focusing on the human host include targeted and mass chemotherapeutic administration with Praziquantel and public health education programs. There have been successes with Mass chemotherapeutic interventions, with a campaign in Ecuador showing a reduction in porcine cysticercosis from 11.4% to 2.6% one year after intervention (Cruz *et al.*, 1989) and a Mexico intervention showing a 75% reduction in anti-cysticercus antibodies 2 years post intervention (Sarti *et al.*, 2000).

However, there is a potential that mass treatment in areas with poor latrine provision
may result in wider dissemination of *T. solium* eggs, in turn increasing transmission to pigs, this is thought to have been the case after a Mexican intervention trial when the prevalence of porcine cysticercosis increased from 6.6% to 11% in the year after treatment (Gonzalez et al., 2003). It is also noted that a risk exists of inducing active neurocysticercosis in occult infections due to the cysticidal nature of the drug (Wood et al., 1996) as has occurred in an intervention in Mexico (Sarti et al., 2000). A modified reed-frost stochastic model developed for the transmission of *T. solium* found mass treatment of the human population alone to only have a short-term effect in reducing Taeniasis and porcine cysticercosis prevalence, which then quickly revert to pre-treatment levels (Kyvsgaard et al., 2007).

Targeted use of chemotherapy in humans is, however the preferred control option for many scientists working on this parasite (Pawlowski, 2008). A strategy has been proposed whereby the existing health systems are used to identify and treat *Taenia* carriers (confirmed or suspected), any person in contact with a confirmed cysticercosis case and those people in contact with late onset epilepsy in combination with mass treatment in targeted groups such as slaughter workers (Pawlowski, 2008). In addition to these categories of people, a demonstration of the clustering of *Taenia* carriers around heavily infected pigs suggests that rapid screening of pig populations by lingual palpation may identify areas suitable for drug administration (O’Neal et al., 2012).

*Public Health Education*

Public health education initiatives are an important aspect of control (Pawlowski, 2008) and have shown some success in reducing the disease burden in pigs. One intervention in Mexico reducing the prevalence in pigs (based on Ab-ELISA) from 5.2% to 1.2% a year after the intervention (Sarti et al., 1997) and a study in Tanzania showing a reduction in incidence when pigs were followed up 10-12 months after the study (Ngowi et al., 2008). In both studies knowledge of the parasite increased, but changes to observed risk behaviors did not match the increased knowledge obtained (Ngowi et al., 2008; Sarti et al., 1997).

Unfortunately, one potential negative impact of the intervention in Tanzania was that selling of infected meat from the control group actually increased, thereby putting the non-intervention group at increased risk (Ngowi et al., 2008). Eliciting behavior change through health education may be constrained by resource scarcity, and it may therefore be that assistance with infrastructural development is required alongside education.
Health education can be an important component of a control strategy due to the wider societal benefits which may be derived from it, the effectiveness of education alone, however in the control of \textit{T. solium} is unclear (Lightowlers, 2010a).

With so many control options available for this parasite, validated and cost-effective control strategies, which may contain a variety of these options, is a priority for the global community and it is pledged that such a strategy should be ready by 2015 (World Health Organization, 2012a). A combined strategy of three rounds of mass treatment with niclosamide in humans, five rounds of oxfenbendazole treatments in pigs and vaccination of pigs with TSOL18 has already been scaled up to a population of approximately 80,000 in Northern Peru which has thus far sustained focal elimination for over one year (Gilman \textit{et al.}, 2012). The strategies required in different geographical locations may vary depending on local conditions, but these are promising results which will hopefully soon be replicated across the globe.

1.3 Thesis Outline

The overarching theme of this thesis is to better understand the epidemiological status of \textit{T. solium} in western Kenya. I do this through 7 chapters, as described below:

**Chapter 2 on page 33.** Describes the design of the two main studies which provided the data utilised in this thesis. These studies were conducted in western Kenya between January 2010 and July 2012 and were conducted under the auspices of the 'People, Animals and their Zoonoses' (PAZ) project. As Chapters 3 to 6 have been prepared as manuscripts there is necessarily some repetition in the methodology sections of each chapter.

**Chapter 3 on page 51.** Quantifies the risk to humans of acquiring a \textit{T. solium} cysticercosis infection from pork butchered and sold in western Kenya using a stochastic risk analysis model. The model was parameterised with data obtained during my field work and from the literature when the required data were not available. This chapter illustrates the high risk of a person acquiring a taeniasis infection from consuming pork prepared in western Kenya and investigated the efficacy and cost-effectiveness of a selection of control strategies.

**Chapter 4 on page 87.** Determines the prevalence of \textit{T. solium} Taeniasis and cysticercosis infections in the human population of western Kenya and of \textit{T. solium} cys-
ticercosis in the pigs owned by study participants. This chapter also establishes the spatial epidemiology of these infections and the risk factors associated with their acquisition.

Chapter 5 on page 121. Investigates the performance of a novel, user-friendly, rapid diagnostic test for *T. solium* cysticercosis infections. The novel diagnostic was produced as a collaborative venture between the University of Edinburgh and the International Livestock Research Institute in response to a need for diagnostic assays which can be easily and cheaply deployed in under-resourced areas. This chapter also obtains Bayesian estimates of the prevalence of *T. solium* in two porcine populations (western Kenya and central Uganda) taking into account the test parameters used to make the diagnoses. The chapter demonstrates the endemic status of *T. solium* cysticercosis in the two populations studied and the potential utility of the novel diagnostic test.

Chapter 6 on page 141. Determines the homerange of a free-ranging pig in western Kenya and the time these pigs spend interacting with environmental features. This study was designed to increase our understanding of how the husbandry of pigs may affect their risk of becoming infected by *T. solium* and other zoonotic and non-zoonotic pathogens.

Chapter 7 on page 165. This chapter summarises the main findings of the data chapters and discusses the relevance of these findings to the ongoing global challenge to control this parasite and the research agenda now required.
Chapter 2

Study Design

Elements of this chapter have been submitted to BMC Infectious Diseases as:  Fèvre, E.M., Thomas, L.F., de Glanville, W.A., Cook, E.A.J., Kariuki, S., Wamae, N.C., A framework for the study of zoonotic diseases of livestock and their keepers in the Lake Victoria Crescent small-holder crop-livestock production system.
This PhD forms part of a larger project which investigated a range of zoonotic diseases within livestock and their keepers in western Kenya; the 'PAZ' Project (People, Animals and their Zoonoses). The PAZ study is a cross-sectional study of cattle, goats, pigs and humans, in the Western and Nyanza provinces of Kenya funded by the Wellcome Trust and is a collaboration between the University of Edinburgh, the International Livestock Research Institute (ILRI) and the Kenya Medical Research Institute (KEMRI). This chapter discusses the overall study design on which this thesis is based, the analytical techniques used for each data chapter are discussed within the relevant chapters.

2.1 Ethical Approval

Ethical approval for aspects of the study pertaining to humans was granted in March 2010 and reviewed annually by the ethical review board of KEMRI (SC1701) and all activities were undertaken in accordance with the approved protocols. Animal sampling was carried out under the umbrella approval of ILRI by trained veterinarians or animal health assistants in accordance with best practice. The ethical permission for this study allowed the sampling of any consenting person over 5 years of age, though in order to reduce the risks to any participants, women in the third trimester of pregnancy were excluded from the study, as were any participants whom the medical team deemed at risk of harm from venapuncture, e.g. due to extreme anemia. If a participant was excluded from sampling for health reasons the medical team referred this participant to a local health centre. Once entered into the study every participant was identified by a unique identifying number and never by name, hence ensuring anonymity of all data.

Prior to any data collection each human participant in the study was required to sign, or mark with a thumb print, an informed consent document (ICD) as included in Appendix .1 on page 178. The steps before signing the ICD involved ascertaining the appropriate language for communication, an explanation of the project, the sampling procedure and emphasising that participation is entirely voluntary. If the individual was happy to participate they signed or marked the ICD, of which one copy was retained by the project and one copy was provided to the participant. 46 individuals refused to participate in the project, with 16 individuals citing fear of giving blood as a reason for non-participation and 30 not disclosing their reasons for non-participation.
2.2 Study Area

The studies on which this thesis is based were located in the Western and Nyanza provinces of Kenya. This area, part of the Lake Victoria Crescent ecosystem, is bordered by Uganda to the west, Lake Victoria to the south, Mount Elgon to the north and Rift Valley Province to the east. The area is occupied predominately by members of the Luo, Luhya and Teso tribes. The area has biannual rains, occurring in March-May and August-October and supports a predominately mixed crop-livestock production system with an average farm size of 0.5ha (Kristjanson et al., 2004). The region is one of the most deprived in Kenya, with as much as 70% of the population of Busia district in Western Province falling below the absolute poverty line, which is based upon “local costs of a basket containing minimum food (calories per adult equivalent) and non-food requirements” (Thornton et al., 2002). Demographic and health surveys carried out within Western and Nyanza province have found high HIV/AIDS prevalence, of 25-28% within Western and Nyanza provinces (Kristjanson et al., 2004). This site was chosen as being representative of the Lake Victoria crescent zone, characterised by a high human population density and large number of small-scale, mixed crop-livestock units.

2.3 Cross-Sectional Study of Homesteads

The study site for the cross-sectional study of homesteads, covered approximately 9,000sq Km of land, encompassing twenty four divisions within the Western and Nyanza Provinces, which fell within thirteen administrative districts. The logistical constraints of the project limited the study site to a 45km radius from a previously established laboratory in Buisa Town. Every Kenyan sublocation of which at least 50% of the landmass fell within this radius was included in the study.

The sampling technique used for the cross-sectional survey of homesteads was a stratified cluster sample, whereby the primary unit of sampling is the sublocation and secondary unit of sampling being the homestead, encompassing all eligible members (human, porcine, caprine and bovine) within it. The sampling technique involved taking a random sample of homesteads from each sublocation in the study area, stratified according to cattle population density.

The sample frame for this study was derived from information on livestock population density for 2009 obtained from the Divisional livestock production office (DPLO). The
CHAPTER 2. STUDY DESIGN

The data obtained was at the divisional level, as per the 2000 administrative boundaries, and were estimations based upon a 2005 livestock census inflated by 10% per year. The total study site has a livestock population of approximately 557,418 cattle and 68,484 pigs according to the 2009 data from the DPLO. Data from the comprehensive Kenyan Human Population Census of 2009, which include livestock numbers were not available at the design stage of this project.

The sample size was powered to account for the lowest expected prevalence in the study, being 2% for *Coxiella burnetti*, the etiological agent for Q fever in cattle, with a maximum error of 5%. The design effect, to account for the likely increase in standard error due to clustering was set to 5. The human, caprine and porcine sample size were incidental to the cattle sample size of 2300, which were expected to be recruited from 412 homesteads.

A two-stage cluster design was used, with the primary sampling unit being the sublocation, the smallest administrative unit in Kenya. All 164 sublocations which fell >50% within the study area were included, and within each sublocation the secondary sampling unit, the homestead, were chosen via a random sampling method. An assumption was made of equal distribution of cattle between sublocations across a division and therefore the proportion of homesteads to be sampled in each sublocation was allocated on the basis of the proportion of cattle found in each.

Using the ILRI geographical information systems (GIS) unit (http://www.ilri.org/gis/) coverages for Kenya, each sublocation was mapped using ArcMap\textsuperscript{TM} version 9.1 and the extension Hawths Tools (Beyer, 2004) was used to generate a master and back-up set of random points within the polygon representing each sublocation. The co-ordinates of each point were entered into a Garmin eTrex\textsuperscript{©} hand held geographical positioning system (GPS) unit via DNR Garmin 5.4.1 extension for ArcView (Minnesota Department for Natural Resources, 2008).

In the field we located each position indicated by the GPS unit and identified the nearest homestead to this position. If more than one homesteads fell equally close to the point then priority was given in the order north-east-south-west. If no homestead fell within 300m of a point, or the homestead refused to participate then that location was discarded and the nearest point from the back-up set was located in replacement. The location of each of the recruited homesteads in the study site is illustrated in figure 2.1 on the facing page.

Each homestead identified this way was visited, with the chief or assistant chief for the
Figure 2.1: Map of PAZ Study Site with the Location of all Recruited Homesteads and Indicating the Location of the Study Site within Kenya
This map was produced using ArcMap\textsuperscript{TM} version 9.1 with geographical data provided by ILRI GIS unit [http://www.ilri.org/gis/](http://www.ilri.org/gis/) and overlaid with the location of homesteads collected in the field using a hand held Garmin\textregistered eTrex GPS unit.
sublocation, the aims and objectives of the study were explained and the consent to participate obtained from the head of the homestead. An appointment was then made for the team to visit for data collection the following week and stool collection pots were left with the homestead to be collected on the day of sampling.

2.3.1 Data Collection for Homestead Survey

Questionnaires were developed for use in this study, each of which were pre-tested extensively before being deployed in the field. Pre-testing was performed first within the research team, then amongst members of the study population from a variety of locations. The questionnaires were written in English and translated into local languages at the point of administration, by the animal health technicians and medical officers administering the questionnaires. Pre-testing of the questionnaires was carried out within the study site in a range of local languages. The questionnaires were programmed for use on Palm Operating System (Palm OS) Personal digital assistant (PDA) using Pendragon Forms 5.1 (Pendragon Software Corporation, Libertyville, IL) for direct entry of answers into a Palm unit. Careful training in the administration of questionnaires and use of the palm units was provided to the field team during the pre-test period. Figure 2.2 on the next page shows one of the Palm units being deployed in the field to scan in a unique ID bar code.

A general household questionnaire was administered to the head of the household, individual questionnaires covering individual risk factors and health status were administered to every person participating in the study and individual animal questionnaires relating to each pig sampled were administered to the person with chief responsibility for the animals. During the administration of the homestead questionnaire geographical co-ordinates for each homestead sampled were gathered using a handled Garmin® eTrex GPS unit. Direct observation was used to record the presence of a latrine on a homestead, the nature of the latrine (open pit/ partially or fully enclosed) and evidence of its use and of scavenging by animals, was verified by physical examination of the latrine where smell, pathways leading to the door, animal footprints etc were used to verify the status.

A concise clinical exam was undertaken by either a veterinarian (Lian Thomas or William deGlave), or a trained animal health assistant, for every animal in the study and each human participant was examined by a clinical officer or community health worker. All clinical examinations were standardised according to the protocol
2.3. CROSS-SECTIONAL STUDY OF HOMESTEADS

Clinical examination of pigs was undertaken with the pigs restrained using a pig snare around the snout, the pigs were observed for abnormal demeanor, skin and hair coat condition, body condition score and the presence of ectoparasites. A lingual examination was conducted by inserting a wooden stick into the mouth and withdrawing the tongue using a swab. The ventral side of the tongue was palpated for the presence or absence of cysticerci as seen in Figure 2.3 on the following page after which blood samples were taken.

Human participants were examined for signs of anemia and jaundice and organomegaly, had their height, weight and upper arm circumference measured and blood samples were then taken from consenting participants. For children under 12 years of age the legal guardian, preferably the mother, was asked to sit with the participant during administration of the questionnaire in order to either provide clarification, or in the case of the youngest children, to provide answers to the questions.
Biological Sample Collection

Cranial vena cava blood samples were obtained from pigs as shown in Figure 2.4 on the next page using BD Vacutainers® and 1.5” 18G needles. 24ml of blood were collected into two 10ml plain tubes and a 4ml EDTA tube. Marginal ear vein samples were collected from pigs using a blood lancet and haematocrit tube and thick and thin blood films were prepared in the field. Venous blood samples from human subjects were obtained using Crystalock™ tourniquets and BD Vacutainer® Safety-lok™ blood collection set 21G or 23G from the forearm vein as shown in Figure 2.4 on the facing page. A maximum of 25ml of blood were collected in plain, heperinised and Ethylenediaminetetraacetic acid (EDTA) test tubes and thick and thin blood smears were prepared from remaining venous blood in the collection set.

Stool samples were taken from each pig, by digital extraction. Stool samples were collected from all consenting human subjects in 30ml sample pots provided during the recruitment visit to the household. Instruction was given at the recruitment visit on hygienic procedures for stool sample collection. All biological samples were identified with a unique bar code which was scanned into the Palm OS PDA and were stored in a cool box on ice for transport to the laboratory in Busia. Figure 2.5 on the next page illustrates a typical set up for data collection in the field and the results of a productive day.
2.3. CROSS-SECTIONAL STUDY OF HOMESTEADS

Figure 2.4: Venous Blood Sample Collection from Pigs and Humans

Figure 2.5: A Typical Sampling Set-up and Samples Ready to Transport to the Laboratory
2.3.2 Data Management for Homestead Study

Each homestead, person and animal within the study was identified by a unique bar code to which every sample could be traced and from which the homestead, sublocation and division of each individual can be identified. No record was kept of peoples’ names to ensure that anonymity was maintained. Questionnaire and clinical data were entered directly onto Palm OS PDAs programmed using Pendragon Forms 5.1 and downloaded daily into Microsoft Office Access® 2007. The Access Database was backed up into a compressed zip file and this file was then backed up onto 3 different computers (in Busia, Nairobi and Edinburgh) and online using the ‘Drop Box’ file storage system.

2.4 Survey of Porcine Slaughter Facilities

A survey was undertaken between February and August 2010 at porcine slaughter facilities (referred to hereafter as “slabs”) with the aim to obtain information on the *T. solium* status of pigs entering the food chain in the study area, to compliment the data collected through the household cross-sectional study.

2.4.1 Study Population

The population investigated in this study are those pigs being slaughtered at government registered slaughter slabs found within our study divisions. The divisions included within this study area conform to the following eligibility criteria; fall at least 50% within a 45km radius of Busia, Western province, Kenya, the presence of at least one registered slaughter slab and have a pig population (as estimated by the DPLO) which comprised more than 2% of the total pig population in the ‘PAZ’ study area. These eligible divisions were; Amagoro, Amakura, Budalangi, Butula, Chakol, Funyula, Matayos, Nambale, Ugunja and Ukwala. The study site and location of slaughter facilities sampled are shown in Figure 2.6 on the facing page.

A sample size of 320 was calculated using the WinEpiscope 2.0 software (Thrusfield *et al.*, 2001) using an expected prevalence of 14% as was previously found in three divisions around Busia (Githgia *et al.*, 2006), with accuracy of +/− 5% and a confidence interval of 99%. The sampling was stratified by division proportionally to the percentage of the pig population expected to be found in each division. Slaughter slabs were identified by contacting the government meat inspector for that division and the day of
Figure 2.6: Map of study site showing pig population density and location of registered slaughter slabs
This map was produced using ArcMap\textsuperscript{TM} version 9.1 with geographical data provided by ILRI GIS unit \url{http://www.ilri.org/gis/} overlaid with 2009 pig population data obtained from the DPLO of each division and the location of registered slaughter slabs collected in the field using a hand held Garmin\textsuperscript{®} eTrex GPS unit.
highest through-put each week identified as the day to visit each facility for sampling. Every pig slaughtered on the day of sampling was included in the study and each slab was visited on repeated occasions until the sample quota for that division was filled. If more than one registered slaughter slab was identified in any one division each slab was sampled from, with priority given to those slabs with highest through-put. Examples of typical slaughter slabs found in the study area can be found in Figure 2.7.
2.4.2 Data Collection and Management for Slaughter Slab Survey

At each visit the following data were collected for every pig which was slaughtered that day: Division of origin of pig, name of owner (used to identify the pig), result of lingual palpation as described in Section 1.2.3 on page 9 and the official meat inspection result as provided to us by the meat inspector on duty that day. Jugular venous blood samples were obtained from pigs using BD Vaccutainers® and 1.5" 18G needles into two 10 ml plain tubes. The tubes were identified with a unique pig number and stored in a cool box on ice for transport to the laboratory.

The data set for this study was manually entered and stored in a Microsoft Office Access® 2007 database which was backed up onto 3 different computers (in Busia, Nairobi and Edinburgh) and online using the 'Drop Box' file storage system.

2.5 Laboratory analysis

Initial sample processing was performed at the Busia laboratory within 24hrs of collection. Separate laboratories were used for human and animal sample processing.

2.5.1 Processing of Porcine Samples

Porcine blood collected into the plain tubes was centrifuged at 3000 rpm for 20 minutes at room temperature and the sera was aliquoted into Nalgene® 2 ml cryovials with bar codes and stored immediately at $-40^\circ C$ for later serological analysis. Blood collected in EDTA was used for haematocrit determination by the Hemocue® method, manual packed cell volume (PCV) and total protein (TP) determination and inspection of the buffy coat for *Trypanosoma* spp. The remaining EDTA blood sample was stored at $-40^\circ C$. Thick and thin blood films were stained with giemsa stain in the laboratory and inspected by microscopy for the presence of haemoparasites. Fecal material was used for a fecal worm egg count carried out using the McMasters egg counting slide and the Kato-Katz technique (Ash *et al.*, 1994; Forrey and Forrey, 2001; Hansen and Perry, 1994). The processing of porcine samples is illustrated in a flow chart in Figure 2.8 on the next page.
2.5.2 Processing of Human samples

Human blood collected into the plain tubes was centrifuged at 3000 rpm for 20 minutes at room temperature and the sera used firstly for the Brucella IgG/IgM LFA (Smits et al., 2003) and the remainder aliquoted into Nalgene® 2ml cryovials with bar codes and stored immediately at $-40{}^\circ C$ for later serological assays. Blood collected in EDTA was used for haematocrit determination by the Hemocue® method, manual PCV and TP determination and inspection of the buffy coat for Trypanosoma spp. Thick and thin blood films were stained with giemsa stain in the laboratory and inspected by microscopy for the presence of haemoparasites.

Fecal material was analysed for detection of intestinal helminths and protozoa using a formal ether concentration method and the Kato-Katz technique, complemented by a Ziehl-Neelsen staining method for Cryptosporidium parvum. The techniques are described in the WHO bench aid manual for intestinal parasites 1994 and in “District laboratory Practices in Tropical Countries” (Cheesbrough, 2006).

After the initial processing of faecal samples the remainder was stored in 5% formal saline with 0.3% Tween®20 at room temperature for later analysis by copro-antigen
ELISA (Allan et al., 1996) for the detection of secretory antigens of *Taenia* spp.

### 2.5.3 Copro-Antigen ELISA for Taeniasis in Humans

Human faecal samples stored in 5% formal saline with 0.3% Tween®20 were analysed for the presence of *Taenia* spp. antigens as described by Allan et al., (Allan et al., 1996) and optimised by Pierre Dorny & Sarah Gabriel of ITM Belgium (per. comms.). Samples were prepared by mixing equal volumes of the faecal sample and Phosphate buffered saline (PBS), soaking for one hour with intermittent shaking. The samples were then centrifuged for 30 minutes at 2000g, and the faecal supernatant aliquoted for analysis.

The negative control panel consisted of 63 non-meat eating people from within the study site. All were negative for *Taenia* spp. eggs on microscopy, 26 of the negative controls had no GI parasites and of the remaining 37 samples all were positive for at least one GI parasite, including, *Schistosoma mansoni*, *Giardia* spp., *Ascaris* spp., *Entaemeba* spp., *Iodamoeba* spp., hookworm and *Strongiloides* spp. The positive control samples were collected from two self-reported *Taenia* spp. carriers who expelled an adult worm on treatment with Niclosamide (2g orally as a single dose), followed 2 hr later by a purgative dose of castor oil (4tsp), this procedure was carried out in controlled conditions under the supervision of a community health officer. Negative and positive control samples were prepared as above. Each sample was run in duplicate, with 2 negative controls, 2 positive controls and duplicate substrate and conjugate controls per plate to check for the validity of each plate run.

A flat bottomed Nunc Maxisorp® plate was coated for 1 hour at 37°C with 100μl IgG polyclonal (Supplied by ITM, Belgium) at 2.5μg/ml in 0.05M Carbonate-Bicarbonate coating buffer (Sigma-Aldrich, C3041), with 100μl coating buffer only in the substrate control. The plate was then washed once in PBS-0.05% Tween®20 before blocking for 1 hour at 37°C with PBS-0.05% Tween®20 with 2% heat inactivated New Born Calf Serum (Invitrogen, 16010-167). After blocking, the solution was emptied and the plate tapped to dry, following which 100μl of sample was added to each well with blocking buffer used in the substrate and conjugate control wells.

The samples were incubated for 1 hr at 37°C before washing 5x in PBS-0.05% Tween®20. Detection used 100μl of biotinilated polyclonal at 2.5μg/ml in blocking buffer with blocking buffer only in the substrate control wells. After 1 hr incubation at 37°C the plate was washed 5x in PBS-0.05% Tween®20. 100μl of streptavidin horseradish per-
oxidase (Jackson immunoresearch) at 1:10,000 dilution was added to each well and incubated for 1hr at 37 before a further 5x washes with PBS-0.05% Tween®20. 100μl of OPD (DAKO) was then added to each well and incubated for 15minutes at 30°C in the dark before the reaction was stopped with 50μl 0.5M H₂SO₄. The plate was then read with an ELISA plate reader (BioTek Synergy-HT) at 492 and 655nm.

The mean optical density (OD) of all negative samples (63) plus 3x standard deviation were used to calculate the cut off value (Somers et al., 2006) of OD = 0.874. Samples with mean OD values over the cut-off were considered to be positive. These calculations were applied automatically to the results in a Microsoft Excel® spreadsheet and the results of the assay for each samples were recorded in an Microsoft Access® database.

2.5.4 Serology for *T. solium* in humans and pigs

**HP10 Antigen ELISA**

Porcine and human sera samples, having been transported to the ILRI laboratories in Nairobi on dry ice and stored at -80, were analysed for the presence of circulating *Taenia* spp. antigens using the HP10 (Harrison et al., 1989) Ag-ELISA as discussed in Section 1.2.3 on page 7. The method used is described briefly here.

Five negative controls, obtained from an indoor farrow- finish unit in the UK, a country that is free from *T. solium*, were run on each plate along with two positive controls, obtained from experimental infections of cattle with *T.saginata* provided by Dr Leslie Harrison (University of Edinburgh) and five substrate controls.

Flat bottom Immulon® 4HB X ELISA plates were coated with 100μl 50% saturated (NH₄)₂SO₄ precipitate MAb HP10 at 10μg/ml diluted in carbonate-bicarbonate buffer 9.6 pH (Sigma C3041). After being coated overnight at 4°C the plate was washed twice in 0.9% NaCl with 0.05% Tween®20 using a manual plate washing technique and non-reacted binding sites blocked for one hour at room temperature using PBS 7.3 pH (Sigma P4417) with 1% Bovine serum albumin (BSA) (Sigma A4503) and 0.05% Tween®20 (Merck-Schuchardt) after which the plate was washed three times. 100μl of sera, diluted 1:1 with PBS/BSA/Tween®20, was added to each well, incubated for one hour at 37°C, washed three times as before, then MAb-HP10 conjugated with biotin at μg/ml in PBS/BSA/Tween®20 was added for 1hr at 37°C. After washing three times, 100μl per well of Streptavidin horseradish peroxidase (sigma S5512) conjugate at 1μl in PBS/BSA/Tween®20 was incubated for 1hour at 37°C, followed by a further three
2.5. **LABORATORY ANALYSIS**

washes before 3,3, 5,5- Tetramethylbenzidine substrate (Sigma T8665) was added for 15 minutes at room temperature covered with foil, the reaction stopped with 0.2M 2M H$_2$SO$_4$ and read in an ELISA plate reader (BioTek Synergy-HT) at 450nm.

A correction factor shown in equation (2.1) (Harrison *et al.*, 2005) was used to correct for plate-to-plate and day-to-day variations, this correction factor refers to the first plate of a screening run performed with the same negative and positive control sera.

\[
\text{Correction factor} = \frac{P_0 - N_0}{P_t - N_t}
\]  

where:

- $P_0$ = mean of positive control sera plate 1
- $N_0$ = mean of negative control sera plate 1
- $P_t$ = mean of positive control sera test plate
- $N_t$ = mean of negative control sera test plate

Applying the correction factor to every samples OD provides a standardisation of samples across the full run. Cut-off values were determined by using the mean corrected OD of all negative controls run during the full screen plus three standard deviations. Any sera sample with a corrected OD value over this cut off was counted as being positive.

**T. solium Antigen Capture Lateral Flow Assay**

A novel LFA for the detection of circulating *T. solium* antigen was performed as follows: after briefly vortexing, 40 µl of sera was pipetted onto the sample well of a LFA cassette and read after 15 minutes incubation at room temperature. If a control line only was seen the sample was considered to be negative, and if two lines (control and test) were seen the sample was considered to be positive. If no control line appeared the test was considered to be invalid and was repeated. The LFA prototype is shown in Figure 5.2 on page 126.

All serological testing was carried out on anonymous samples, in accordance with the ethical permission granted to us. Laboratory results were entered into a Microsoft Office Access® 2007 database, and later joined to the individual and homestead level data. Statistical analysis of is described in each relevant data chapter.
Chapter 3

Risk of Transmission of *Taenia solium* from Pork Consumed in Western Kenya

Following a positive pre-submission enquiry, this chapter has been submitted to *PLoS Neglected Tropical Diseases* as:

3.1 Abstract

3.1.1 Background

The tapeworm *Taenia solium* has been identified as a neglected zoonotic disease of public health importance (World Health Organization, 2007, 2010, 2012a; World Health Organization/DFID-AHP, 2005), with larval infection of the CNS, neurocysticercosis, being the attributable cause for approximately 1/3 of epilepsy cases in endemic areas (Ndimubanzi *et al.*, 2010). The global burden of cysticercosis is estimated to be 7 DALYs lost per 100,000 people, (Murray *et al.*, 2013), but within the developing countries this is likely to be a vast under-estimate, with 9DALYs lost per 1000 people being recently estimated in Cameroon (Praet *et al.*, 2009).

The consumption of undercooked infected pork meat perpetuates the parasitic life-cycle through the establishment of adult tapeworm infections in the community. Carriers of *T. solium* tapeworms are a risk to themselves (OR = 9.2 (1.2, 136.9)) (Praet *et al.*, 2009) and to others living in close proximity to them (Lescano *et al.*, 2009; ONeal *et al.*, 2012). It therefore must be a public health priority to reduce the risk associated with pork consumption in the developing world.

In order to develop mitigation strategies for this infection it is important to understand the risks associated with pork production, preparation and consumption as is currently practiced. Models provide one way of reducing a complex system to a series of parameters which we can then manipulate to simulate the effect of various mitigation strategies.

3.1.2 Methods and Findings

This paper discusses a quantitative food chain risk assessment, used to determine the risk posed by pork consumed in western Kenya in terms of *T. solium* infection. This model was informed using data from two field studies carried out in western Kenya, supplemented when needed by the available literature and expert opinion.

This model indicates that any one pork meal consumed in western Kenya has a probability of 0.061 (95% C.I. 0.039 0.081) for being infected with at least one *T. solium* cysticercus and therefore infectious to humans. This equates to approximately 810,000 potentially infective events occurring each year in a population of 3 million. The propagation of the tapeworm life cycle places the wider population at risk of acquiring neu-
3.2. INTRODUCTION

The zoonotic tapeworm *Taenia solium*, has a two host life cycle, with humans as the definitive host, and pigs as an intermediate host. Humans are infected after consumption of viable cystercerci in under-cooked pork and harbor the adult tapeworm, in an infection known as taeniasis. Gravid proglottids, containing thousands of infective eggs, detach from the adult tapeworm and are excreted in faeces in an intermittent

rocystercerosis through environmental contamination with eggs and proglottids from the human tapeworm carriers.

The effect of 15 potential mitigation strategies were modeled, to determine the reduction in risk they could effect as well as their cost-effectiveness in terms of incremental cost-effectiveness ratio (ICER). A combined intervention, consisting of improving the meat inspection service, providing a pig vaccine campaign, a pre-slaughter diagnostic assay and health education has the potential to reduce the number of infective meals consumed in the area by 95.3% (95% C.I. 89.6-99).

In the absence of a well staffed and resourced meat inspectorate, the adoption of a pre-slaughter diagnostic assay, or a pig vaccination campaign were found to be the most cost effective strategies modeled, at an ICER of $0.25 (0.2-0.35) and $0.47 (0.033-0.73) per infective meal avoided respectively. If statutory legislation on meat inspection was enforced, however, the adoption of a porcine vaccination campaign was found to lead to a potential saving to farmers with an ICER of $-0.04 (-0.44-0.25) per infective meal avoided, due to the prevention of economic losses through carcass condemnation.

3.1.3 Conclusion

This model indicates a worryingly high risk of *T. solium* infection associated with pork consumption in western Kenya, highlighting an important public health issue. We have been able to model potential intervention measures for this parasite indicating the utility of improved diagnostic ability for pig farmers and traders or the provision of a pig vaccine. We emphasise the benefit that stringent meat inspection can have in providing an economic driver for farmers to adopt control policies. We hope that this model and the data presented here encourage the global public health community to engage in some of the proposed control strategies for this dangerous food-borne parasite.

3.2. Introduction

The zoonotic tapeworm *Taenia solium*, has a two host life cycle, with humans as the definitive host, and pigs as an intermediate host. Humans are infected after consumption of viable cystercerci in under-cooked pork and harbor the adult tapeworm, in an infection known as taeniasis. Gravid proglottids, containing thousands of infective eggs, detach from the adult tapeworm and are excreted in faeces in an intermittent
fashion. Ingestion of these eggs, by either pigs or humans, results in the larval stage penetrating the intestinal wall, moving through the lymph and blood vessels to encyst in muscle, eyes or the CNS as cysticerci (Garcia et al., 2003). Infection of the CNS, neurocysticercosis, manifests predominately as epileptic seizures and is thought to be responsible for 29% of epilepsy cases in endemic regions (Ndimubanzi et al., 2010).

*T. solium* cysticercosis has been identified as an important disease predominately in Latin America (Ndimubanzi et al., 2010), Asia (Rajeshkhar et al., 2003) and across much of Africa (Phiri et al., 2003; Zoli et al., 2003) although the nature of global travel and migration puts all countries at risk of infection. This is highlighted by cases of neurocysticercosis being diagnosed in the United States, predominately in immigrants from Latin America with histories indicating that infection was acquired from endemic areas (ONEal et al., 2012; Schantz and McAuley, 1991; Sorvillo et al., 2011).

The global burden of cysticercosis has been estimated for the first time, and the parasite is thought to be responsible for a global total of 503,000 (379,000-663,000) disability adjusted life years (DALYs), or 7 DALYs lost per 100,000 people, annually (Murray et al., 2013). The burden of this disease lies disproportionally on the developing nations, such as Cameroon, where the burden was estimated to be over 100 times greater at 9 DALYs per 1000 people (Prat et al., 2009). Human prevalence of circulating *T. solium* antigen of 10.3% in Burkino Faso (Carabin et al., 2009) and 40.8% of circulating anticystercerci antibodies in Burundi (Nsengiyumva et al., 2003) indicate the widespread infection with and exposure to this parasite in other sub-Saharan African countries, where an estimated 1.90 to 6.16 million people (of a population of approximately 850 million) may be infected (Winkler, 2012).

The consumption of undercooked, infected pork is a key step in the perpetuation of the parasite life cycle, and represents a major public health hazard, with people acquiring a taeniasis infection then representing a focus of infection for life-threatening cysticercosis (Lescano et al., 2009) and indeed the consumption of pork (Schantz et al., 1994) and the inability to recognise infected meat (Cao et al., 1997) are risk factors significantly associated with human cysticercosis (OR = 1.6 (1.2-2.4) and 11.7 (1.9—∞) respectively).

Addressing porcine infection and reducing the infection pressure entering the food chain is therefore an important issue for public health practitioners in order to reduce the burden of this neglected tropical disease. Understanding the current risks posed by the pork industry in developing counties can be achieved by a process of risk analysis; whereby risks are identified and described, qualitatively and quantitatively assessed
3.2. INTRODUCTION

and then communicated and mitigated.

The principal of risk analysis allows scientific, justifiable and transparent decisions to be made regarding the risks associated with food products and is a key component of the Codex Alimentarius framework. Codex Alimentarius is a joint FAO/WHO commission whose role is to protect consumer safety in its member states in such as way that trade can be conducted in an environment where consistent food safety standards are enforced for all countries, removing the potential for non-tariff barriers to trade (Dawson, 1995).

A stochastic, quantitative risk assessment, as part of a risk analysis process, allows us to incorporate quantitative data and the uncertainty and variability that surrounds these data, in order to establish an quantitative estimate of risk and a probability interval around that estimate.

In order to reduce the public health burden of this parasite, design and validation of control activities are urgently required. Several potential control strategies have been described, including but not limited to: mass drug administration with a taeniacide (Allan et al., 1997; Sarti et al., 2000), targeted human anthelmintic treatment of taenia carriers or high risk categories (Pawlowski, 2008) treatment of pigs with oxfenbendazole (Gonzalez et al., 1997; Sikasunge et al., 2008), vaccination of pigs (Lightowlers, 1999, 2010b), improvements in the meat inspectorate, which is currently under-staffed and under-funded in many regions (Kagira et al., 2010) and health education of communities, for improvement in pig husbandry and hygiene practices (Ngowi et al., 2008; Pawlowski, 2008; Wohlgemut et al., 2010). The technology in many cases is available, though in regions where resources in both the human health and veterinary sector are scarce a system of prioritisation of control initiatives is needed, based upon efficacy and cost.

A method of prioritising control options, when the goal is to maximise health benefits for the whole of the target population utilising constrained resources is a cost-effectiveness analysis (Detsky and Naglie, 1990). In order to compare different strategies and prioritise the use of scarce resources the cost-effectiveness analysis must take into account the incremental effectiveness obtained for each unit increase in cost (Detsky and Naglie, 1990).

The aim of the work presented here was to estimate the risk to humans of *T. solium* infection from the pork currently entering the food chain and to allow the impact of potential intervention strategies to be evaluated and compared. To this end a stochastic
risk assessment model with Monte Carlo simulation was built and informed by data gathered in the field in western Kenya and available through the literature to estimate the probability of any one pork meal consumed in western Kenya being infective with \textit{T. solium}. The model was then used to assess the potential effectiveness of a variety of interventions and a cost-effectiveness analysis was performed in order to prioritise these interventions.

The costs considered in this analysis are those incurred by pig farmers in the region and include only direct financial outlay in US\$, except in the case of health education attendance where opportunity costs of attendance were considered. Effectiveness is considered in terms of an infective meal prevented and the cost-effectiveness analysis considered the ratio of cost to effectiveness of a proposed intervention against the 'do nothing' strategy of the status quo.

3.3 Material and Methods

3.3.1 Risk Question

A stochastic risk assessment model was built to answer the following question: What is the risk of any one pork meal consumed in western Kenya being contaminated with viable cysticerci of \textit{Taenia solium}?

3.3.2 Model inputs

Each parameter in the model was informed either by field data or from the literature where field data were lacking.

The field data were obtained from two complementary studies in western Kenya, both forming part of the “People, Animals \& their Zoonoses” study (PAZ) (Doble and Fèvre, 2010). The study site is largely representative of the Lake Victoria Crescent ecosystem. It is bounded to the west by the border with Uganda, to the south by Lake Victoria and to the North by Mount Elgon. The study area used for both of these studies, indicating the location of homesteads and porcine slaughter facilities is shown in Figure 3.1 on the facing page.

The first study was a community based cross-sectional study collecting data from humans and their livestock from 416 randomly selected homesteads within this study area.
Figure 3.1: Map of Study Area Showing Recruited Homesteads and the Location of Porcine Slaughter Facilities
This map was produced using ArcMap™ version 9.1 with geographical data provided by ILRI GIS unit http://www.ilri.org/gis/ and overlaid with the location of homesteads and slaughter facilities collected in the field using a hand held Garmin® eTrex GPS unit.
between July 2010 and July 2012. During which questionnaire data were collected on a wide range of homestead and individual level risk factors (Doble and Fèvre, 2010).

The second study was based at porcine slaughter facilities within the pig-owning divisions of this study area, where a pig owning division was defined as those containing over 2% of the total pig population of the study area and together these 10 divisions account for over 80% of the total pig population.

A total of 343 serum samples from pigs were collected from all porcine slaughter facilities (hereafter slabs) in the study site identified for us by the district veterinary office, between February and August 2010. A minimum sample size of 319 pigs was calculated to be required, using WinEpiscope 2.0 (Thrusfield et al., 2001) and based upon an assumed T. solium prevalence of 14% (Githgia et al., 2006) with 5% precision and 99% probability level. Slabs were visited on the day of highest through-put as identified by the meat inspector or slab owner and all pigs being slaughtered on the day of visit were sampled. Facilities were re-visited until a quota proportional to the percentage of the total pig population found in that division had been filled.

Pigs were restrained with a pig snare behind the canine teeth and anterior vena cava blood samples (Muirhead, 1981) were collected in BD Vacutainer® 10 ml plain tubes and transported to the laboratory on ice, where they were centrifuged at 3000 rpm for 20 minutes at room temperature. Sera were then separated into 2 aliquots in 2 ml labeled cryovials and stored at −40 °C until they were transported on dry ice to the ILRI facility in Nairobi where they were stored at −80 °C for between 2-7 months before analysis by the HP10 Ag-ELISA (Harrison and Parkhouse, 1989). The prevalence estimate obtained from this analysis was then adjusted for the diagnostic test parameters as previously estimated using a Bayesian framework, to be 70.4% sensitivity and 66.1% specificity (Krecek et al., 2011). 343 pigs were sampled during the course of this study, of which 167 tested positive on HP10 Ag-ELISA, a prevalence of 48.7% (95% C.I. 43.3-54.1%), which when adjusted to account for the diagnostic test parameters estimates a true prevalence of 39.7% (95% C.I. 25.3-54.2%).

Meat inspection results were obtained by contacting the meat inspector on duty for that slaughter facility at the end of the day and requesting his report on all pigs slaughtered. The investigators for this study did not interfere in any way with the process of meat inspection as we wished to obtain a true reflection of the activities carried out. A further communication was made with all the meat inspectors involved at the conclusion of the study during which data were gathered on carcass condemnation for any reason during the time that this survey was taking place. During the course of the study it was
reported that no pig carcass was condemned on meat inspection for any reason.

3.3.3 Risk Assessment Model

A stochastic risk assessment model using Monte Carlo simulation was built using the @Risk (Palisade corp. USA) add-on for Excel (Microsoft Corp. USA). The risk of any one pork meal being infective was the sum of the different scenario probabilities as shown in equation (3.1).

\[
P(\text{meal infective}) = P(\text{meal infective | Scenario 1}) \times P(\text{Scenario 1}) \\
+ P(\text{meal infective | Scenario 2}) \times P(\text{Scenario 2}) \\
+ P(\text{meal infective | Scenario 3}) \times P(\text{Scenario 3}) \\
\ldots + P(\text{meal infective | Scenario 9}) \times P(\text{Scenario 9})
\]

(3.1)

Where:

Scenario 1 = Pig is formally slaughtered, is lightly infected and is not detected at meat inspection
Scenario 2 = Pig is formally slaughtered, is lightly infected, is detected at meat inspection and condemned
Scenario 3 = Pig is formally slaughtered, is heavily infected and is not detected at meat inspection
Scenario 4 = Pig is formally slaughtered, is heavily infected, is detected at meat inspection and condemned
Scenario 5 = Pig is formally slaughtered/ uninfected and is not detected at meat inspection
Scenario 6 = Pig is formally slaughtered, is uninfected, is detected at meat inspection (false positive) and condemned
Scenario 7 = Pig is informally slaughtered and is lightly infected
Scenario 8 = Pig is informally slaughtered and is heavily infected
Scenario 9 = Pig is informally slaughtered and is uninfected

and all scenarios are mutually exclusive and add to all possible scenarios of pork consumption in the study site.
With:

\[ P(\text{Scenario} 1) = (P(\text{Pig formally slaughtered}) \times P(\text{Pig infected}) \times P(\text{Pig lightly infected}) \times P(\text{Pig not detected at meat inspection})) \]

ect....

The model structure can be seen in Figure 3.2 on the next page and the parameters of each model input are described in Table 3.1 on page 62.

The model using the Auto function in @Risk, a function which runs sufficient iterations that all input parameters have converged. A basic sensitivity analysis was performed to determine the influence of 9 input parameters (P9 & P10 as described in Table 1 were excluded from the sensitivity analysis as \( P = 0 \)) on the output “Probability that pork meal is infective at consumption”. The sensitivity analysis was performed using the built in functions with 105 simulations of 10,000 iterations, monitoring % changes in baseline of the input parameters from -100% of baseline to +100% of baseline.
Figure 3.2: Structure of Risk Model
The mirror image structure of this model accounts to the two parallel food chains existing in this area, the formal, legalised, chain (above) and the informal black market (below), our risk assessment accounts for both these chains.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Probability that:</th>
<th>Source</th>
<th>Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>Pig was slaughtered informally</td>
<td>Cross-sectional data&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Beta(4,62)</td>
</tr>
<tr>
<td>1-P1</td>
<td>Pig was slaughtered formally</td>
<td>Cross-sectional data</td>
<td>1-Beta(4,62)</td>
</tr>
<tr>
<td>P2</td>
<td>Pig is infected (formal slaughter)</td>
<td>Slaughter-slab survey&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Beta(140,150)</td>
</tr>
<tr>
<td>P3</td>
<td>Pig is infected (informal slaughter)</td>
<td>Literature (Praet et al., 2010)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Beta(140,150)</td>
</tr>
<tr>
<td>P4</td>
<td>Pig is lightly infected</td>
<td>Literature (Sciutto et al., 1998)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Beta(6,13)</td>
</tr>
<tr>
<td>1-P4</td>
<td>Pig is heavily infected</td>
<td>Literature (Sciutto et al., 1998)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Beta(6,13)</td>
</tr>
<tr>
<td>P5</td>
<td>Infected pig detected at meat inspection (as currently performed in the study area)</td>
<td>Meat inspection reports</td>
<td>Beta(1,31874)</td>
</tr>
<tr>
<td>1-P5</td>
<td>Infected pig is not detected at meat inspection</td>
<td>1-Beta(1,31874)</td>
<td></td>
</tr>
<tr>
<td>P6</td>
<td>Uninfected pig is detected at meat inspection (false positive)</td>
<td>Slaughter-slab survey&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Beta(1,148)</td>
</tr>
<tr>
<td>1-P6</td>
<td>Uninfected pig is not detected at meat inspection</td>
<td>1-Beta(1,148)</td>
<td></td>
</tr>
<tr>
<td>P7</td>
<td>Any one meal is infected (lightly infected pig)</td>
<td>Literature (Sciutto et al., 1998)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>Beta(44,56)</td>
</tr>
<tr>
<td>P8</td>
<td>Probability any one meal is infected (heavily infected pig)</td>
<td>Literature (Sciutto et al., 1998)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>Beta(5,1)</td>
</tr>
<tr>
<td>P9</td>
<td>Any one meal is infected (uninfected pig)</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>P10</td>
<td>Any one meal is infected (pig is condemned)</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>P11</td>
<td>Pork is eaten undercooked</td>
<td>Cross-sectional study&lt;sup&gt;g&lt;/sup&gt;</td>
<td>Beta(234,1062)</td>
</tr>
<tr>
<td>1-P11</td>
<td>Pork is eaten well cooked</td>
<td>1-Beta(234,1062)</td>
<td></td>
</tr>
<tr>
<td>P12</td>
<td>Pig population of study area</td>
<td>District Livestock and Production Officer</td>
<td>Pert(50000,54727,60000)</td>
</tr>
<tr>
<td>P13</td>
<td>Proportion of pigs available for slaughter</td>
<td>Cross-sectional study data&lt;sup&gt;h&lt;/sup&gt;</td>
<td>Beta(101,78)</td>
</tr>
<tr>
<td>P14</td>
<td>Number of meals supplied per pig</td>
<td>Literature (Levy et al., 2009)&lt;sup&gt;i&lt;/sup&gt;</td>
<td>Beta(4,2,13,5)</td>
</tr>
</tbody>
</table>

Table 3.1: Description of Model Parameters

<sup>a</sup> 3 out of 69 pig owning homesteads practice home slaughter,  
<sup>b</sup> prevalence of cysticercosis as detected by HP10 Ag-ELISA adjusted for diagnostic test parameters (40.5%),  
<sup>c</sup> indicates no significant difference between prevalence at formal and informal slaughter,  
<sup>d</sup> Dissection data from experimental infections,  
<sup>e</sup> None of the pigs testing positive to HP10 were condemned at meat inspection,  
<sup>f</sup> Extrapolation from dissection of experimentally infected pigs,  
<sup>g</sup> those reporting a preference for consuming pork 'red', 'still pink', 'raw' considered to be undercooked,  
<sup>h</sup> proportion of weaned pigs in study population considered available for slaughter in a year,  
<sup>i</sup> Average slaughter weight/pig 22.5kg and estimation of pork meal between 50g-250g
3.3.4 Intervention strategies

Four individual intervention strategies were modeled followed by combinations of these strategies. The points of the risk model affected by these interventions are shown in Figure 3.4 on page 69 and the input parameters for each intervention strategy are found in Figure 3.4 on page 69.

The four key interventions modeled were:

1. Improved meat Inspection

At present meat inspection as carried out in the study area is not detecting any infected pigs. The legislation governing meat inspection is in place in Kenya through the Meat Control Act (Cap 365) (Government of Kenya, 2012). Under this legislation the carcass and viscera of pigs is judged unfit for human consumption if cysticerci are found on inspection (Government of Kenya, 2012).

There is a network of meat inspectors working within western Kenya, though this service has been reported to be under-staffed and under-resourced, which may be resulting in this poor performance (Kagira et al., 2010). We modeled this intervention to bring the standard of meat inspection to that reported in other African countries. Meat inspection, although not very sensitive, is very specific and has been estimated, with respect to cysticercosis, to have a sensitivity of approximately 40% and specificity of 99% as carried out in Zambia (Phiri et al., 2006).

The cost of meat inspection to the farmer is based upon the findings of a previous study in western Kenya (Levy et al., 2009), with the cost of whole carcass condemnation based upon the average kill-out weight in the region (Levy et al., 2009) and the current pork price (per. Obs). The cost of pig transport was based upon a typical fare on a boda-boda (bicycle taxi) as the majority of pigs are transported by bike (Kagira et al., 2010) as illustrated in Figure 3.3 on the next page.

It would be necessary to ensure that if the statutory framework is enforced for meat inspection, that farmers do not turn to informal markets to slaughter their pigs. The cost to the government of improving the meat inspectorate would also therefore need to include the cost of policing clandestine slaughter.
2. Pre-slaughter diagnostic test

Serologic diagnostic tests are available for cysticercosis, including the antigen-capture ELISA based upon the HP10 MAb (Harrison et al., 1989). This, and other ELISA tests, require laboratory infrastructure and a degree of technical expertise to run, making them difficult to perform in some developing country settings (Fleury et al., 2007; Petti et al., 2005) and the format is not suitable for screening of pigs prior to slaughter.

We have designed and developed an LFA which utilises the HP10 MAb. The aim of this work is to develop a user-friendly, rapid, diagnostic kit that can be used with little technical knowledge to detect infected pigs. The test is currently in prototype form with an initial run of 2000 having been manufactured by a private sector firm. The test is discussed in more detail in Chapter 5 on page 121.

We envisage that one use of this assay would be for pig traders to screen pigs prior to purchase allowing uninfected pigs to be preferentially purchased for slaughter. The infected pigs may then be treated with Oxfendazole (30mg/kg), returning 8-10 weeks after to purchase the treated pigs, at which point the lesions will have resolved (Gonzalez et al., 1998).

In this model, we make the assumption that the lateral-flow assay is expected to perform
with similar diagnostic parameters as the HP10 ELISA, with high specificity (98.6%) and a sensitivity dependent on infection level, from 46.1% in lightly infected pigs to 83.7% in heavily infected pigs (Sciutto et al., 1998).

The cost of the pen-side test, although not currently in commercial production, has been estimated by Phil Toye (ILRI), based upon other similar technologies. The cost of anthelmintic treatment was obtained from local agri-vet businesses in Nairobi and the cost of pig transport was based upon a typical fare on a boda-boda.

3. Vaccination

A recombinent oncosphere-antigen based vaccine (TSOL18) has been developed for the protection of pigs against *T. solium* infection (Lightowlers, 2010b) and has been shown to provide 99-100% protection against experimental challenge (Flisser et al., 2004; Gonzalez et al., 2005). A field trial has been undertaken in Cameroon, with pigs receiving three immunisations with the TSOL18 vaccine, with a 30mg/kg dose of Oxfenbendazole at the time of the second immunisation. This protocol completely eliminated infection in the study pigs when slaughtered at 12 months of age (Assana et al., 2010).

Vaccination of pigs against *T. solium* would break transmission of the parasite, without the need to change husbandry practices, it also would provide protection to consumers despite the use of informal slaughter practices without adequate meat inspection (Lightowlers 1999). We modeled the efficacy of this vaccine as 99-100% and the vaccine coverage to allow for a minimum of 10% coverage, maximum of 100%, with a most likely figure of 70% coverage.

The cost of the vaccination is hard to estimate as production has not yet been scaled up to industrial levels (Lightowlers, 2010b), the cost of anthelmintic treatment was obtained from local agri-vet businesses in Nairobi and the cost of the vaccination has been estimated at $2.4 by Phil Toye (ILRI) who has prior experience in vaccine production.

To encourage farmers to purchase this vaccine there would need to be an incentive to do so. This could be achieved either through the punitive route of enforced condemnation of infected carcasses, or through incentivisation such as the addition of a vaccine for production limiting diseases such as porcine reproductive and respiratory syndrome virus (PRRS).
4. Health Education

Educating the population about the dangers of *T. solium*, how it is transmitted and the steps they can take to protect themselves is an important aspect of any control policy. Within this model we have looked at the impact of one benefit of education, the reduction in consumption of infective meat. A health education intervention in Tanzania reported a 20% reduction in consumption of infective meat (Ngowi *et al.*, 2008) and this figure was used to model the impact of health education on *T. solium* transmission.

The cost of a health education intervention was estimated as the opportunity cost of 4hrs work (Ngowi *et al.*, 2007), with the hourly wage cost in western Kenya being modeled based upon the range of minimum wages for different jobs encountered in the study site (Wage Indicator Network, 2012). In this model we have assumed provision of health education to the head of every homestead in the study area, as opposed to only pig farmers, in order to achieve a blanket reduction in the consumption of infected meat.

In Tanzania the incidence rate of porcine cysticercosis did reduce significantly, however there was also an increase in the sale of infected pigs by farmers. This makes any extrapolation into the prevalence of infection in pigs entering the food chain difficult and was therefore not accounted for in the current model. No account has been made either in this model of cost of compliance with improved pig husbandry practices, such as pen building, as in Tanzania no improvement was observed in such practices (Ngowi *et al.*, 2008).

Every combination of the above four intervention strategies were also modeled, giving a total of 16 possible interventions.

The risk of any one pork meal being infective after intervention was again the sum of the different scenario probabilities as shown in equation (3.2).

\[
P(\text{meal infective}) = P(\text{meal infective} | \text{Scenario 1v}) \times P(\text{Scenario 1v}) \\
+ P(\text{meal infective} | \text{Scenario 2v}) \times P(\text{Scenario 2v}) \\
+ P(\text{meal infective} | \text{Scenario 3v}) \times P(\text{Scenario 3v}) \\
+ \cdots + P(\text{meal infective} | \text{Scenario 24v}) \times P(\text{Scenario 24v})
\]  

(3.2)
Where;
Scenario1v = Pig is vaccinated, presents for formal slaughter, is lightly infected, is detected prior to purchase and is not slaughtered

Scenario2v = Pig is vaccinated, presents for formal slaughter, is lightly infected, is not detected prior to purchase and is not detected at meat inspection

Scenario3v = Pig is vaccinated, presents for formal slaughter, is lightly infected, is not detected prior to purchase is detected at meat inspection and condemned

Scenario4v = Pig is unvaccinated, presents for formal slaughter, is lightly infected, is detected prior to purchase and not slaughtered

Scenario5v = Pig is unvaccinated, presents for formal slaughter, is lightly infected, is not detected prior to slaughter and is not detected at meat inspection

Scenario6v = Pig is unvaccinated, presents for formal slaughter, is lightly infected, is not detected prior to slaughter, is detected at meat inspection and condemned

Scenario7v = Pig is vaccinated, presents for formal slaughter, is heavily infected, is detected prior to purchase and is not slaughtered

Scenario8v = Pig is vaccinated, presents for formal slaughter, is heavily infected, is not detected prior to slaughter and not detected at meat inspection

Scenario9v = Pig is vaccinated, presents for formal slaughter, is heavily infected, is not detected prior to slaughter, is detected at meat inspection and is condemned

Scenario10v = Pig is unvaccinated, presents for formal slaughter, is heavily infected, is detected prior to purchase and not slaughtered

Scenario11v = Pig is unvaccinated, presents for formal slaughter, is heavily infected, is not detected prior to slaughter and not detected at meat inspection

Scenario12v = Pig is unvaccinated, presents for formal slaughter, is heavily infected, is not detected prior to slaughter, is detected at meat inspection and is condemned
Scenario 13v = Pig is vaccinated, presents for formal slaughter, is uninfected, is detected prior to slaughter (false positive) and is not slaughtered

Scenario 14v = Pig is vaccinated, presents for formal slaughter, is uninfected, is not detected prior to slaughter is not detected at meat inspection

Scenario 15v = Pig is vaccinated, presents for formal slaughter, is uninfected, is not detected prior to slaughter, is detected at meat inspection (false positive) and is condemned

Scenario 16v = Pig is unvaccinated, presents for formal slaughter, is uninfected, is detected prior to slaughter (false positive) and not slaughtered

Scenario 17v = Pig is unvaccinated, presents for formal slaughter, is uninfected, is not detected prior to slaughter and not detected at meat inspection

Scenario 18v = Pig is unvaccinated, presents for formal slaughter, is uninfected, is not detected prior to slaughter, is detected at meat inspection (false positive) and is condemned

Scenario 19v = Pig is vaccinated, presents for informal slaughter, is lightly infected

Scenario 20v = Pig is unvaccinated, presents for informal slaughter, is lightly infected

Scenario 21v = Pig is vaccinated, presents for informal slaughter and is heavily infected

Scenario 22v = Pig is unvaccinated, presents for informal slaughter and is heavily infected

Scenario 23v = Pig is vaccinated, presents for informal slaughter and is uninfected

Scenario 24v = Pig is unvaccinated, presents for informal slaughter and is uninfected

The parameters for the interventions modeled can be seen in Table 3.2 on page 70.
3.3. MATERIAL AND METHODS

Figure 3.4: Aspects of Risk Model Affected by Intervention Strategies
Illustrates the predominant effect on the formal pork trade.
<table>
<thead>
<tr>
<th>Intervention &amp; Parameter</th>
<th>Source</th>
<th>Distribution</th>
<th>Costs (US$ 2012)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Meat Inspection (current strategy)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P5. Infected pig detected at inspection</td>
<td>Meat inspection reports</td>
<td>Beta(1,31874)</td>
<td>Inspection $0.88/pig&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Condemnation of pig $38&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Transport condemned pig $1.2&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Vaccination</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P15. Pig is vaccinated</td>
<td>Estimation of vaccine coverage</td>
<td>Pert(0.1,0.7,1)</td>
<td>Vaccination $2.4&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>P16. Vaccinated pig is infected</td>
<td>Intervention trial (Assana et al., 2010)</td>
<td>Uniform(0,0.01)</td>
<td>Oxfenbendazole $1.6&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Improved Meat Inspection</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P18 Infected pig is detected</td>
<td>Literature (Phiri et al., 2006)</td>
<td>1-(Beta(148,1))</td>
<td>Inspection $0.88&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Beta(13,18)</td>
<td>Condemnation of pig $38&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Transport of pig $1.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>P19 Uninfected pig is detected</td>
<td></td>
<td>Beta(13,18)</td>
<td></td>
</tr>
<tr>
<td><strong>Pre-slaughter diagnostic</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P20. Lightly infected pig detected</td>
<td>Literature (Sciutto et al., 1998)</td>
<td>Beta(9,9)</td>
<td>Diagnostic test $1.2&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Beta(145,27)</td>
<td>Transport of rejected pig $1.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>P22. Uninfected pig detected</td>
<td></td>
<td>1-Beta(46,1)</td>
<td></td>
</tr>
<tr>
<td><strong>Health Education</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P23 Meat is undercooked</td>
<td>Literature (Ngowi et al., 2007)</td>
<td>Beta(187,1274)</td>
<td>4hrs opportunity cost&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Uniform($1.2-$5.6)</td>
</tr>
</tbody>
</table>

Table 3.2: Input Parameters for Intervention Strategies

<sup>a</sup> (Levy et al., 2009)  <sup>b</sup> Based on 22.5kg average kill-out weight (Levy et al., 2009) @ $1.7/kg (Field Data),  <sup>c</sup> personal observation,  <sup>d</sup> expert opinion,  <sup>e</sup>(Ngowi et al., 2007)
3.3.5 Cost-Effectiveness Analysis

The model was also used to perform an incremental cost-effectiveness analysis, with each strategy evaluated in terms of an incremental cost-effectiveness ratio (ICER), being the incremental cost per extra unit of outcome. In this instance our outcome of interest is infective meal avoided. The incremental cost-effectiveness ratio is calculated according to the equation (3.3) (Detsky and Naglie, 1990).

\[
ICER = \frac{(\text{Cost of strategy} - \text{Cost current strategy})}{(\text{Effectiveness of strategy} - \text{Effectiveness current strategy})}
\]

(3.3)

Where: Costs are in US$ at 2012 values.

Meat inspectors in western Kenya condemned no pig carcass for any reason during the course of our study, but all people presenting pigs for slaughter at registered facilities were required to pay for the services of an inspector. It is this situation that we model as our current (do nothing) strategy against which other strategies are compared.

The costs of each intervention in the model are borne by the farmer, apart from health education for which the opportunity costs (time) are borne by each homestead in the study site. For farmers to bear the cost of a public health intervention, such as these suggested, there needs to be an incentive or regulatory framework to encourage them to do so. Meat inspection, as laid out in law, provides such a regulatory framework, whereby farmers are penalised for presenting unsafe meat to slaughter by the risk of it being condemned.

Meat inspection as is currently carried out in western Kenya is not providing this driver, and increasing the stringency of this service may be a pre-requisite for adoption of any other control strategy. We therefore also investigated the cost-effectiveness, in terms of ICER of strategies which combine an improved meat inspection service with the other control strategies.

The costs relating to each intervention can be found in Table 3.2 on the facing page and the calculations to determine the costs of each intervention strategy can be found in Appendix .2 on page 181. The model was run using the auto functions, which runs sufficient iterations to converge all inputs. A sensitivity analysis was then run to monitor the change in the output “probability meal is infective at consumption under intervention 15” (vaccination campaign, pre-slaughter diagnostic, improved meat inspection and health education) brought about by changes from baseline in the input
parameters relating to the interventions, excluding those already monitored in the previous sensitivity analysis. The sensitivity analysis used 100 simulations of 10,000 iterations each.

3.4 Results

3.4.1 Estimated current infection risk from pork consumed in western Kenya

After 50,000 iterations all parameters in the model had converged. The model predicted that with the current prevalence of *T. solium* (as determined by HP10 ELISA) in pigs entering the food chain, any one pork meal consumed (after cooking) in western Kenya has a probability of 0.061 (95% C.I.0.039 0.081) of containing at least one viable *T. solium* cysticercus, and therefore being infective to humans. The probability distribution for this output is shown in Figure 3.5. This equates to approximately 810,000 potentially infective meals consumed across this study site, with a population of approximately 3million, in the course of one year. Meat inspection, as is currently practiced in western Kenya is responsible, according to the model, for avoiding only 23 infective meals a year.

Figure 3.5: Probability Distribution Showing Risk of any one Pork Meal being Infected with *T. solium* at Consumption.
3.4.2 Sensitivity analysis

A sensitivity analysis was performed with 125 simulations of 1000 iterations, monitoring the effect of each input on the outcome “Risk of any one pork meal being infected with *T. solium* at consumption”. The analysis suggested that the parameter with greatest influence on the outcome was the probability that a pig is infected with *T. solium* if formally slaughtered, followed by the probability that a meal is undercooked, then the probability that any one pork meal is infected if originating from a heavily infected pig. The effect of 100% changes from baseline in the 9 input parameters monitored in the sensitivity analysis are shown in Figure 3.6 and can be found in Appendix .3 on page 185, Table 1 on page 185.

![Figure 3.6: Sensitivity Analysis Indicating Change in Output “Probability Pork Meal is Infective at Consumption” Relating to % Change in Input Parameters from Baseline.](image)

The vertical axis shows us the risk of a pork meal being infective in relation to the change in input parameter described in the horizontal axis, being the % change from the baseline parameter.
3.4.3 Effect of intervention strategies

The effect that each intervention strategy would have on the output, probability that meal is infective at consumption, and the incremental cost-effectiveness ratio associated with each intervention are shown in Table 3.3 on the facing page.

The most effective intervention in terms of percentage of infectious meals avoided was the combination of a pig vaccine, pre-slaughter diagnostic test, improved meat inspection and health education, which the model predicted would reduce the number of infective meals consumed in the study site by 95.3% (95% C.I. 89.6-99%). The cost-effectiveness of this intervention, according to the incremental cost-effectiveness ratio (ICER) is $1.5 (95% C.I. 0.81-2.54) per infective meal avoided.

Single effective interventions were: the provision of a pre-slaughter diagnostic with oxfenbendazole treatment of postive pigs, which has the potential to reduce the number of infective meals consumed by 72.6% (95% C.I. 62.1-80.9%) and porcine vaccination, with the potential to reduce the number of infective meals consumed by 64.2% (95% C.I. 30.6-91.8%). This assumes that vaccination coverage of 70% could be achieved, but at higher vaccination coverage there is the potential to reduce the number of infective meals to zero, as shown in Figure 3.7 on page 76.
### Table 3.3: Effect of Intervention Strategies on Probability of a Pork Meal being Infectious with *T. solium* at Consumption

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Risk meal infective after cooking</th>
<th>Percentage infective meals avoided (%)</th>
<th>Total Cost Thousand(US$)</th>
<th>Incremental cost-effectiveness Ratio(US$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current Meat Inspection</td>
<td>0.061 (0.039-0.081)</td>
<td>NA</td>
<td>50.7 (46.7-53.7)</td>
<td>NA</td>
</tr>
<tr>
<td>Improved Meat Inspection (MI)</td>
<td>0.037 (0.022-0.054)</td>
<td>39.4 (23.9-56.1))</td>
<td>515 (329-724)</td>
<td>1.3 (0.95-1.9)</td>
</tr>
<tr>
<td>Pre-slaughter diagnostic (PSD)</td>
<td>0.02 (0.01-0.024)</td>
<td>72.6 (62.1-80.9)</td>
<td>224 (186-257)</td>
<td>0.25 (0.2-0.35)</td>
</tr>
<tr>
<td>Vaccination (Vac)</td>
<td>0.022 (0.005-0.046)</td>
<td>64.2 (30.6-91.8)</td>
<td>333 (182-476)</td>
<td>0.47 (0.033-0.73)</td>
</tr>
<tr>
<td>Health Education (H.Ed)</td>
<td>0.043 (0.028-0.058)</td>
<td>28.8 (15.2-40.6)</td>
<td>794 (336-1,308)</td>
<td>2.9(1-6)</td>
</tr>
<tr>
<td>MI &amp; H.Ed</td>
<td>0.026 (0.015-0.039)</td>
<td>56.8 (42.4-70.2)</td>
<td>1,258 (738-1,824)</td>
<td>2.3 (1.2-4.1)</td>
</tr>
<tr>
<td>PSD &amp; H.Ed</td>
<td>0.012 (0.007-0.0174)</td>
<td>80.5 (71.9-87.0)</td>
<td>967 (507-1,485)</td>
<td>1.2 (0.55-2.3)</td>
</tr>
<tr>
<td>Vac &amp; H.Ed</td>
<td>0.016 (0.003-0.03)</td>
<td>74.5 (49.2-94.2)</td>
<td>1,072 (576-1,613)</td>
<td>1.8 (0.85-3.47)</td>
</tr>
<tr>
<td>MI &amp; Vac</td>
<td>0.013 (0.003-0.029)</td>
<td>78.3 (55.8-95.2)</td>
<td>508 (417-610)</td>
<td>0.6 (0.4-1.03)</td>
</tr>
<tr>
<td>MI &amp; Vac &amp; H.Ed</td>
<td>0.01 (0.002-0.02)</td>
<td>84.6 (67.7-96.6)</td>
<td>1,251 (770-1,779)</td>
<td>1.5 (0.79-2.74)</td>
</tr>
<tr>
<td>Vac &amp; PSD</td>
<td>0.006 (0.0013-0.013)</td>
<td>90 (79.4-97.8)</td>
<td>443 (322-563)</td>
<td>0.5 (0.3-0.7)</td>
</tr>
<tr>
<td>Vac &amp; PSD &amp; H.Ed</td>
<td>0.004 (0.001-0.009)</td>
<td>93.3 (85.9-98.5)</td>
<td>1,186 (699-1,719)</td>
<td>1.3 (0.67-2.31)</td>
</tr>
<tr>
<td>MI &amp; PSD</td>
<td>0.011 (0.006-0.017)</td>
<td>81.6 (72.8-88.5)</td>
<td>1,416 (1,130-1,721)</td>
<td>1.8 (1.32-2.75)</td>
</tr>
<tr>
<td>MI &amp; PSD &amp; H.Ed</td>
<td>0.008 (0.005-0.012)</td>
<td>86.9 (80-92.1)</td>
<td>2,159 (1,583-2,778)</td>
<td>2.6 (1.7-4.2)</td>
</tr>
<tr>
<td>MI &amp; PSD &amp; Vac</td>
<td>0.004 (0.0008-0.009)</td>
<td>93.4 (85.6-98.6)</td>
<td>618 (524-737)</td>
<td>0.6 (0.45-1.0)</td>
</tr>
<tr>
<td>MI &amp; PSD &amp; Vac &amp; H.Ed</td>
<td>0.003 (0.0006-0.006)</td>
<td>95.3 (89.6-99)</td>
<td>1,361 (884-1,893)</td>
<td>1.5 (0.81-2.54)</td>
</tr>
</tbody>
</table>
Figure 3.7: Effect of Porcine Vaccination Coverage on the Risk of a Pork Meal being Infective with *Taenia solium* at Consumption Where each dot is the result from an individual iteration of the stochastic model described

The most cost-effective interventions based on the ICER are: provision of a pre-slaughter diagnostic with treatment of positive pig $0.25/ meal avoided (95% C.I.0.2-0.35), pig vaccination $0.47/ infective meal avoided (95% C.I.0.033-0.73), combination of vaccination and pre-slaughter diagnostic $0.5/ infective meal avoided (95% C.I.0.3-0.7), pig vaccination and improved meat inspection $0.6 (95% C.I. 0.4-1.03) and the combination of pig vaccination, pre-slaughter diagnostic and improved meat inspection combined $0.6 (95% C.I. 0.45-1.0). The ICER for each of these five intervention options are expressed graphically in Figure 3.8 on the facing page.
Figure 3.8: **Box Plot Showing Incremental Cost Effectiveness Ratio (ICER) for the Top 5 Intervention Strategies**

1 = Vaccination, 2 = Pre-slaughter diagnostic, 3 = Vaccine & pre-slaughter diagnostic, 4 = Vaccination & improved meat inspection, 5 = Vaccine, pre-slaughter diagnostic & improved meat inspection

3.4.4 Sensitivity Analysis, Intervention Strategies

The sensitivity analysis was performed with 270 simulations of 500 iterations, which was above the number required for the model to converge. The analysis monitored the effect on the output *Probability that pork meal is infective upon consumption* of a +/- 100% change from baseline in input parameters. The effect of these changes can be seen in Figure 3.9 on the next page and values found in Appendix .4 on page 186, Table 2 on page 186. The inputs with the most influence over the outputs in the intervention scenario are the probability of a pig being vaccinated and the probability of a heavily infected pig being detected using a pre-slaughter diagnostic.
Figure 3.9: Sensitivity Analysis Indicating Change in Output “Probability Pork Meal is Infective at Consumption” Relating to % Change in Intervention Input Parameters from Baseline

3.4.5 Risk of Pork Consumption and Effectivness of Interventions at Varying Baseline Prevalence

The structure of the model described here does not take into account the change in prevalence over time and does therefore not account for the changing cost-effectiveness of interventions as prevalence is reduced in the porcine population over the course of interventions. It is therefore interesting to investigate how the incremental cost of each infective meal avoided will be affected by an increasingly lower baseline prevalence. To this end the model was re-run using 3 estimates of base-line prevalence in the porcine population and the effect of this on the ICER was evaluated at each. The prevalence values were: Low (2.7%), Medium (21.6%) and High (39.7%). The results of these models, in terms of risk of *T. solium* transmission through pork consumption and the cost effectiveness of intervention strategies can be found in Tables 3.4 and 3.6.
Baseline Prevalence | Risk of infection from pork at consumption
--- | ---
Low | 0.004 (95% C.I. 0.002-0.006)
Medium | 0.021 (95% C.I. 0.012-0.029)
High | 0.061 (95% C.I.0.039-0.081)

Table 3.4: Risk of Infection from Pork at Consumption at Different Porcine Prevalence Levels

3.4.6 Cost effectiveness analysis in the presence of an improved meat inspection service

The ICER was also calculated for interventions combining an improved meat inspection service with the pre-slaughter diagnostic, pig vaccination, health education or a combination thereof. In this case ICER was calculated using Improved meat inspection as the alternative strategy and these results can be seen in Table 3.5. This analysis indicates that at initiation of an intervention while the baseline prevalence of porcine cysticercosis is high, the combination of a pig vaccine with an improvement in the meat inspection service will actually provide a saving to farmers (ICER $-0.04 (-0.43-0.25)) for each infective meal avoided.

<table>
<thead>
<tr>
<th>Intervention Strategy*</th>
<th>ICER (95% C.I.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
</tr>
<tr>
<td>Health education (H.ed)</td>
<td>79.6 (20-218)</td>
</tr>
<tr>
<td>Vaccine (Vac)</td>
<td>15.1 (7-29.1)</td>
</tr>
<tr>
<td>Vac &amp; H.ed</td>
<td>45.3 (15.6-107.3)</td>
</tr>
<tr>
<td>Pre-slaughter diagnostic (PSD)</td>
<td>4.5 (1.8-21.1)</td>
</tr>
<tr>
<td>PSD &amp; H.ed</td>
<td>167.8 (165.1-170.5)</td>
</tr>
<tr>
<td>PSD &amp; Vac</td>
<td>11.9 (5-25.2)</td>
</tr>
<tr>
<td>PSD &amp; Vac &amp; H.ed</td>
<td>35.9 (13.4-79.2)</td>
</tr>
</tbody>
</table>

Table 3.5: ICER for Control Strategies, Utilising the Improved Meat Inspection Alone as the Alternative Strategy

*all strategies include improved meat inspection
## Table 3.6: ICER for Control Strategies at Different Prevalence Levels

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Incremental cost-effectiveness Ratio (US$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
</tr>
<tr>
<td>Current Meat Inspection</td>
<td>NA</td>
</tr>
<tr>
<td>Improved Meat Inspection (MI)</td>
<td>1.9 (1.0-4.38)</td>
</tr>
<tr>
<td>Pre-slaughter diagnostic (PSD)</td>
<td>2.05 (1.02-3.99)</td>
</tr>
<tr>
<td>Vaccination (Vac)</td>
<td>9.87 (4.4-22.5)</td>
</tr>
<tr>
<td>Health Education (H.Ed)</td>
<td>48.9 (13-132)</td>
</tr>
<tr>
<td>MI &amp; H.Ed</td>
<td>14.7 (7.8-61.6)</td>
</tr>
<tr>
<td>PSD &amp; H.Ed</td>
<td>18.9 (6-44.7)</td>
</tr>
<tr>
<td>Vac &amp; H.Ed</td>
<td>28.3 (10.1-66.7)</td>
</tr>
<tr>
<td>MI &amp; Vac</td>
<td>7.8 (3.6-16.4)</td>
</tr>
<tr>
<td>MI &amp; Vac &amp; H.Ed</td>
<td>23.94 (9.1-53.3)</td>
</tr>
<tr>
<td>Vac &amp; PSD</td>
<td>7.43 (3.3-15.2)</td>
</tr>
<tr>
<td>Vac &amp; PSD &amp; H.Ed</td>
<td>21.95 (8.6-47.8)</td>
</tr>
<tr>
<td>MI &amp; PSD</td>
<td>3.2 (1.6-7.1)</td>
</tr>
<tr>
<td>MI &amp; PSD &amp; H.Ed</td>
<td>18.9 (6.7-42.6)</td>
</tr>
<tr>
<td>MI &amp; PSD &amp; Vac</td>
<td>7.6 (3.5-15.4)</td>
</tr>
<tr>
<td>MI &amp; PSD &amp; Vac &amp; H.Ed</td>
<td>21.8 (8.7-46.7)</td>
</tr>
</tbody>
</table>
3.5 Discussion

This stochastic risk model has enabled us to express, quantitatively, the risk which pork entering the food chain in western Kenya is posing to consumers, in terms of infection with the zoonotic tape worm *T. solium*.

Any model is obviously an approximation of reality and the current study is no different. Some of the parameters used in the model could be updated as suitable data become available to allow us to simulate a situation closer to that of reality. For instance, the distribution of infection burden is obviously a continuous gradient in pigs, although the data available to us at present allowed only a split into “lightly” and “heavily” infected pigs. Better understanding of the expected impacts of interventions, e.g. the reduction in porcine prevalence due to health education, will allow a more accurate evaluation of the ICUR for these proposed interventions. In the absence of better available data, however, this model allows us to improve our understanding the epidemiology of this parasite in this setting and to explore the impact and cost-effectiveness of some potential mitigation strategies.

Pork is a high risk product according to this model. With the current input parameters, there is a 0.061 (95% C.I. 0.039-0.081) risk that any one pork meal consumed in western Kenya is infected with a viable *T. solium* cysticercus, and therefore infectious to humans. This equates to approximately 810,000 potentially infectious events occurring in this study site in any one year, in a region with a human population of 3 million.

In Chapter 4 on page 87 it is shown that people who consume pork in this study area are 67% more likely to be positive for taeniasis infection on copro-ag ELISA than those who do not (OR = 1.67, 95% C.I. 1.05-2.63, *p*=0.029).

Several interventions have the potential to drastically reduce the risk of pork to consumers. 96.3% (95% C.I. 89.6-99.0) of potentially infected meals could be avoided through an intervention combining an improved meat inspectorate, pre-slaughter diagnostic, vaccination of pigs and health education of the community.

Single component intervention strategies such as a pre-purchase diagnostic test (leading to refusal of a trader to purchase pigs and provision of oxfendazole treatment), or vaccination of the pig population (with a mean of 70% coverage) had the potential to reduce the risk of exposure to *T. solium* by 72.6% (95% C.I. 62.1-80.9%) and 64.2% (95% C.I. 30.6-91.8%), respectively. As indicated by the sensitivity analysis performed, the effectiveness of these interventions is strongly related to a. the sensitivity of the
pre-slaughter diagnostic assay and b. the vaccination coverage in the population.

Prioritisation of intervention strategies must take into account the costs involved with the strategies. The costs modeled in this study are those costs which can be directly attributed to the farmers in the study area (be that direct or opportunity costs) and does not estimate the “global” costs of such interventions such as administration and personnel costs.

The most efficient interventions in terms of the incremental cost-effectiveness ratio (ICER, $/infective meal avoided) are the provision of a pre-slaughter diagnostic ($0.25, 95% C.I. 0.20-0.35), vaccination of pigs ($0.47, 95% C.I. 0.03-0.73), or a combination of the two interventions ($0.5, 95% C.I. 0.3-0.7).

If we expect pig producers to bear the cost of such interventions, there needs to be an incentive, or regulatory framework to encourage farmers to adopt them. Stringent meat inspection is already a legal requirement, but, due to an under-staffed and under-resourced meat inspectorate, appears not to be enforced in western Kenya (Kagira et al., 2010) although farmers are still expected to pay for the services of an inspector (Levy et al., 2009) (& per.obs).

This analysis indicates that should stringent meat inspection be enforced, ensuring the condemnation of infective meat, in areas with high prevalence of *T. solium*, farmers would benefit financially by adopting the use of a vaccination, at an ICER of -$0.04 (95% C.I.-0.43-0.25) per infective meal avoided. The cost to the government, of ensuring that this service is carried out to the required standards is beyond the scope of this work. The aim of meat inspection, however, is to “provide safe and wholesome meat for human consumption” (Herenda and Chambers, 1994) and the cost of improving the meat inspectorate service is not therefore, completely borne by cysticercosis control, but in providing a wider public good.

The use of a pre-slaughter diagnostic has the advantage over meat inspection in that pigs can be treated and slaughtered at a later date, thereby preserving the farmers income. There are still oppurtunity costs bourne by the farmer from the inability to sell a pig on a given day, as well as the cost of keeping the pig for the 8-10 weeks before it can be re-presented for slaughter.

As many farmers keep pigs in order to quickly release capital at times of need (e.g. for school and medical bills) (Lekule and Kyvsgaard, 2003), the inability do so may encourage farmers to use informal markets. In order to prevent this it would be interesting to investigate the potential for a payment scheme, administerd through pork traders,
where a down-payment is placed on a pig which tests positive on LFA, with a contract signed between farmer and trader to buy the pig at a later given date. This would provide security to farmers, discouraging the use of informal markets whilst retaining enough disincentive for a positive result to encourage adoption of better pig husbandry techniques.

Whichever intervention strategy is chosen it is important to continue to enforce them even as the prevalence of infection in the porcine host falls. At a 2.7% prevalence of infection there are still approximately 54,000 potentially infective meals consumed in the study site in one year. The relative cost of avoiding these events, however, become more expensive as the prevalence of porcine infection drops. This study shows that the ICER for the intervention strategies rise dramatically as the porcine prevalence drops. At the lowest prevalence investigated, improvement in the meat inspection services has the lowest ICER ($1.9, 95% C.I. 1-4.38).

The addition of components such as porcine vaccination and pre-slaughter diagnostics over a baseline strategy of improved meat inspection become more expensive as the porcine prevalence falls. At this point additional incentives may be required to retain farmers in such programs.

Health education is vital to the success of any control strategy for *T. solium*. Despite the ICER analysis indicating that health education as a control strategy is highly expensive in comparison with other options, it is likely to be vital for the implementation of any of the alternative strategies we suggest.

An educated consumer is also able to influence food production practices through the willingness to pay a price premium for pork perceived to be safe (Röhr *et al.*, 2005). Obviously a poor community such as this in western Kenya is predominately “price-sensitive” and maybe unable to pay such a premium for standard food products. Pork, however, is a relatively new product in east Africa, is more expensive than beef and is therefore potentially consumed by wealthier families. In Kigali (Rwanda) pork is known as “Benz” (as in Mercedes Benz), denoting its status as a premium product (Peters, 2008). It is therefore anticipated that as disposable incomes increase, an educated consumer base willing to pay a price premium, may provide an incentive to farmers to adopt control policies, as well as potentially encouraging the use of the formal market opposed to the informal, “black” market.

The presence of *T. solium* in the porcine population combined with the poor risk mitigation shown by the pork industry as presently structured in western Kenya poses
a significant public health hazard and requires concerted activity from policy makers and other stakeholders alike to address.

The risk of acquiring a neurocysticercosis infection is increased not only in those who have a history of an adult *T. solium* infection (Sarti *et al.*, 1992) but also in those living within the vicinity of a taeniasis case (Lescano *et al.*, 2009; O’Neal *et al.*, 2012). The implication of this is that any one person acquiring an adult *T. solium* infection has the potential to infect many more people with neurocysticercosis, as well as providing a source of infective material that can be consumed by pigs, propagating the parasitic life-cycle.

Eliminating the source of human *T. solium* infection by reducing the risk of infective pork entering the food chain, or ensuring safe preparation of pork has the potential to make a drastic impact on the disease burden imposed by cysticercosis infections.

### 3.6 Conclusion

A quantitative risk assessment such as that presented here provides a transparent and reproducible way of assessing both the current state of risk from a food product, as well as a method by which intervention strategies can be evaluated. The model suggests that one pork meal being consumed in western Kenya has a 0.06 probability of containing at least one infectious *T. solium* cysticercus. This is far beyond what we would consider an acceptable level risk.

We have evaluated a variety of intervention strategies in terms of their ability to reduce the number of potentially infective events which occur each year in the study area and for their cost-effectiveness in terms of ICER. The most cost-effective control measure may be obtained if statutory legislation is enforced, whereby the addition of a pig vaccination campaign has the ability to reduce the number of infected meals consumed by 78.3% (95% C.I. 55.9-95.3).

In areas where porcine prevalence is high this intervention may actually provide a saving to farmers of $-0.04 (95% C.I. -0.43-0.25) per infective meal avoided, by avoiding economic losses due to carcass condemnation. The economic advantage of vaccination campaigns fall as the prevalence in pigs drops and at low prevalences improving meat inspection services alone is the most cost effective intervention strategy.

The biggest barrier to achieving *T. solium* control through the pig production system,
is the uptake of these technologies by pig farmers and traders. Effective incentives for producing “clean” pigs or dis-incentives for presenting infective pigs at slaughter are required for the success of these programs, both of which may be achieved through the enforcement of current legislation and the education of communities about the risks posed by this parasite. The global public health community must therefore act to encourage the adoption of such strategies and work towards making suitable technologies, such as vaccinations and easy-to-use diagnostics, available to those who need them.
Chapter 4

Prevalence and Risk Factors for *T. solium* Infections in the Human and Porcine Populations of Western Kenya
CHAPTER 4. PREVALENCE AND RISK FACTORS FOR TAENIA SOLIUM

4.1 Abstract

4.1.1 Background

The zoonotic helminth Taenia solium is one of the 17 “neglected tropical diseases” for which a “road map for control” has been devised, with validated control strategies being required, ready to roll out by 2020 (World Health Organization, 2012a). Baseline epidemiological data is still missing, with few studies having investigated the prevalence of T. solium cysticercosis and taeniasis in the human host at a community level (Johansen and Mejer, 2010). In order to address this paucity of data, a cross-sectional study has been undertaken which investigated the epidemiology of this parasite in both the human and porcine host.

4.1.2 Methodology & Results

An extensive, community-based cross-sectional study was undertaken between July 2010 and July 2012, across several districts of western and Nyanza provinces of Kenya (Doble and Fèvre, 2010) into which 2113 human participants and 93 pigs were recruited from 416 homesteads. The prevalence of human cysticercosis, as detected by HP10 antigen ELISA was found to be 6.6% (95% C.I. 5.5-7.7%) and taeniasis, as detected by Copro-antigen ELISA to be 19.9% (95% C.I. 18.2-21%). Porcine cysticercosis was found in 17.2% (95% C.I. 10.2-26.4%) of pigs. The study area was also found to be endemic with many of the other neglected tropical diseases, namely Hookworm (31.7%, 95% C.I. 29.7-33.8%), Trichuriasis (10.0%, 95% C.I.8.8-11.4%), Ascariasis (8.4%, 95% C.I. 7.3-9.7%) , Schistosomiasis (6.8%, 95% C.I. 5.8-7.9%), amoebiasis (34.8%, 95% C.I. 32.8-36.9) and strongyloidiasis (3.2%, 95% C.I. 2.5-4.0%).

4.1.3 Conclusion

This study has demonstrated that all stages of the life cycle of T. solium are present in this region of western Kenya, and that the area is co-endemic for many of the neglected tropical diseases. The data presented here provide justification for an integrated control strategy considering multiple pathogens.
4.2 Background

The zoonotic helminth, *Taenia solium*, has a two host life cycle with humans as the definitive host, who, after consumption of under-cooked pork, harbor the adult tapeworm in an infection known as taeniasis. Gravid segments, proglottids, containing approximately 50,000 eggs with infective embryophores, detach from the adult tapeworm and are excreted in faeces in an intermittent fashion (Garcia *et al.*, 2003).

Ingestion of eggs by either pigs or humans results in the larval stage encysting in tissues as cysticerci. Porcine cysticercosis is characterised by cysts found in the heart, diaphragm, masseters thigh and loin musculature and was recognised as early as 380BC (Hawk *et al.*, 2005). Humans, as an aberrant intermediate host, can also harbor cysticerci both in muscle but also in ocular and nervous tissues, although the reason for the predilection for the central nervous system in man is, as yet, unknown (Sciutto *et al.*, 2000). Large numbers of larval stages encysting in muscles can cause stiffness, pain and muscular pseudohypertrophia (Garcia *et al.*, 2003). Ocular cysticercosis can cause disturbances in vision, but the greatest morbidity is that caused by neurocysticercosis, the most common manifestation of which is epileptic seizures (Quet *et al.*, 2009).

Neurocysticercosis is thought to be responsible for up to 29% of all epilepsy cases in endemic areas (Ndimubanzi *et al.*, 2010), with between 1.9-6.16 million people being affected in sub-Saharan Africa (SSA) alone (Winkler, 2012). The Global Burden of Disease study 2010 (GBD2010) has for the first time estimated a DALY score, for *T. solium* which suggests that globally the parasite is responsible for 503,000 (379,000-633,000) DALYs lost, or 7 (5-10) DALYs lost/100,000 people (Murray *et al.*, 2013).

In recognition of the burden this parasite imposes in the developing world, *T. solium* has been recently included in a road-map for the control of 17 previously neglected tropical diseases in which the global community declares that by 2015 a validated control strategy will have been devised, and control will be scaled up in selected African, Asian and Latin American countries by 2020 (World Health Organization, 2012a).

The epidemiological picture for this parasite in sub-Saharan Africa (SSA) is becoming clearer, especially in the porcine population, and it appears that apart from in Muslim areas, *T. solium* is present in practically all countries in the region (Phiri *et al.*, 2003; Shey-Njila *et al.*, 2003). Estimates of porcine cysticercosis prevalence in SSA vary greatly within the region, although many countries appear to have hyper-endemic status, with prevalence estimates of up to 56.7% being reported (Krecek *et al.*, 2011). Human cysticercosis infections have also been reported in the majority of SSA coun-
ties, although valid epidemiological data from community based studies are scarce and under-reporting at a country level appears to be high.

A Gap Analysis completed at the 2nd meeting of the European working group on taeniasis/cysticercosis identified a requirement for more data on the prevalence and risk factors for the parasite in humans (Johansen and Mejer, 2010). In order to devise and implement control strategies which are valid at a regional level, the gaps in our epidemiological knowledge about the parasite need to be filled. This chapter aims to address this paucity of data for western Kenya.

4.3 Methods and Materials

This study forms part of a larger project which investigated a range of zoonotic diseases within livestock and their keepers within Western Kenya; the 'PAZ' Project (People, Animals and their Zoonoses) (Doble and Fèvre, 2010). This study was located within the Western and Nyanza provinces of Kenya, the study site and location of recruited homesteads can be found in Figure 2.1 on page 37 and is described in detail in Section 2.2 on page 35.

The study consisted of an extensive cross-sectional survey, described in Section 2.3 on page 35, in which the unit of study was the homestead, with all eligible persons (over the age of 5yrs and not in the 3rd trimester of pregnancy), and pigs (excluding pregnant animals in their 3rd trimester and suckling piglets) being recruited into the study.

4.3.1 Data collection at the homestead

Selected homesteads were recruited into the study on permission of the head of each homestead. Each eligible person in the homestead was then recruited once informed consent had been provided by the individual or, in the case of children under 14 yrs, by the parent or guardian. The data collection is described in detail in Section 2.3 on page 35 and the key data utilised in this chapter are briefly described here.

A homestead level questionnaire was administered to the head of the household. This questionnaire collected data in the following categories:

- Animal ownership, herd level husbandry and veterinary care
- Homestead water provision and treatment
4.3. METHODS AND MATERIALS

- Access to healthcare
- Wealth indicators

Each recruited individual then partook in an individual level questionnaire, with the following categories:

- Demographic data
- Potential exposures to zoonotic diseases
- Self reported health data

At the end of the questionnaire, each participant underwent a physical examination by a community health officer or clinical officer and blood and faecal samples were collected.

For each pig recruited into the study, questionnaire data were collected from the person reporting most involvement with the care of the animal. Data were collected on:

- age, breed & sex of pig
- clinical history of the pig, including prophylactic treatments

and was followed by a brief physical examination and collection of blood and faecal samples.

4.3.2 Laboratory analysis of samples

Preliminary processing of all samples and microscopy for the detection of hemoparasites and gastrointestinal parasites in humans and pigs was performed at the field laboratory in Busia, western Kenya. At a later date anonymous, second-level testing was carried out at the ILRI laboratory, Nairobi, Kenya. Sample collection, processing and analysis are all described in detail in Chapter 2 on page 33, Section 2.5 on page 45. Detection of *Taenia* spp. was carried out using the following techniques:

- Microscopy (Formal ether concentration & Kato-Katz technique)
- HP10 Antigen-capture ELISA (Harrison *et al.*, 1989)
Copro Antigen-capture ELISA (Allan et al., 1996)

Sera samples from humans testing positive for taeniasis on copro-Antigen ELISA and a random selection of negative samples were sent to the Center for Disease Control (CDC) laboratory in Washington DC, USA, for testing by rES33 EITB for the detection of *T. solium* taeniasis. The analysis was performed by Dr John Noh in the laboratory of Dr Patty Wilkins according to the protocol described by Levine et al., (Levine et al., 2007).

### 4.3.3 Statistical Analysis

Field and laboratory data were entered into Microsoft Access® databases and statistical analysis was carried out using the “R” environment for statistical computing (R Development Core Team, 2005) with the packages “lme4” (Bates et al., 2012), “plyr” (Wickham, 2011), “epicalc” (Chongsuvivatwong, 2011) and “epiR” (Stevenson et al., 2013).

The diagnostic assays used for the detection of cysticercosis and taeniasis are not 100% sensitive or specific and the estimates of their performance can be found in Table 4.1 on the facing page. The “epi.prev” function in epiR (Stevenson et al., 2013) was used to incorporate the estimated sensitivity and specificity of these diagnostic assays into the estimates of prevalence. Confidence intervals for the prevalence estimates for all data were calculated to take account for the clustered nature of the data (individuals within homesteads) using the “epi.conf” function in epiR (Stevenson et al., 2013) where the design effect was calculated as shown in Equation (4.3.3).

\[
\text{design} = ICCx(\bar{n} - 1) + 1
\]

Where: ICC = intra-cluster correlation, calculated using the 'ICCbare' function from the ICC package (Wolak et al., 2012), and \(\bar{n}\) = mean number of individuals per cluster (4.1)
<table>
<thead>
<tr>
<th>Diagnostic Assay</th>
<th>Host &amp; Pathogen</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HP10 Ag ELISA</td>
<td>Cysticercosis&lt;sup&gt;2&lt;/sup&gt;</td>
<td>70.4 (52.7-84.7)</td>
<td>66.1 (0.446 0.851)</td>
<td>(Krecek et al., 2011)</td>
</tr>
<tr>
<td></td>
<td>Cysticercosis&lt;sup&gt;1&lt;/sup&gt;</td>
<td>84.8 (74.4-95.2)</td>
<td>94 (90.2-97.8)</td>
<td>(Fleury et al., 2007)</td>
</tr>
<tr>
<td>Lingual palpation</td>
<td>Cysticercosis&lt;sup&gt;2&lt;/sup&gt;</td>
<td>7.3 (0.008-0.151)</td>
<td>80.8 (0.710-0.904)</td>
<td>(Krecek et al., 2011)</td>
</tr>
<tr>
<td>Copro-Ag ELISA</td>
<td>Taeniasis&lt;sup&gt;1&lt;/sup&gt;</td>
<td>84.5 (61.9-98)</td>
<td>92 (90-93.8)</td>
<td>(Praet et al., 2013)</td>
</tr>
<tr>
<td>Microscopy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Formal ether concentration</td>
<td>Taeniasis&lt;sup&gt;1&lt;/sup&gt;</td>
<td>52.5 (11.1-96.5)</td>
<td>99.9 (99.5-100)</td>
<td>(Praet et al., 2013)</td>
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<tr>
<td></td>
<td>Schistosomiasis&lt;sup&gt;1&lt;/sup&gt;</td>
<td>85.0 (78.391.6)</td>
<td>100</td>
<td>(Glinz et al., 2010)</td>
</tr>
<tr>
<td></td>
<td>Amebiasis&lt;sup&gt;1&lt;/sup&gt;</td>
<td>39.1 (27.6-51.6)</td>
<td>100</td>
<td>(Nesbitt et al., 2004)</td>
</tr>
<tr>
<td></td>
<td>Strongyloidiasis&lt;sup&gt;1&lt;/sup&gt;</td>
<td>57.1 (37.2-75.5)</td>
<td>100</td>
<td>(Anammart et al., 2010)</td>
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<td></td>
<td>Hymenolepis spp.&lt;sup&gt;1&lt;/sup&gt;</td>
<td>88.6 (73.3-96.8)</td>
<td>100</td>
<td>(Ahmadi and Paklad, 2007)</td>
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<td></td>
<td>Blastocystic hominis&lt;sup&gt;1&lt;/sup&gt;</td>
<td>20.5 (9.8-36.9)</td>
<td>100</td>
<td>(Suresh and Smith, 2004)</td>
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<td>Kato-Katz technique</td>
<td>Asariasis&lt;sup&gt;1&lt;/sup&gt;</td>
<td>88.1 (84.2-91.5)</td>
<td>100</td>
<td>(Levecke et al., 2011)</td>
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<tr>
<td></td>
<td>Trichuriasis&lt;sup&gt;1&lt;/sup&gt;</td>
<td>82.6 (78.1-86.3)</td>
<td>100</td>
<td>(Levecke et al., 2011)</td>
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<td></td>
<td>Hookworm&lt;sup&gt;1&lt;/sup&gt;</td>
<td>78.3 (73.3-82.7)</td>
<td>100</td>
<td>(Levecke et al., 2011)</td>
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<td></td>
<td>Fascioloiasis&lt;sup&gt;1&lt;/sup&gt;</td>
<td>11.9 (5.3-22.2)</td>
<td>100</td>
<td>(Martínez-Sernañez et al., 2011)</td>
</tr>
<tr>
<td>McMasters technique</td>
<td>Asariasis&lt;sup&gt;2&lt;/sup&gt;</td>
<td>68.4 (61.3-75)</td>
<td>100</td>
<td>(Vadlejch et al., 2013)</td>
</tr>
<tr>
<td></td>
<td>Trichuriasis&lt;sup&gt;1&lt;/sup&gt;</td>
<td>80.3 (75.7-84.4)</td>
<td>100</td>
<td>(Levecke et al., 2011)</td>
</tr>
<tr>
<td></td>
<td>Coccidiosis&lt;sup&gt;3&lt;/sup&gt;</td>
<td>93.3 (90.6-95.5)</td>
<td>100</td>
<td>(Vadlejch et al., 2013)</td>
</tr>
<tr>
<td></td>
<td>Strongyles&lt;sup&gt;5&lt;/sup&gt;</td>
<td>73</td>
<td>84</td>
<td>(Andersen et al., 2012)</td>
</tr>
<tr>
<td></td>
<td>Strongyloides&lt;sup&gt;6&lt;/sup&gt;</td>
<td>86 (67-95)</td>
<td>100</td>
<td>(Marra et al., 2010)</td>
</tr>
<tr>
<td>Hematocrit concentration</td>
<td>Trypanosomiasis&lt;sup&gt;2&lt;/sup&gt;</td>
<td>13 (4.9-26.3)</td>
<td>100 (89.6-100)</td>
<td>(Ogunsannmi et al., 2000)</td>
</tr>
<tr>
<td></td>
<td>Trypanosomiasis&lt;sup&gt;1&lt;/sup&gt;</td>
<td>86 (79-93.3)</td>
<td>100</td>
<td>(Wery and Mulumba, 1989)</td>
</tr>
<tr>
<td>Ziehl-Neelsen staining</td>
<td>Cryptosporidium parvum&lt;sup&gt;1&lt;/sup&gt;</td>
<td>83.7 (64-91.8)</td>
<td>98.9 (97.6-99.7)</td>
<td>(Morgan et al., 1998)</td>
</tr>
<tr>
<td>Thin &amp; Thick Smear</td>
<td>Malaria&lt;sup&gt;1&lt;/sup&gt;</td>
<td>95 (91.8)</td>
<td>97 (95100)</td>
<td>(Ohrt et al., 2007)</td>
</tr>
<tr>
<td></td>
<td>Theileriosis&lt;sup&gt;4&lt;/sup&gt;</td>
<td>77 (70-83)</td>
<td>100</td>
<td>(Martin-Sanchez et al., 1999)</td>
</tr>
<tr>
<td>SD Bioline</td>
<td>HIV&lt;sup&gt;1&lt;/sup&gt;</td>
<td>100 (99.1-100)</td>
<td>99.4 (98.8-99.7)</td>
<td>(Lyamuya et al., 2009)</td>
</tr>
</tbody>
</table>

Table 4.1: Estimates of Sensitivity and Specificity for Diagnostic Assays Used in this Study

<sup>1</sup>human data, <sup>2</sup>porcine data, <sup>3</sup>avian data, <sup>4</sup>cattle data, <sup>5</sup>equine data, <sup>6</sup>rodent data
Risk factors for Taenia spp. infections were identified using multivariate logistic regression models built in R. Variables were first excluded from analysis if they were strongly correlated with another variable of interest, for example the use of borehole water in the wet and dry season were strongly associated ($\chi^2 = 1960, p < 0.001$), and so only the wet season water sources was used and the number of people in a homestead was strongly associated with the number of dwellings in that homestead ($t = 55.2, p < 0.001$) and so the number of dwellings in the homestead was excluded from the analysis.

Predictor variables were then assessed for association with the outcomes of interest using chi-square for categorical data and t-test for continuous data. The association of predictor variables with the disease status, both of individuals and of the homestead unit, were tested in a univariate fashion, with those factors with a $p$ value $< 0.2$ being then used to fit a multivariate logistic regression model. For individual level models variables were included at both an individual and a homestead level, whereas for the homestead models only homestead level data were included.

To account for the clustered nature of the data (i.e. individuals within homes) a multivariate mixed effects logistic regression model was fitted using the lme4 package (Bates et al., 2012) with homestead_id included as a random effect, no random effect was included when modeling disease status at the homestead level.

Models were built using a forwards step-wise selection, beginning with an empty model and adding each variable in turn, retaining those who remained significant in the model. Model selection for mixed-effect models (for individual level risk factors) was done manually, with the homestead level models selected using the 'step' function of the 'glmer' package. The model with the smallest Akaike second-order information criterion (AIC) is then selected as the working model, from which the odds ratios, confidence intervals and $p$ values were extracted.

4.3.4 Spatial Analysis of Taeniasis and Cysticercosis Cases

All homesteads in the study were included in a spatial point pattern in the R environment for statistical computing (R Development Core Team, 2005), each marked with the status of the homestead (negative or positive) in regards to taeniasis, human cysticercosis or porcine cysticercosis. The SpatStat package (Baddeley and Turner, 2005) was used to calculate Ripleys K function (Dixon, 2002), to look for clustering of cases of each disease against the null hypothesis of no-clustering, as determined by the random labeling technique, where the underlying distribution of homesteads is taken
into account, rather than assuming a null distribution of complete spatial randomness (Dixon, 2002). This analysis can tell us whether clustering exists in the spatial pattern, but not where this clustering occurs.

4.4 Results

4.4.1 Descriptive Data

Between July 2010 and July 2012, 2113 people from 416 homesteads were recruited into the study. The study site exemplifies a typical mixed crop-livestock ecosystem, with 97.6% (95% C.I. 95.6-98.8) of homes growing crops, and 66.8% (95% C.I. 62.1-71.3) of homes keeping livestock, rising to 87.3% (95% C.I. 83.7-90.3) of homes when poultry are included. Cattle are kept by 55.3% (95% C.I. 50.4-60.1) of homesteads, cats by 48.8% (95% C.I. 43.9-53.7), small ruminants by 35.6% (95% C.I. 31-40.4) and dogs by 35.1% (95% C.I. 30.5-39.9). 16.6% (95% C.I. 13.1-20.5) of homesteads owned pigs at the time of this study. Pig keeping was found to be significantly ($p =0.019$) clustered using the Ripleys K function against a null hypothesis of random labeling, as shown in Figure 4.1 on the next page.

The average number of people living in a homestead unit is 7.3 (95% C.I. 6.9-7.7), with homes which keep livestock being significantly larger (8.14 people, 95% C.I. 7.6-8.6) than the average non-livestock keeping homestead (5.62, 95% C.I. 5.1-6.2), $p <0.0001$. A significant difference was also found between the average size of pig-keeping homesteads (9.53 people, 95% C.I. 8.5-10.6) compared to non-pig-keeping homes (6.85, 95% C.I. 5.4-7.3), $p <0.0001$.

The homesteads in the study population have on average 2.5 (95% C.I. 2.3-2.6) inhabited dwellings, 75% (95% C.I. 70.5-79.1) have access to a latrine, of which the majority (64.4%, 95% C.I. 58.8-69.7%) are partially enclosed, 29.2% (95% C.I. 24.2-34.6) of which were completely enclosed and 6.4% (95% C.I. 4.9-7.7) were open pit latrines.

The most common sources of homestead water were boreholes or springs across both the wet and dry seasons, with other sources of water being rivers, wells, piped, pond or dam and roof capture. Water is treated before consumption in 58.9% (95% C.I. 54-63.7) of homes, with 72.7% (95% C.I. 66.6-78.1) of homesteads which do treat drinking water, using chlorination (water guard or aquatabs), 31% (95% C.I. 25.3 - 37.2) filtering and 9% (95% C.I. 5.7-13.3) boiling. (N.B multiple types of water treatment could be
CHAPTER 4. PREVALENCE AND RISK FACTORS FOR TAENIA SOLIUM

Figure 4.1: Ripley’s K-test for Spatial Randomness, Pig-keeping Homes
Pig keeping homes can be seen to fall outside the confidence interval of the null hypothesis (random labeling) at distances of \( r = 0.05 - 0.15 \) (\( p = 0.019 \)) and showing the distribution of pig-keeping and non pig-keeping homes.
4.4. RESULTS

Much of the land utilised by the study population would be considered marginal, being at risk of natural disasters, with 19.9% (95% C.I. 16.2-24.1) of homesteads having experienced flooding (to an extent that it damaged crops) in the last 12 months and 21.9% (95% C.I. 18-26.2) of homesteads having experienced a drought (which killed crops) in the last 12 months.

**Human descriptive data**

The population comprises of four major tribal groups, being: the Lyhya tribe, making up 50.2% (95% C.I. 45.2 - 55.3) of the population, the Luo, Teso and Samia make up 21.9% (95% C.I. 17.5-26.3), 14.5% (95% C.I. 10.7 - 18.2) and 12.6% (95% C.I. 9.1 - 15.8) of the population respectively with the remaining 0.6% (95% C.I. 0.15-1.36) of the population comprising of members of a variety of other tribes not indigenous to the area, including Kalenjin, Kisii, Kikuyu, Pokot, Saboat and Turkana. The major religion in the study area is Christianity (95.8%, 95% C.I. 94.1-97.5), including Roman Catholic, Pentecostal, Protestant, Baptist and other Christian denominations, with a small proportion of Muslims, (1.9%, 95% C.I. 0.5-3.4) and members of tribal religions (0.05%, 95% C.I. 0-0.13). 46.2% (95% C.I. 44.6-47.8) of participants were male and the average age in the population is 23.5 years (95% C.I. 22.7 - 24.4). The population pyramid for study participants is shown in Figure 4.2 on the following page.

Almost half (46.8%, 95% C.I. 41.5-45.5) of the study population are currently students. Of the remaining population, the majority (57.4%, 95% C.I. 54.4-60.5) described their major occupation as farming. Farming is more common (P<0.0001) in women over 20yrs (71.9%, 95% C.I. 66.8-77.1) than men over 20yrs (58.2%, 95% C.I. 53.1-63.2). The majority of the population have achieved at least a pre-school level of education, with only 9.4% (95% C.I. 7.7-11) having had no education and 2.4% (95% C.I. 1.5-3.3) having achieved a tertiary level of education or above.

The majority of the population (86.2%, 95% C.I. 84.6-87.9) are meat eaters, beef being consumed by 85.4% (95% C.I. 93.5-87.2) of the population and 65.6% (95% C.I. 62.4-68.9) of the population consuming pork. The frequency of consumption for beef and pork are shown in Table 4.2 on the next page. Older (over 20yrs) people are more likely to eat meat, including beef (p<0.0001) than those under 20yrs. Pork is significantly more likely to be consumed by older men than any other group of people (p<0.0001). The majority (89.8%, 95% C.I. 88.2-91.4) of meat eating participants obtain meat from
CHAPTER 4. PREVALENCE AND RISK FACTORS FOR TAENIA SOLIUM

Figure 4.2: Population Pyramid for Human Population

formal channels (butchery or market), with 15.9% (95% C.I. 13.1-18.7) obtaining meat through informal channels (from neighbors, family or their own animals).

A small proportion of the population (15%, 95% C.I. 13.8-18) are aware that you can obtain diseases from animals, with men over 20yrs most likely to be aware of this risk (p <0.0001). Only 3.2% (95% C.I. 2.3-4.1) of the population are aware of the possibility of contracting tapeworm from meat. 5% (95% C.I. 4.1-6.1) of the population report having had a tapeworm infection at one point. Open defecation is practised at least some of the time by 36.4% (95% C.I. 33-39.9) of the population, with open defecation practised more often by people under 20yrs (p<0.001). Occurrence of potential risk factors for exposure to *T. solium* in humans are summarised in Table 4.3 on page 100.

<table>
<thead>
<tr>
<th>Frequency of consumption</th>
<th>Prevalence % (95% C.I.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily</td>
<td>0.8 (0.2-1.4)</td>
</tr>
<tr>
<td>At least once weekly</td>
<td>37 (33.9-40)</td>
</tr>
<tr>
<td>At least once monthly</td>
<td>51.3 (47.6-54.9)</td>
</tr>
<tr>
<td>At least once yearly</td>
<td>6 (4.2-7.7)</td>
</tr>
<tr>
<td>On special Occasions</td>
<td>2.1 (1.1-3.1)</td>
</tr>
</tbody>
</table>

Table 4.2: Frequency of Meat Consumption
124 individuals tested positive for HIV on the SD Bioline HIV-1/2 Fast 3.0 test strips (Standard Diagnostics Inc, Korea). When adjusted for the diagnostic test sensitivity and specificity and for the clustered nature of the data the population prevalence is estimated at 5.9% (95% C.I. 4.6-7.3%). HIV infection is more common people over 20 years of age ($p<0.001$) and more common in females over 20yrs (13.2%, 95% C.I. 10.4-16) than males over 20yrs ($p<0.001$).

A summary of all parasitic infections identified in the study population can be found in Table 4.4 on page 101.
## Table 4.3: Prevalence of potential Risk Factors for *Taenia* spp. Exposure Reported by Study Participants

<table>
<thead>
<tr>
<th>Exposure**</th>
<th>Female Sub-Adult</th>
<th>Female Adult</th>
<th>Male Sub-Adult</th>
<th>Male Adult</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>Consume meat (Any)**</td>
<td>78.4 (72.4-84.4)</td>
<td>94.1 (91.2-97)</td>
<td>77.7 (73.3-82)</td>
<td>97.3 (95.6-98.8)</td>
<td>86.2 (84.6-87.9)</td>
</tr>
<tr>
<td>Consume pork</td>
<td>60.1 (52.4-67.8)</td>
<td>62.1 (56.1-68.1)</td>
<td>64 (57.6-70.3)</td>
<td>80.4 (73.1-87.7)</td>
<td>65.6 (62.4-68.9)</td>
</tr>
<tr>
<td>Consume undercooked pork</td>
<td>5.5 (3.7-7.4)</td>
<td>5.7 (2.8-8.6)</td>
<td>8.9 (5.6-12.4)</td>
<td>8.8 (4.7-13)</td>
<td>7.1 (5.5-8.8)</td>
</tr>
<tr>
<td>Consume pork with cysticerci**</td>
<td>9 (6.6-11.5)</td>
<td>7.2 (5.1-9.3)</td>
<td>12.8 (10.1-15.6)</td>
<td>14.2 (10.8-17.6)</td>
<td>10.5 (9.2-11.8)</td>
</tr>
<tr>
<td>Consume beef**</td>
<td>77.1 (70.2-84)</td>
<td>93.3 (90.2-96.4)</td>
<td>76.4 (70.3-82.6)</td>
<td>96.8 (95.1-98.5)</td>
<td>85.4 (83.5-87.2)***</td>
</tr>
<tr>
<td>Consume undercooked beef</td>
<td>5.9 (3.5-8.3)</td>
<td>7.4 (4.3-10.5)</td>
<td>8.4 (5.4-11.5)</td>
<td>8.1 (3.7-12.4)</td>
<td>7.6 (6-8.8)</td>
</tr>
<tr>
<td>Consume meat prepared outside of the homestead**</td>
<td>60.8 (54.5-67.1)</td>
<td>79.2 (75.4-83)</td>
<td>65.1 (59.7-70.)</td>
<td>90.4 (86.8-94)</td>
<td>64.9 (62.8-66.9)</td>
</tr>
<tr>
<td>Drink animal blood</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Do not treat drinking water</td>
<td>17.3 (11.2-23.4)</td>
<td>20.3 (16.1-24.6)</td>
<td>19.9 (14.4-25.5)</td>
<td>20.8 (14.2-27.5)</td>
<td>19.5 (16.6-22.5)</td>
</tr>
<tr>
<td>Report open defecation**</td>
<td>40.2 (33.4-47)</td>
<td>26.2 (19.9-32.4)</td>
<td>49 (42.2-55.8)</td>
<td>28.7 (20.2-37.3)</td>
<td>36.4 (33-39.9)</td>
</tr>
<tr>
<td>Engage in hunting**</td>
<td>0.7 (0-1.9)</td>
<td>0.7 (0-1.3)</td>
<td>14.3 (10.3-18.2)</td>
<td>8.8 (6.1-11.6)</td>
<td>5.9 (4.8-7.1)***</td>
</tr>
<tr>
<td>Slaughtering animals**</td>
<td>8 (4.5-11.4)</td>
<td>19.2 (14.4-24.0)</td>
<td>12.7 (9.4-16)</td>
<td>26.4 (20.7-32)</td>
<td>16 (13.6-18.3)</td>
</tr>
<tr>
<td>Self-reported tapeworm infection</td>
<td>4.2 (2.3-6.2)</td>
<td>4.4 (2.7-6)</td>
<td>4.9 (2.8-7)</td>
<td>7.7 (5.1-10.3)</td>
<td>5.1 (4.1-6.1)</td>
</tr>
<tr>
<td>Self reported worm infection (any)**</td>
<td>25 (18.9-31.2)</td>
<td>18.6 (14.3-22.8)</td>
<td>29 (23.7-34.2)</td>
<td>18.7 (14.1-23.3)</td>
<td>23.3 (21-25.6)</td>
</tr>
<tr>
<td>Unaware of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zoonotic diseases**</td>
<td>90.2 (86.3-94)</td>
<td>83.6 (79.5-87.7)</td>
<td>88.4 (85.2-91.6)</td>
<td>74.6 (70.4-78.9)</td>
<td>89.1 (87.3-90.9)</td>
</tr>
<tr>
<td>Taeniasis risk from meat**</td>
<td>98.7 (97.7-99.7)</td>
<td>95.1 (92.7-97.5)</td>
<td>98.8 (97.9-99.7)</td>
<td>94.1 (89.9-98.2)</td>
<td>96.8 (95.9-97.7)</td>
</tr>
</tbody>
</table>

*Confidence intervals adjusted for clustering in data, **Significant difference between stratified prevalence P<0.05, **p<0.01, ***p<0.001, ***includes participants who reported that they used to engage in activity.
### 4.4. RESULTS

**Prevalence (95% C.I.)***

<table>
<thead>
<tr>
<th>Infection**</th>
<th>Dx.***</th>
<th>Female</th>
<th>Male</th>
<th>Unadjusted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sub-Adult</td>
<td>Adult</td>
<td>Sub-Adult</td>
<td>Adult</td>
</tr>
<tr>
<td>Cysticercosis</td>
<td>HP10</td>
<td>3 (1.6-4.8)</td>
<td>1.4 (0.3-2.4)</td>
<td>0.2 (0-0.5)</td>
</tr>
<tr>
<td>Taeniasis</td>
<td>CAg</td>
<td>12 (8.8-15.2)</td>
<td>13.6 (10.7-16.6)</td>
<td>20.7 (16.6-24.7)</td>
</tr>
<tr>
<td></td>
<td>FE</td>
<td>0.2 (0-0.6)</td>
<td>0.9 (0.1-1.6)</td>
<td>0 (0-1.1)</td>
</tr>
<tr>
<td>Strongyloidiasis</td>
<td>FE</td>
<td>0.3 (0-0.8)</td>
<td>5.7 (3.8-7.7)</td>
<td>4.4 (2.7-6.1)</td>
</tr>
<tr>
<td>Schistosomiasis</td>
<td>FE</td>
<td>7.2 (4.7-9.6)</td>
<td>5.7 (3.7-7.7)</td>
<td>8.4 (5.8-11)</td>
</tr>
<tr>
<td>Fascioliasis</td>
<td>KK</td>
<td>1.5 (0.5-2.5)</td>
<td>0 (0-5.7)</td>
<td>0 (0-5.8)</td>
</tr>
<tr>
<td>Trichuriasis</td>
<td>FE</td>
<td>15.2 (11.9-18.5)</td>
<td>13 (10.2-14.9)</td>
<td>12.2 (9.2-15.2)</td>
</tr>
<tr>
<td>Ascariasis</td>
<td>KK</td>
<td>12.6 (9.5-15.7)</td>
<td>8.6 (6.3-10.8)</td>
<td>12 (9.1-15)</td>
</tr>
<tr>
<td>Hookworm</td>
<td>KK</td>
<td>28.5 (24.4-32.7)</td>
<td>38.5 (34.4-42.6)</td>
<td>32.3 (28.2-36.5)</td>
</tr>
<tr>
<td>Giardiasis</td>
<td>FE</td>
<td>10.2 (7.7-12.8)</td>
<td>2.3 (1.1-3.4)</td>
<td>15.2 (12.2-18.3)</td>
</tr>
<tr>
<td><em>Hyminolepis</em> spp.</td>
<td>FE</td>
<td>0.4 (0-0.9)</td>
<td>0 (0-0.7)</td>
<td>0.4 (0-1)</td>
</tr>
<tr>
<td>Amoebiasis</td>
<td>FE</td>
<td>97.9 (96.7-99.2)</td>
<td>97.2 (95.8-98.6)</td>
<td>85 (82-88)</td>
</tr>
<tr>
<td><em>Blastocystis</em> spp.</td>
<td>FE</td>
<td>2.8 (1.2-4.5)</td>
<td>2.5 (1.2-3.7)</td>
<td>2.6 (0-17.4)</td>
</tr>
<tr>
<td>Cryptosporidiosis</td>
<td>ZN</td>
<td>0 (0.0-0.3)</td>
<td>0 (0.0-0.8)</td>
<td>0 (0-0.6)</td>
</tr>
<tr>
<td>Trypanosomiasis</td>
<td>Tn</td>
<td>0.04 (0-0.08)</td>
<td>0 (0-0.8)</td>
<td>0 (0-0.8)</td>
</tr>
<tr>
<td>Malaria</td>
<td>Tk</td>
<td>46.5 (42.2-50.7)</td>
<td>8.9 (6.5-11.3)</td>
<td>46.4 (42.2-50.6)</td>
</tr>
</tbody>
</table>

Table 4.4: **Prevalence of Human Parasitic Infections Detected in the Study Site**

*Adjusted for test sensitivity & for clustering. **Significant difference between stratified prevalence\(^1\) P<0.05, \(^2\)p<0.01, \(^3\) p<0.001, ***CAg = Copro-Ag ELISA n = 2003, HP10 = HP10 Ag-ELISA n = 2094, FE = formal-ether concentration n=2056, ZN = Ziehl Neelson n=2057, KK = Kato-Katz n=2011, Tn = thin smear n = 2111, Tk = thick smear n= 2113*
Porcine Descriptive Data

Pig owning homesteads (n=68) had an average herd size of 2.6 pigs (95% C.I. 2.3-3.2). Pigs were kept in order to sell piglets or to sell slaughter-weight animals for meat, with only one farmer reporting keeping pigs for their own consumption. Approximately 1/3 of pigs were bred on the homestead, with the majority (71%) being purchased from another homestead.

Pig husbandry was consistent across the dry and wet seasons (p = 1 for all variables). The most common production system was found to be tethering, practiced by 92.5% (95% C.I. 83.4-97.5) of farmers. Free range pig production is practiced by 13.2% (95% C.I. 6.2-23.6) of farmers, with only 2.9% (95% C.I. 0.4-10.2) of farmer using a pig pen, despite 16.2% (95% C.I. 8.4-27.1) having a pen available, the spatial ecology of pigs kept under free-range systems was investigated further as part of this thesis, see Chapter 6 on page 141.

92.6% (95% C.I. 83.7-97.6) of pig farmers utilised waste food (from their own home, neighbors homes or business premises) to feed their pigs, with only 1.5% (95% C.I. 0-8) of farmers using commercial feedstuffs for pigs. Of those farmers feeding waste food, 65% (95% C.I. 52-76.7) reportedly cook this waste before feeding to the pig.

Almost half (47.8%, 95% C.I. 34.8-59.6) of pig farmers report use of prophylactic worm control, although 97% (95% C.I. 79.2-99.2) of these farmers cannot name the drug used. 43.3% (95% C.I. 31.2-56) of farmers use prophylactic tick treatment, 34.5% (95% C.I. 17.9-54.3) using Amitraz, whilst many (44.8%, 95% C.I. 26.4-64.3) again are unable to name the drug used. The frequency of prophylactic treatments given by farmers are shown in Table 4.5 on the next page. No vaccines of any type were provided to pigs by farmers.

The majority (58.8%, 95% C.I. 46.2-70.6) of pig farmers purchase drugs from the agrovet, 31% (95% 20.2-43.3)did not know where to purchase drugs for pigs and 4.4%(95% 1-12.4) farmers use a private vet to purchase drugs. Roughly half (51.5%, 95% C.I. 39-63.8) of farmers reported no problems with their pigs, of those who reported problems with pigs these were: poor weight gain (16.2%, 95% C.I. 8.4-27.1), sudden mortality (14.7%, 95% C.I. 7.3-25.4), worms (4.4%, 95% C.I. 1-12.4), coughs (5.9%, 95% C.I. 1.6-14.4) and others (5.9%, 95% C.I. 1.6-14.4).

Ninety three pigs were found to be eligible for sampling during the study. 96.8% (95% C.I. 93.1-100) of pigs were reported to be a local breed with the others being cross-bred
### 4.4. RESULTS

<table>
<thead>
<tr>
<th>Frequency of treatment</th>
<th>Worm Control % (95% C.I.)</th>
<th>Tick Control % (95% C.I.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weekly</td>
<td>NA</td>
<td>1.5 (0.7-7.8)</td>
</tr>
<tr>
<td>Once a month</td>
<td>2.9 (0.4-10.8)</td>
<td>2.9 (0.4-10.1)</td>
</tr>
<tr>
<td>q. 2 months</td>
<td>2.9 (0.4-10.8)</td>
<td>7.1 (0.9-23.5)</td>
</tr>
<tr>
<td>q. 3 months</td>
<td>10.1 (4.2-19.8)</td>
<td>3.6 (0.1-18.3)</td>
</tr>
<tr>
<td>q. 6 months</td>
<td>2.9 (0.4-10.8)</td>
<td>3.6 (0.1-18.3)</td>
</tr>
<tr>
<td>On professional advice</td>
<td>2.9 (0.4-10.8)</td>
<td>3.6 (0.1-18.3)</td>
</tr>
<tr>
<td>Pigs thin</td>
<td>8.7 (3.3-18)</td>
<td>NA</td>
</tr>
<tr>
<td>See worms in faeces/ticks on animal</td>
<td>1.5 (0-7.8)</td>
<td>18.8 (10.4-30.1)</td>
</tr>
<tr>
<td>If money available</td>
<td>5.8 (1.6-14.2)</td>
<td>7.2 (2.4-16.1)</td>
</tr>
<tr>
<td>other</td>
<td>7.2 (2.4-16.1)</td>
<td>NA</td>
</tr>
<tr>
<td>Do not use</td>
<td>52.2 (39.7-64.6)</td>
<td>56.7 (39.7-64.6)</td>
</tr>
</tbody>
</table>

Table 4.5: Frequency of Prophylactic Treatments Provided to Pigs in the Study Area

With an improved breed. Of the sampled pigs 55.9% (95% C.I. 45.2-66.2) were female, and 24.4% (95% C.I. 12.4-40.3) of male pigs were castrated. The mean age of sampled pigs was 7.2 (95% C.I. 6.2-8.2) months as estimated by the farmer (age range 1-24 months).

The pigs in this study had a mean body condition score (BCS) of 2.63 (95% C.I. 2.5-2.7), with male pigs having a higher BCS (2.7) than female animals (2.5), \((p = 0.004)\). This average BCS indicates that the sampled population were of moderate to good condition (Holness \textit{et al.}, 1991). The mean packed cell volume (PCV) of 34.19% (95% C.I. 33.0-35.4%) and total protein of 8.3 g/dl (95% C.I. 8.13-8.6), indicate that the sampled population have blood parameters within normally expected limits (Iowa State University, 2013).

29% (95% C.I. 15.8-42.3) of pigs had been provided with prophylactic treatment within the last year, with 20.4% (95% C.I. 7.5-33.4) of pigs receiving treatment for endoparasites and 6.5% (95% C.I. 0-14.1) of pigs receiving treatment for ectoparasites. 8.6% (95% C.I. 3.8-16.2) of sampled pigs (8) had recently (within 6 months) been ill of which 3 (37.5%) had been treated: 2 with levamisole, 1 with antibiotics, 1 with engine oil

The majority (79.6%, 95% C.I. 68.2-91) of pigs were found to be infested with ectoparasites (ticks and/or lice). The only hemoparasites detected in the study were \textit{Trypanosoma vivax} and \textit{Theileria} spp.. Gastrointestinal parasites identified included \textit{Strongyloides} spp., Strongyles, Coccidia, \textit{Trichuris} spp., and \textit{Ascaris} spp. The prevalence of various porcine parasites observed in this study are summarised in Table 4.6 on the following page.
Infection | Dx. Assay* | Positive | Prevalence (95% C.I.)**
--- | --- | --- | ---
Adult ticks (all spp.) | PE | 57 | 61.3 (47.9-74.6)
Lice | PE | 61 | 65.6 (55-75.1)
*Trypanosoma vivax* | BCT | 3 | 24.8 (16.4-34.8)
*Theileria* spp. | Tk | 1 | 1.5 (0-3.6)
*Strongyloides* spp. | MM | 30 | 58 (39.9-76)
Strongyles | MM | 55 | 100 (100-100)
Coccidia | MM | 33 | 58.3 (44.9-70.9)
*Trichuris* spp. | MM | 15 | 31.7 (20.2-44.9)
*Ascaris* spp. | MM | 28 | 68.3 (55-79.7)
*T. solium* | HP10 | 16 | 0 (0-5.8)
| LP | 9 | 79.6 (69.4-89.8)

Table 4.6: **Prevalence of Porcine Parasites in the Study Site**

*P.E = physical exam n=93, MM = McMasters n = 60, KK = Kato-Katz n= 59, Tk = thick smear n=87, HCT = Hematocrit concentration n= 93, HP10 = HP10 Ag ELISA n = 93, LP = lingual palpation n=93, **Adjusted for diagnostic test sensitivity/specificity and effect of clustering

### 4.4.2 Risk factors for *Taenia solium* Cysticercosis in Humans

The prevalence of human *T. solium* cysticercosis as determined by the HP10 antigen ELISA was estimated to be 6.6% (95% C.I. 5.6-7.8%), with 169 out of 2094 individuals testing positive. When the observed prevalence at an individual level was adjusted to account for the sensitivity and specificity of the HP10-antigen ELISA for the detection of neurocysticercosis and the clustered nature of the data, the true prevalence is estimated to be 0.81% (95% C.I. 0-2.3%), which equates to a potential 24,300 people living with cysticercosis infections in this population of approximately 3 million people.

Table 3 in Appendix .5 on page 187 describes the modelling process for HP10 positivity. The final model was chosen using backward selection based on the lowest AIC value, the selected model can be seen in Equation (4.2).

\[
\text{cysti} \sim \text{sex} + \text{Hemocue} + \text{eat\_meat} + \text{ther} + (1 \mid \text{homestead\_id}) \tag{4.2}
\]

Where cysti = result of HP10 antigen ELISA, sex = gender of participant, Hemocue = hemoglobin reading in g/dl and eat\_meat = non-meat eater or eat meat outside homestead (other) and \((1 \mid \text{homestead\_id}) = \text{random effect.}\n
As shown in Table 4.7 on the facing page, there were no significant variables found for human HP10 positivity in this study.
### 4.4. RESULTS

<table>
<thead>
<tr>
<th>Variable</th>
<th>Level</th>
<th>Odds Ratio</th>
<th>95% C.I.</th>
<th>( p \text{ value} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sext</td>
<td>Female</td>
<td>1</td>
<td>0.54</td>
<td>0.29-1.01</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eat meat outside homestead</td>
<td>No</td>
<td>1</td>
<td>0.77</td>
<td>0.35-1.7</td>
</tr>
<tr>
<td></td>
<td>non-meat eater</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td></td>
<td>0.63</td>
<td>0.25-1.6</td>
</tr>
<tr>
<td>Hemocue</td>
<td>3.5</td>
<td>1</td>
<td>0.85</td>
<td>0.71-1.02</td>
</tr>
<tr>
<td></td>
<td>each additional g/dl</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4.7: **Odds Ratio for an Individual Testing Positive on HP10 Antigen-ELISA for Human Cysticercosis Evaluated by Multivariate Logistic Regression**

The homestead prevalence of cysticercosis as detected by HP10 antigen ELISA (a homestead was considered to be positive if > 1 member of that homestead tested positive on HP10 ELISA) was estimated to be 17.9% (95% C.I. 14.3-21.9%). The logistic regression model, chosen by forwards selection by the 'stepAIC' function in the R 'Stats' package (R Development Core Team, 2005), with an AIC value of 364, is shown in Equation 4.3. The variables significantly associated with a homestead having at least one person positive on HP10 ELISA were; the homestead sources water from a well (OR=2.3, 95% C.I. 1.2-4.5, \( p =0.014 \)), at least one person in the homestead had self-reported a tapeworm infection (OR=1.8, 95% C.I. 1.0-3.1, \( p =0.042 \)), and the homestead has engaged with other studies (OR=2.1, 95% C.I. 1.1-4.1, \( p =0.026 \)). All variables which were retained in the multivariate model can be found in Table 4.8 on the next page.

\[
\text{hscysti} \sim \text{other.stud} + \text{well} + \text{tapes} + \text{taenia.status} + \\
+ \text{open} + \text{piped} + \text{pork}
\]  

(4.3)

Where; hscysti = Homestead HP10 status, other.stud = homestead engaged in other studies, open = people in homestead report open defecation, piped = homestead uses piped water, well = homestead uses well water, tapes = at least one person on homestead reports a tapeworm infection, taenia.status = at least one person on the homestead tested positive on CoproAg ELISA, pork = at least one person on homestead consumes pork.
CHAPTER 4. PREVALENCE AND RISK FACTORS FOR TAENIA SOLIUM

<table>
<thead>
<tr>
<th>Variable</th>
<th>Level</th>
<th>Odds Ratio</th>
<th>95% C.I.</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Use well water</td>
<td>Do not use well water</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>2.3</td>
<td>1.2-4.5</td>
<td>0.014*</td>
</tr>
<tr>
<td>Use piped water</td>
<td>Do not use piped water</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>0.3</td>
<td>0.8-3.2</td>
<td>0.12</td>
</tr>
<tr>
<td>Open defecation reported</td>
<td>No</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>1.6</td>
<td>0.8-3.2</td>
<td>0.15</td>
</tr>
<tr>
<td>Engagement in other programs</td>
<td>No</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>2.1</td>
<td>1.1-4.1</td>
<td>0.026*</td>
</tr>
<tr>
<td>Homestead positive for Taenia</td>
<td>No</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>1.5</td>
<td>0.9-2.6</td>
<td>0.15</td>
</tr>
<tr>
<td>Tapeworm infection reported</td>
<td>No</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>1.8</td>
<td>1.0-3.1</td>
<td>0.042*</td>
</tr>
<tr>
<td>Pork eaten by homestead member</td>
<td>No</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>2.6</td>
<td>0.9-7.8</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Table 4.8: Odds Ratio for Household Being Positive on HP10 Antigen-ELISA for Human Cysticercosis Evaluated by Multivariate Logistic Regression

4.4.3 Risk Factors for Taeniasis in Humans

Taeniasis (as caused by *T. solium* or *T. saginata*) was diagnosed by microscopy following formal-ether concentration, in 4 of 2059 samples (0.19% (95% C.I. 0.07-0.5%), which, when adjusted for the sensitivity and specificity of microscopy and for clustering in the data suggests a true prevalence of 0.18% (0-0.76%). Using the, more sensitive copro-antigen ELISA, 400 of 2003 human study participants were found to be positive for taeniasis, a prevalence of 19.9% (95% C.I, 18.2-21.8%), which equated to an adjusted (for diagnostic parameters and clustering) prevalence of 13.9% (95% C.I, 11.3-16.6%) when test sensitivity and specificity were considered.

As discussed in Section 1.2.3 on page 11 the copro-antigen ELISA is not species specific (Allan *et al.*, 2003), and in order to determine which species of *Taenia* was present in the study area the positive samples were tested by the CDC in Washington, USA, using the rEs33 EITB. This assay has high sensitivity and specificity for the detection of *T. solium* taeniasis. No *T. solium* taeniasis was detected in the 500 samples tested, giving a prevalence estimate of 0% (95% C.I. 0-0.9%).

The selection of a mixed-effects logistic regression model for teniasis in humans was performed manually in a forwards direction and the selection process and AIC values can be found in Table 4 in Appendix .6 on page 189. The model selected based upon the lowest AIC value (1440) can be seen in Equation (4.4.3) and the Odds Ratios returned by this mixed-effect logistic regression model can be found in Table 4.9 on page 108.
Significant risk factors for copro-antigen ELISA positivity were found to be: belonging to the Luo tribe (OR = 2.1, 95% C.I 1.2-3.7, \( p = 0.008 \)), being a non-christian (OR = 2.6, 95% C.I. 1-6.5, \( p = 0.05 \)), participating in skinning animals (OR = 2.9, 95% C.I. 1.3-6.6, \( p = 0.01 \)) and being positive for the intestinal parasite *Strongyloides* spp. larvae on microscopy (OR = 3.5, 95% C.I. 1.5-7.7, \( p = 0.002 \)).

Variables which were found to be significantly protective for taeniasis were as follows; being positive for the intestinal parasite *Iodamoeba butschlii* on microscopy (OR = 0.5, 95% C.I 0.3-0.8, \( p = 0.01 \)) and belonging to the Teso tribe (OR = 0.4, 95% C.I 0.2-0.9, \( p = 0.01 \)).

\[
\text{Taenia} \sim \text{tribe} + \text{strong} + \text{iodamoeba} + \text{dwellings} + \text{skin} + \text{religion} + \text{lat} + (1 | \text{homestead\_id})
\]

where; Taenia = Individual positive on copro-ag ELISA, tribe = tribal affiliation, religion = religious affiliation, skin = individual engaged in skinning animals, iodamoeba = individual positive for *Iodamoeba* spp., strong. = individual positive for *Strongyloides* spp., lat = individual uses a latrine, dwellings = no. dwellings on homestead (1 | homestead\_id) = random effect

\[ (4.4) \]

The homestead prevalence of taeniasis was found to be 40.4% (95% C.I. 35.6-45.3%) the variable found to be a significant risk factor on multivariate logistic regression was any one person in the homestead being positive on HP10 (OR = 2.5, 95% C.I. 1.3-5.3, \( p = 0.009 \)). Variables found to be protective were: homestead uses piped water (OR = 0.3, 95% C.I. 0.1-0.8, \( p = 0.012 \)) and the homestead uses well water (OR = 0.3, 95% C.I. 0.2-0.7, \( p = 0.006 \)). The model selected using the ‘stepAIC’ function in R in a forwards direction, can be seen in Equation(4.5) and the output of the model can be found in Table 4.10 on page 109.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Level</th>
<th>Odds Ratio</th>
<th>95% C.I.</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tribe</td>
<td>Luhya</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Luo</td>
<td>2.1</td>
<td>1.2-3.7</td>
<td>0.008*</td>
</tr>
<tr>
<td></td>
<td>Teso</td>
<td>0.4</td>
<td>0.2-0.9</td>
<td>0.017*</td>
</tr>
<tr>
<td></td>
<td>Other</td>
<td>0.6</td>
<td>0.3-1.2</td>
<td>0.18</td>
</tr>
<tr>
<td>Religion</td>
<td>Christian</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Non-christian</td>
<td>2.6</td>
<td>1 - 6.5</td>
<td>0.05 *</td>
</tr>
<tr>
<td>Skinning animals</td>
<td>No</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>2.9</td>
<td>1.3-6.6</td>
<td>0.01*</td>
</tr>
<tr>
<td>Number of dwellings on homestead</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>every extra dwelling</td>
<td>1.1</td>
<td>0.97-1.4</td>
<td>0.119</td>
</tr>
<tr>
<td>Latrine use</td>
<td>No</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>6.3</td>
<td>0.5-80.3</td>
<td>0.15</td>
</tr>
<tr>
<td><em>Iodamoeba butschlii</em></td>
<td>Negative</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>0.50</td>
<td>0.3-0.8</td>
<td>0.01*</td>
</tr>
<tr>
<td><em>Strongyloides</em> spp. larvae</td>
<td>Negative</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>3.5</td>
<td>1.5-7.7</td>
<td>0.002*</td>
</tr>
</tbody>
</table>

Table 4.9: Odds Ratio for Individuals Testing Positive on Copro-Antigen ELISA for Human Taeniasis Evaluated by Multivariate Logistic Regression

*significant variable

\[
\text{hstaenia} \ no.cattle + \ piped + \ well \\
+ \ cysti.status + \ pork.sum + \ slaught
\]  

(4.5)

Where; hstaenia = Homestead copro-Ag ELISA status, no.cattle = no. cattle kept on homestead, piped = homestead uses piped water, well = homestead uses well water, cysti.status = homestead HP10 ELISA status, pork.sum = no. people in homestead who eat pork, slaught = at least one person in homestead slaughters animals.
### Table 4.10: Odds Ratio for Households Testing Positive on Copro-Antigen ELISA for Human Taeniasis Evaluated by Multivariate Logistic Regression

<table>
<thead>
<tr>
<th>Variable</th>
<th>Level</th>
<th>Odds Ratio</th>
<th>95% C.I.</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. cattle in homestead</td>
<td>0</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>every extra cow</td>
<td>1.06</td>
<td>0.99-1.14</td>
<td>0.13</td>
</tr>
<tr>
<td>Piped water</td>
<td>No</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>0.22</td>
<td>0.05-0.72</td>
<td>0.024*</td>
</tr>
<tr>
<td>Well water</td>
<td>No</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>0.32</td>
<td>0.12-0.73</td>
<td>0.010*</td>
</tr>
<tr>
<td>Use animal health assistant</td>
<td>No</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>2.66</td>
<td>1.18-6.3</td>
<td>0.02*</td>
</tr>
<tr>
<td>Any one person HP10 positive in homestead</td>
<td>No</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>1.8</td>
<td>0.9-3.7</td>
<td>0.09</td>
</tr>
<tr>
<td>No. people slaughtering animals</td>
<td>0</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Each additional person</td>
<td>1.54</td>
<td>0.9-2.7</td>
<td>0.114</td>
</tr>
<tr>
<td>Any one person eats pork in homestead</td>
<td>No</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>1.1</td>
<td>1.00-1.24</td>
<td>0.04*</td>
</tr>
</tbody>
</table>

* significant factors
4.4.4 Risk Factors for *Taenia solium* Cysticercosis in the Porcine Host

The prevalence of porcine cysticercosis as detected by HP10 Antigen ELISA was found to be 17.2% (95% C.I. 10.2-26.4%), which, as is the case with the human data, estimates a zero prevalence (95% C.I. 0-5.8%) when adjusted for test sensitivity and specificity. Lingual palpation detected cysts in 9 pigs, an apparent prevalence of 9.8% (95% C.I. 5.2-17.4%).

The estimated sensitivity of this diagnostic tool is very low (7.3%, 95% C.I. 0.8-15.1) (Krecek *et al.*, 2011) and so the estimated prevalence when adjusted for test se/sp and the effect of clustering is 79.6% (95% C.I. 69.4-89.8%). This is likely to be an overestimation for active infections due to difficulties in distinguishing between viable and degraded cysts using this method. Due to the poor performance of lingual palpation as a diagnostic tool no further analysis was performed with these data.

A mixed-effect logistic regression model was built to investigate the risk factors for HP10 positivity, the model, selected using a forwards selection method for the lowest AIC value, can be seen in Equation (4.4.4) and the model selection process with related AIC values can be found in Table 5 in Appendix .7 on page 191.

\[
\text{pycsti} \sim \text{sheep} + TP + (1 | \text{homestead}_{id})
\]

(4.6)

Where: pycsti = porcine HP10 result, TP = total protein, sheep = homestead keeps sheep.

The output from the mixed-effect logistic regression model can be found in Table 4.11 on the next page, the only variable significantly associated with increased risk of HP10 positivity, was found to be increasing total protein reading (OR = 2, 95% C.I. 1.1-3.6, \(p = 0.02\)).

Homestead prevalence of porcine cysticercosis, as measured by HP10 Ag-ELISA, was found to be 23.6% (95% C.I 13.2-37.1%) of pig-owning homesteads. Using a forwards
### Table 4.11: Odds Ratio for a Pig Testing Positive on HP10 Antigen-ELISA Evaluated by Multivariate Logistic Regression

*significant variable

<table>
<thead>
<tr>
<th>Variable</th>
<th>Level</th>
<th>Odds Ratio</th>
<th>95% C.I.</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Keep Sheep</td>
<td>No</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>2.5</td>
<td>0.7-8.0</td>
<td>0.14</td>
</tr>
<tr>
<td>Total protein</td>
<td>6g/dl</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>each additional 1g/dl</td>
<td>2</td>
<td>1.1-3.6</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Selection based upon AIC value (using the 'step' function in the R 'stats' package (R Development Core Team, 2005)) only a homestead having knowledge of tapeworm transmission remained in the model at an AIC of 60.6, but was not significant (OR = 4.1 95% C.I. 0.9-19.7 p =0.08).
4.4.5 Spatial Distribution of *Taenia* spp. infections

Ripleys K function was returned for each data set (Human cysticercosis and taeniasis and porcine cysticercosis) at the homestead level using the random labeling hypothesis. Only porcine cysticercosis, as detected by HP10 ag-ELISA was found to have a clustered distribution, with homesteads owning a positive pig being significantly clustered ($p =0.019$) above the null hypothesis provided by random labeling of all pig owning homes, as shown in Figure 4.3. Human infections did not have a distribution significantly different from the null hypothesis of random labeling of all recruited homesteads.

![Ripleys K-test for Clustering of Homesteads with at least one Pig Testing Positive on HP10 ELISA](image)

Figure 4.3: Ripleys K-test for Clustering of Homesteads with at least one Pig Testing Positive on HP10 ELISA
A significant ($p =0.019$) clustering was observed over spatial randomness, as measured by random labeling
4.5 Discussion

This is the first study in Kenya to investigate the prevalence and risk factors for Taenia solium infections of humans in a community-based setting, and the first to consider the epidemiology of the parasite in both the human and porcine hosts. The prevalence of active *T. solium* cysticercosis in humans as detected by the HP10 antigen ELISA, adjusted for test sensitivity/specificity and the effect of clustering, was found to be 0.81% (95% C.I. 0.2-1.5%). This indicates that within this population of approximately 3 million people there may be 24,300 people with active *T. solium* infections.

There may be 3400-4380 people within our study site suffering from epileptic seizures and the physical and mental health burdens associated with this condition. This figure is based upon an approximation that 20% of *T. solium* infections in humans are symptomatic (Pal, 2000), with 70-90% of these cases presenting as epilepsia (Pal, 2000). Epilepsy inflicts a serious burden upon those who suffer from it, through physical injury, cognative disorders, poor mental health and social stigmatisation (de Boer *et al.*, 2008). Social exclusion of epileptics, and perhaps even their whole families, appears to be most pronounced in the poorer, less educated, regions of the world (Newton and Garcia, 2012).

The prevalence of human cysticercosis has rarely been investigated in community based studies, as opposed to within particular risk-groups such as epileptics. Previous studies which have done using antigen-ELISA for the detection of active *T. solium* infections in sub-Saharan Africa have found prevalences of 0.7% (95% C.I. 0.4-0.9%) in Cameroon (Nguekam *et al.*, 2003), 0-10.3% in Bukino Faso (Carabin *et al.*, 2009), 4.8% (95% C.I. 4.1-7.5) in Zambia (Mwape *et al.*, 2012) and 21.6% (95% C.I. 18.3-25.0) in the Democratic Republic of the Congo (D.R. Congo) (Kanobana *et al.*, 2011). These are apparent prevalences, reported without adjustment for diagnostic test parameters. The unadjusted prevalence of human cysticercosis of 6.6% (95% C.I. 4.8-8.4) found in this study indicates that the status of *T. solium* endemnicity in western Kenya is similar to that observed in Zambia.

No variables were found to be significantly associated with HP10 Ag ELISA positivity in this population, including any of those variables which have previously been reported to be risk factors such as gender (Carabin *et al.*, 2009; Kanobana *et al.*, 2011), pork consumption (Carabin *et al.*, 2009) or village of origin (Carabin *et al.*, 2009; Nguekam *et al.*, 2003).
At the homestead level, however the use of well water (OR = 2.3m 95% C.I. 1.2-4.5, \( p =0.014 \)), engagement with development programs (OR = 2.1, 95% C.I. 1.1-4.1, \( p =0.03 \)) and tapeworm infection being reported on the homestead (OR = 1.8, 95% C.I. 1.0-3.1, \( p = 0.04 \)) were found to be significant risk factors for any one person on a homestead being tested positive on HP10 Ag-ELISA.

It is difficult to determine the etiological pathway for a couple of these risk factors, we may hypothesise that well water could have been contaminated with faecal material, therefore exposing homestead members to potential infection with \textit{T. solium} eggs. Engagement in development programs may indicate homesteads of lower socioeconomic status which are targeted by organisations.

More easily explained as a risk factor is the presence in the homestead of one or more people reporting tapeworm infections. The presence of a \textit{T.solium} tapeworm infection in a homestead is a previously reported risk factor for cysticercosis infection, with seroprevalence gradients existing for human cysticercosis around \textit{Taenia} carriers (Lescano \textit{et al.}, 2009). This is due to the likely hood of either auto-infection from the carrier of himself, or the infection of other homestead members through contamination of food products or water.

The prevalence of taeniasis infections in this population (13.9%, 95% C.I. 11.3-16.6%) is not significantly different from that detected in Papua, Indonesia (8.6% (95% C.I. 3.2-19.7) (Margono \textit{et al.}, 2003), though is significantly \( (p <0.001) \) higher than reported in some other endemic communities including; Vietnam (0.6%, 95% 0.2-1.6) (Somers \textit{et al.}, 2006), Peru (1.5%, 95% C.I. 0.6-3.4) (O’Neal \textit{et al.}, 2012) and Zambia (6.3, 95% C.I. 4.58.1) (Mwape \textit{et al.}, 2012).

This estimate may have been inflated through the inclusion of false positives. Cross-reactions which have previously been reported for the copro-Ag ELISA include: \textit{Ascaris lumbricoides}, \textit{Trichuris trichiuria}, \textit{Hymenolepis nana} and parasitic protozoa (Rodríguez-Hidalgo, 2003). In order to reduce the influence of such cross-reactions on these results a large (63) panel of faecal samples with a variety of parasitic infections were used to calculate the cut-off value as discussed in Section 2.5.3 on page 47 although \textit{Trichuris} spp. and \textit{Hymenolepis} spp. were not represented.

The copro-Ag ELISA cannot differentiate between different \textit{Taenia} spp. (Allan \textit{et al.}, 1990) and the \textit{T. solium} specific rEs33 ETIB was therefore used to test 500 sera samples. This testing estimated a \textit{T. solium} taeniasis prevalence of (0%, 95% C.I. 0-0.9%). This esimtate is not significantly different from those estimated in Zambia (0.6%, 95% C.I. 0-0.9%).
0.11.7% (Praet et al., 2013) or in a migrant Hispanic community in the USA (1.1%, 95% 0.4-2.7%) (DeGiorgio et al., 2005).

In order to obtain a more accurate estimate of the *T. solium* taeniasis prevalence in the study population a larger number of samples should be tested by the rEs33 EITB. In our community, with a design effect estimated at 3, and an average of 5 participants per cluster, a sample of 1279 should be tested with the rEs33 EITB in order to detect an expected prevalence of 1% + 1%.

*T. solium* carriers are notoriously difficult to detect, even in endemic communities, potentially due to a combination of low prevalence, imperfect diagnostic tests or potential spontaneous expulsion of the worm by the host (Flisser, 2006). It appears, however, that a small number of taeniasis cases can sustain the *T. solium* lifecycle in a community and it has been suggested that in the majority of endemic communities the prevalence is unlikely to be over 4% (Pawlowski et al., 2005).

It is therefore likely that the majority of copro-Ag positive samples in our study are indicative of active infection with *Taenia saginata* as was assumed in Zambia (Praet et al., 2013). This result suggests that the cattle within the study site should be tested for the presence of this parasite as, although of lower public health importance, the presence of *T. saginata* in the cattle population would seriously curtail the economic viability of cattle production in the area.

In contrast to human cysticercosis infections, several risk factors were identified for taeniasis infections in humans.

Tribal and religious differences in the risk of taeniasis infections were identified. In comparison to the Luuya tribe, belonging to the Luo tribe was found to increase the risk of testing positive on copro-Ag ELISA (OR = 2.1, 95% C.I 1.2-3.7, \( p =0.008 \)), whereas belonging to the Teso tribe was found to be protective (OR = 0.4, 95% C.I 0.2-0.9, \( p =0.01 \)).

Pork and beef consumption was found to be significantly \( p =0.008 \) and \( p =0.029 \) respectively) more common in the Luo tribe than other tribes in the study area, and these consumption differences may be responsible for the increased risk within the Luo tribe.

Being non-christian also was found to be a risk factor for taeniasis (OR = 2.6, 95% C.I. 1.0-6.5, \( p =0.011 \)), potentially indicating a protective aspect in of some of the religious prohibitions surrounding meat consumption, either by species or by time of
year. Participating in skinning animals was also found to be significant (OR = 2.9, 95% C.I. 1.3-6.6), and may relate to the potential for consumption of potentially infective and uncooked meat.

Co-infection with the soil-transmitted helminth *Strongyloides* spp. larvae, (which has not been reported to cross-react with the copro-antigen ELISA (Guezala *et al.*, 2009)) was found to be a risk factor for infection with taeniasis as detected by copro-antigen ELISA (OR = 3.5, 95% C.I. 1.5-7.7, \( p = 0.002 \)). Strongyloidiasis has also been identified as one of the neglected tropical diseases (Olsen *et al.*, 2009) and similarly to *T. solium* is found in poor communities where open defecation is commonly practiced.

Conversely, the protozoal parasite *Iodamoeba butschlii*, which is also included in the list of neglected tropical diseases (Hotez and Pecoul, 2010), was found to be protective (OR = 0.5, 95% C.I. 0.3-0.8, \( p = 0.01 \)). This is interesting as it is generally stated that co-morbidity of the neglected tropical diseases is common (Singer and Ryff, 2007), but this finding may suggest an aspect of cross-protection between *I. butschlii* and *Taenia* spp. which could be further investigated.

At the homestead level, two protective factors were found for Copro-Ag positivity, being the use of piped (OR = 0.22, 95% C.I. 0.05-0.72, \( p = 0.024 \)) or well (OR= 0.32, 95% C.I. 0.12-0.73, \( p =0.01 \)) water. It may be that the homesteads with access to piped water also have a higher state of hygiene across the homestead, including the ability to prepare pork correctly, protecting them against *Taenia* spp. infections. Well water is harder to explain, however, as we have seen already that it appeared to be a risk factor for HP10 Ag-ELISA positivity at the homestead level, indicating a potential that the water is contaminated by *Taenia* eggs. These eggs, however, may originate from a neighbouring homestead and it may therefore be that the quality of this water source is not linked to other practices in these homesteads which may protect them against tapeworm infections.

Two variables were found to be significant risk factors for any one person in the homestead being positive on copro-Ag ELISA. These were the use of animal health assistant (AHA) to provide veterinary care in livestock owning homesteads (OR = 2.66, 95% C.I. 1.2-6.3, \( p =0.02 \)) and having at least one person in the homestead who eats pork (OR = 1.1, 95% C.I. 1.0-1.24, \( p =0.04 \)).

Using a AHA to provide veterinary care may possibly be an indication of a poorer homestead who cannot afford a private vet and the consumption of pork is an unsurprising risk factor as pork consumed in the study area appears to be a high risk
product, as previously discussed in Chapter 3 on page 51. The small increase in risk due to pork consumption may reflect the high probability of many Copro-Ag ELISA positive individuals hosting a *T. saginata* worm.

Pork production is becoming an increasingly important contributor to the rural Kenyan economy (Wabacha *et al.*, 2004) and it is therefore important that the pork sold in this region is safe for consumers. Interventions which would reduce the risk of acquiring a *T. solium* infection from pork are discussed in detail in Chapter 3 on page 51.

Porcine cysticercosis, as detected by HP10 antigen ELISA appears to be significantly lower (*p* < 0.001) in the pigs sampled as part of this community-based study 0% (95% C.I. 0-5.8%) than that observed in pigs presented for slaughter in the study area (39.7%, 95% C.I.25.3-54.2%) (see Section 3.3.2 on page 56).

The difference between the prevalence estimates in the two populations may relate to the following factors:

**Insufficient sample size.** As discussed in Section 2.3 on page 35 the sampling strategy used did not specifically target pig owning homes, instead attempting to recruit a truly representative sample of homesteads in the study area. No previous data was available on the proportion of all homes who keep pigs in the area, though the proportion determined by this study (16.9%) was lower than anticipated and the average number of pigs owned by a homestead (2.6) was lower than previously reported (3.6) (Kagira *et al.*, 2010), therefore resulting in a lower than expected sample size.

The difference between expected and observed pig numbers may be due to a couple of key factors. Firstly post-election violence experienced by western Kenya appears to have lead to a dramatic reduction in the number of pigs kept in Busia, with a reduction of 21% in the number of boars and reductions of 40% and 42% in the number of piglets and growing pigs respectively (Dewey *et al.*, 2011). There have also been several outbreaks of African Swine Fever (ASF), a viral infection causing high mortality in pigs, within this area during the time of this study (OIE, 2011).

**Age of pigs.** The two populations are likely to have differed slightly in age, with the average age of pigs recruited into our community based study (7.2 months, see Section 4.4.1 on page 95) being slightly younger than the average age at which pigs are sold for slaughter (9 months (Kagira *et al.*, 2010)). Increasing age of pig was not found to be a significant risk factor in this study, but has been found to be in several previous studies (Carrique-mas *et al.*, 2001; Phiri *et al.*, 2002; Pondja *et al.*, 2010; Sikasunge *et al.*, 2008) which may start to explain some of this difference.
The estimate presented here is not significantly different ($p = 0.09$), from a previous 'homestead-based’ study in Busia district which found a prevalence of 4% (95% C.I. 1.9-6.2%) (Kagira et al., 2010), though is lower ($p =0.042$) than that observed in another 'homestead-based’ pig population in neighbouring Nyanza province (32.8%, 95% C.I. 26.8-39.2%) (Eshitera et al., 2012). In which we would expect the age distribution of pigs to be similar, although the issues regarding sample size still remain.

There are also issues to consider when interpreting porcine $T. solium$ prevalence estimates for the current study. There is uncertainty in the diagnostic parameters of the HP10 ELISA. In order to adjust prevalence estimates I have used the latest published estimates of diagnostic parameters for the test used in this study (see Table 4.1 on page 93). Estimates of sensitivity and specificity of the HP10 ELISA do however, vary widely depending on the strength and location of infection, as indicated in Table 1.1 on page 15.

It is also important to note that although previous literature suggests that the HP10 Ag-ELISA does not cross-react with $T. hydategina$ (Harrison et al., 1989) and that $T. hydategina$ is not an important parasite of pigs in East Africa (Dorny et al., 2004), there have been cases of $T. hydategina$ in pigs reported in Zambia (Dorny et al., 2004) and Tanzania (Ngowi et al., 2004) and the potential for cross-reation has been noted (Leslie Harrison Per. Comms.).

Approximately a quarter (23.6%) of pig herds may be positive for porcine cysticercosis, with at least one pig positive on HP10 ELISA. These positive herds were significantly ($p =0.019$) clustered in comparison to the underlying spatial distribution of pig-owning homesteads, potentially reflecting an underlying clustered distribution of risk factors, although no significant risk factors were identified in this study.

The study area of western Kenya a very impoverished region (Dewey et al., 2011) and there are many indicators in this study that, over and above the issue of $T. solium$, the health of the community is being negatively effected by poor hygiene and sanitation provision. 36.4% (95% C.I. 33-39.9%) of our study participants report open defecation and this is reflected in the high prevalence of the soil transmitted helminths, including: hookworm (31%), $Trichuris$ spp. (11.9%, 95% C.I. 9.8-14) and $Ascaris$ spp. (9.6%, 95% C.I. 1.6-11.6), $Strongyloides$ spp. (5.6%, 95% C.I. 4.7-6.6), $Schistosoma mansoni$ (7.4%, 95% C.I. 5.3-9.6) and the very high prevalence of amoebiasis (89.4%, 95% C.I. 87.6-91.1). All of these parasites have been associated with poverty and poor sanitation (Bethony et al., 2006; De Silva et al., 2003; Hotez, 2008; Hotez and Gurwith, 2011; Hotez and Pecoul, 2010).
These indicators would corroborate the theory that neglected tropical diseases such as *T. solium* are diseases of the poorest members of communities (Hotez *et al.*, 2009, 2007). It is important therefore that when designing intervention strategies for *T. solium* and other NTD’s, economic development of affected communities must be a priority.

4.6 Conclusion

The results of this study indicate that all stages of the *T. solium* life cycle are present in western Kenya, at prevalences within previously reported ranges in sub-Saharan Africa. (as seen in Figure 1.2 on page 20). These data, including the co-endemicity of several neglected tropical diseases including *T. solium*, soil transmitted helminths, *Schistosoma mansoni*, *Strongyloides* spp. and amoebiasis, provide evidence that western Kenya is a region where integrated control of neglected tropical diseases, including *T. solium*, is required.

Several significant risk factors have been identified for *T. solium* infections in humans and pigs, several of which indicate a link between *T. solium* infection and marginalised members of the community, i.e. living in drought prone areas, lack of access to piped water, lack of certain material possessions (e.g. a watch), co-infection with soil-transmitted helminths (*Strongoloides*) and presence of open pit latrines. The linkage between poverty and the neglected tropical diseases indicates that control measures should be aimed at improvements in health infrastructure at a community wide level. The analysis undertaken in this chapter also shows that a large fraction (30.4%) of taeniasis cases may be attributable to pork consumption and therefore, as already discussed in Chapter 3 on page 51 steps are urgently required to improve the safety of pork produced in the region. Suggestions for appropriate control strategies for integrated control of *T. solium* and other NTDs in this region are further discussed in Chapter 7 on page 165.
Chapter 5

Evaluation of a Novel, User-friendly, Diagnostic for *Taenia solium* Infections in Pigs
5.1 Abstract

*Taenia solium* is a neglected zoonotic disease for which epidemiological data is lacking in tropical regions where it causes the highest public health impacts. The current diagnostic tests are not generally suitable for the resource poor settings in which this parasite occurs. This study aimed to evaluate a novel, user-friendly, diagnostic test for this parasite which is currently under development. I evaluate how the new test performs in relation to the HP10 antigen-ELISA on which it is based, and evaluated the diagnostic parameters of both tests in the absence of a gold-standard. The Bayesian framework used for this evaluation also provided us with the prevalence of *T. solium* in two distinct porcine populations in east Africa, adjusted for the performance of the diagnostic tests used.

The novel diagnostic assay was easy to use and was found to have reasonable agreement with the HP10 ELISA in the negative direction, with a Bayesian Agreement Index of 74.2% (95% B.C.I. 71-77%), though only 60% (95% B.C.I. 55-65%) in the positive direction. When evaluated in a no-gold-standard framework it was estimated to perform promisingly, with 82.7% sensitivity (95% B.C.I. 72.5-91.9%) and 87% specificity (95% B.C.I. 80.2-93.4). The results presented here support the view that this prototype assay deserves to be further developed in order to improve it’s diagnostic parameters, with other key issues being; the addition of a blood filter, improvement in assay stability at high ambient temperatures and validation against a true gold standard. An assay such as this has great potential for use in control programs in resource poor regions.

5.2 Introduction

Cysticercosis, infection with the intermediate stage of the tapeworm *Taenia solium* is a zoonotic disease of public health and economic importance, being a leading cause of acquired epilepsy in humans (Commission on Tropical Diseases, 1994) as well as potentially causing substantial losses to pig farmers due to meat condemnation (Phiri *et al.*, 2003). The increasing global popularity of pork as a protein source puts a great many people at risk from contracting this disease. This is exacerbated in developing countries where many factors perpetuate the parasite life cycle, such as the predominance of free-ranging production systems (Poudedet *et al.*, 2002; Sikasunge *et al.*, 2007; Widdowson *et al.*, 2000) and poor latrine provision (Githgia *et al.*, 2006; Mutua *et al.*, 2007; Ngowi *et al.*, 2004). Much attention has been paid to the current epidemic of cysticercosis in
Latin America and although many similar risk factors exist in East Africa, as yet there is inadequate epidemiological data for this region and further research has been called for (Boa et al., 2003; Phiri et al., 2003).

In the porcine host the gold standard diagnoses for cysticercosis is by a complete dissection of the carcass and enumeration of cysts, which is an impractical approach for both epidemiological studies and in the control of this parasite. Although the detection of cysts under the tongue of a live pig (Mutua et al., 2007) has been promoted as a potential screening test for farmers to perform themselves, the sensitivity of the test is reported to be very low (0.7% (Krecek et al., 2011)-16% (Dorny et al., 2004)).

The HP10 MAb used in an antigen-capture ELISA was first developed to detect the secretory glycoproteins from the surface of T. saginata (Harrison et al., 1989) which circulate in the bovine host sera. The MAb was found to have a useful cross-reaction with T. solium, but very little cross-reaction with a variety of other helminth infections, including T. hydatigena (Harrison et al., 1989) and has subsequently been used to detect cysticercosis in cattle, pigs and humans with varying degrees of sensitivity and specificity as is shown in Table 1.1 on page 15. This format of test is, however, difficult for use in many of the regions where T. solium is found, due to the requirement for a well equipped laboratory and experienced staff. It also has limitations in situations where immediate feedback is required and therefore, the provision of a suitable pen-side test with good sensitivity and specificity would be an important tool for T. solium control in pigs.

Similar problems are encountered in human medicine where diagnoses of NCC is made through neuroimaging (Magnetic Resonance Imaging or Computer Tomography) or histological demonstration of the parasite in a brain biopsy (Del Brutto, 2012; Del Brutto et al., 1996). In endemic areas, however, the infrastructure and technical capacity are often lacking and if available may be prohibitively expensive for inflicted people to access (Pal, 2000). Poor diagnostic capacity also limit the utility of serological techniques in these situations, therefore raising the potential for suitable bedside diagnostic kits for human cysticercosis to be used in both clinical and epidemiological situations (Fleury et al., 2013, 2007).

One candidate for such a 'bed-side' or 'pen-side' test would be a lateral flow assay (LFA), these assays rely on the lateral flow of fluid containing the substance of interest (analyte) through a membrane to activate dried reagents within the strip. Lateral flow format assays are widely used for a variety of uses, including detection of pathogens, drugs, hormones and metabolites in medical, veterinary, food and agricultural settings, with
the most commonly known assay of this format being that of the human pregnancy test kit (Posthuma-Trumpie et al., 2009). A sandwhich-capture type lateral flow immuno-assay is comprised of several standard components as detailed here (as summarised from Posthuma-Trumpie, Korf, and van Amerongen (2009)) and illustrated in Figure 5.1 on the next page:

- **Membrane.** The polymeric membrane is the central component of this assay format and is most commonly made of nitro-cellulose. Capillary action will cause fluid to flow through the membrane and different pore sizes can be chosen to facilitate the optimum reaction times

- **Absorptive pad.** An absorptive material, such as a cellulose filter, which will facilitate the fluid flow through wicking action

- **Sample pad.** Onto which the sample will be placed and which will facilitate the even distribution of sample to the conjugate pad

- **Labelling material.** Analyte specific antibodies are labelled, typically with colloidal gold, to allow for detection of the bound analyte

- **Conjugate release pad.** From which labelled antibodies will be released into the sample liquid

- **Detection lines.** At least two detection lines are present. One, the control line, will contain anti-species antibodies and detects the correct flow of the sample fluid through the membrane. The second, test, line contains anti-analyte antibodies sprayed onto the test strip membrane

- **Protective cassette.** To protect the fragile test membrane the LFA’s are generally encased in a protective plastic cassette

As part of a project currently underway at the International Livestock Research Institute, Nairobi, Kenya, I was involved in the design and development of a prototype lateral flow format assay for the detection of *T. solium* cysticercosis infections in the porcine host, using the HP10 MAb (Harrison et al., 1989). As discussed in Chapter 3 on page 51 it is hoped that diagnostic assay may be a component of *T. solium* control in this region. In order to fulfill this role the decision was made to develop an assay which would detect circulating antigen, and therefore active *T. solium* cysticercosis infections. This chapter evaluates the diagnostic performance of this novel, user friendly diagnostic assay in the absence of a gold standard.
Bayesian techniques have been widely adopted by the epidemiological community for use in test parameter estimation and for analysis of epidemiological data (Branscum et al., 2005; Bronsvoort et al., 2010; Krecek et al., 2008; Praet et al., 2013). This approach allows the incorporation of prior information on the test performance alongside the current data obtained from our own testing to inform a posterior probability distribution of the likely test performance. A modified version of the Hui Walter no gold standard model (Hui and Walter, 1980) has been used in this work to evaluate the prototype diagnostic assay. The modeling process also estimates the seroprevalence of porcine cysticercosis in two distinct populations of pigs in east Africa.

5.3 Methods and Materials

5.3.1 Lateral Flow Assay

Lyophilised 50% ammonium-sulphate precipitate of HP10 MAb (Harrison et al., 1989) was labeled with colloidal gold at Arista Biologicals (California, USA). The labeled fraction, and an unlabeled, lyophilised 50% ammonium-sulphate precipitate of the MAb were then provided to Astel Diagnostics (Kampala, Uganda) for production of the LFA. The unlabeled MAb fraction was sprayed onto a nitrocellulose membrane (90CN, MDI,
Ambala, India) at a concentration of 2mg/ml at 0.1l/mm for the test line. The control line consisted of a 1mg/ml goat-anti-mouse immunoglobulin, sprayed at 0.1l/mm. The colloidal gold labeled conjugate was sprayed onto the gold release pad at an OD of 25 (at 540nm) after incorporation of 20% sucrose in conjugate dilution buffer. The sample and gold release pad comprised of paper 8964 (Ahlstrom, CITY, USA). The LFA test strips were enclosed in a plastic cassette and stored sealed in a humidity resistant foil package containing silica desiccant. The test cassettes with the control and test lines are illustrated in Figure 5.2.

5.3.2 Samples

To reduce the reliance of the Bayesian model on the prior distributions, a multiple-test, multiple-population approach was used to increase the number of degrees-of-freedom in the model and to enable identifiability. A collaborative agreement was therefore formed with a research group at Makerere University, Kampala, Uganda, who provided samples from a porcine population outside of our western Kenyan study site to form part of this analysis.

The two porcine populations were drawn from different ecosystems in geographically
5.3. METHODS AND MATERIALS

Figure 5.3: Map of Uganda Showing Study Site. Provided by Dr Zachary Nsadha, Makerere University

distinct locations and comprised of different sub-sets of pigs, one (Kenya) being older pigs presented for slaughter, with the other (Uganda) being representative of the pig population on homesteads, with a wider age distribution. Collection of samples from porcine slaughter facilities in western Kenya, is described in Section 2.4 on page 42 and collection of the Uganda samples was carried out as described briefly here:

The study area was based in those districts adjoining Lake Kyoga in central Uganda with pig populations over 10,000 head (pig population data provided by the Uganda Ministry of Agriculture, Animal Industry and Fisheries in 2008). Within each of these 6 selected districts, one sub-county was randomly selected for the study as shown in Figure 5.3.

The sample size calculation was based on the clustered sample formula (Population services international, 2007) with a design effect of 2 in order to take into account the clustering associated with the design of the study, where an expected two pigs were to be found on each farm, and using an expected prevalence of porcine cysticercosis being 8.5% in Kamuli District, Uganda (Waiswa et al., 2009). The minimum sample size required in each district was calculated to be 60 pigs.

Between March and July 2011, a snow-ball sampling method was used to recruit pigs
from the 6 districts. Selected pigs were cast in dorsal recumbency and using a 20 gauge needle, 10 mls of blood was collected from the cranial vena cava into plain BD Vacutainers® and left at room temperature for 12 hours to allow adequate clotting. The clotted blood was centrifuged to separate the serum which was then collected and stored in cryovials at $-20^\circ$C until laboratory analysis was performed at ILRI, Nairobi.

5.3.3 Serology

Porcine sera from both populations were analysed for the detection of viable *T. solium* cysticerci using the HP10 Antigen ELISA and the our prototype LFA as described in Section 2.5.4 on page 48. There was a minor minor procedural change made when running the samples from Uganda as TMB tablets (Sigma T5525) were substituted for TMB liquid (Sigma T8665) which was unavaliable at the time of analysis.

5.3.4 Stability Testing

Stability testing of the LFA cassettes was conducted as follows. The cassettes were stored within their packaging at room temperature, $37^\circ$C and $4^\circ$C. Cassettes were then tested using three positive sera from experimentally infected bovids, and one negative (uninfected bovine) provided by Dr Leslie Harrison, University of Edinburgh. These same 4 sera were tested at two week intervals for a period of 6 months.

5.3.5 Evaluation of Diagnostic Test Performance

Evaluation of the performance of the LFA was undertaken in three ways. Firstly a sensitivity and specificity of the test was calculated, using the HP10 ELISA as a “pseudo” gold standard as shown in Equations (5.1) to (5.6).

Secondly the Bayesian Agreement Index was calculated using an algorithm adapted from that provided by Graham and Bull (1998) based upon an assessment of the agreement between two independent operators (Feinstein and Cicchetti, 1990). The code for this analysis was provided by Nicolas Praet (ITM, Belgium) and can be found in Appendix .8 on page 192.

Although the Bayesian Agreement Index assumes no gold standard, it does not provide information on the sensitivity and specificity of the tests used, only on the strength of agreement between them (Graham and Bull, 1998).
(Hui and Walter, 1980) was used to estimate the diagnostic test parameters of the HP10 ELISA and the LFA and two population prevalence in the absence of a gold-standard. The sensitivity and specificity estimates returned by the latent class model were then used to calculate likelihood ratios for both the HP10 ELISA and LFA according to Equations (5.3) and (5.4).

$$Se = \left( \frac{\text{True test positives}}{\text{True positives}} \right) \times 100$$ \hspace{1cm} (5.1)

$$Sp = \left( \frac{\text{True test negatives}}{\text{True negatives}} \right) \times 100$$ \hspace{1cm} (5.2)

$$PLR = \left( \frac{\text{Sensitivity}}{1 - \text{Specificity}} \right)$$ \hspace{1cm} (5.3)

$$NLR = \left( \frac{1 - \text{Sensitivity}}{\text{Specificity}} \right)$$ \hspace{1cm} (5.4)

$$PPV = \left( \frac{\text{True test positives}}{\text{Test positives}} \right) \times 100$$ \hspace{1cm} (5.5)

$$NPV = \left( \frac{\text{True test negatives}}{\text{Test negatives}} \right) \times 100$$ \hspace{1cm} (5.6)

**Latent Class Modeling**

Test results were available for both diagnostic tests (HP10 Ag-ELISA and LFA) for both populations. In the absence of a gold-standard the test parameters (sensitivity and specificity) and the prevalence within each population were estimated using a modification of a latent class model proposed by Hui-Walter (Hui and Walter, 1980) for 2 tests and 2 populations (Bronsvoort et al., 2006, 2010). The latent class model put forward by Hui Walter (Hui and Walter, 1980) makes several critical assumptions: (1) that the prevalence in each population is different, (2) that the tests perform identically in each population and (3) that the tests are independent conditional on the animals true status. The assumption of conditional independence is often considered to be violated when different serological tests are compared because the tests are looking for the same antibody response in the animal (Gardner et al., 2000). In this case the two
tests are dependent with reference to detection of cysticercosis in an animal as they are all reliant on detection of the HP10 antigen circulating in the plasma. However, comparison to a diagnostic test system independent of the circulating antigen would either be highly insensitive (such as lingual palpation) or impractical (post mortem examination). If we are prepared to assume that all infections with \( T. solium \) result in release of the HP10 antigen then we can argue that the tests to detect it are independent, conditional on the animal having circulating antigen (rather than being diseased).

The Bayesian version of the Hui-Walter model assumes that for the \( i \)th sub population the counts \((O_i)\) of the different combinations of test results, \(+/+\), \(+/-\), \(-/+\) and \(-/-\) for the two tests, follow a multinomial distribution shown in equation (5.7):

\[
O_i \mid S_e j, j, p_i \sim \text{Multinomial}(P_i, n_i) \text{ for } i = 1, 2, \ldots S \text{ and } j = 1, 2, \ldots T \tag{5.7}
\]

where \( S \) is the number of sub populations, \( T \) is the number of tests and \( P_i \) is a vector of probabilities of observing the individual combinations of test results. Conditioning on the (latent) disease status, these probabilities can be specified using the \( S_e j \) and \( S_p j \) of the tests and the prevalence \((p_i)\) of the sub populations. As an example, for two tests the probability of observing both tests positive in the \( i \)th sub population is given as equation (5.8)):

\[
P(T_1+, T_2+) = S_e 1 \times S_e 2 \times p_i + (1 - S_p 1) \times (1 - S_p 2) \times (1 - p_i) \tag{5.8}
\]

The other probabilities for the two test scenarios may be similarly derived.

In a Bayesian analysis all parameters are expressed as random variables. Hence, prior distributions for the test properties and the prevalence within the sub populations must be specified. A recent publication estimated the performance of the HP10 ELISA for the detection of porcine cysticercosis, also using a Bayesian framework, and estimated the Se to be 70.4% (95% B.C.I. 52.7–84.7%) and Sp to be 66.1% (95% B.C.I. 44.6–85.1%) (Krecek et al., 2011). These estimates were then incorporated into the Bayesian framework as priors for the Se and Sp of the Ag-ELISA to inform posterior estimates. Uninformed, beta(1,1) priors were given to the prevalence estimates for both populations and a wide beta(12,6) prior was assigned to Se and Sp of the LFA, based upon an expert opinion (Per.comms.B.M.deC.Bronsvoort) of where the distribution should lie based upon the performance of the HP10 MAb that was used in the assay. The modelling process relies on “Markov chain Monte-Carlo” (MCMC) simulation of the
probability distribution of an outcome and these simulated chains were given dispersed starting points to ensure that the chains explored the full spectrum of parameter space.

The model was built in the R environment for statistical computing (R Development Core Team, 2005) using the runjags package (Denwood, 2008) to call Just another Gibbs sampler (JAGS) software (Plummer, 2004). The model code for the model can be found in Appendix .9 on page 193. To ensure that sampling took place from the posterior distribution of interest a burn in period of 500,000 iterations was discarded and the Gibbs sampler then sampled from a posterior distribution formed from every 10th iteration of 500,000 total iterations. Convergence of the chain after the initial burn-in was assessed by visual inspection of the time-series plots for the parameters as well as Gelman-Rubin diagnostic plots using three sample chains with dispersed starting values.
5.4 Results

Busia Region, western Kenya

343 sera samples from pigs were collected between February and August 2010 and the point estimate of prevalence for the study region by HP10 Ag-ELISA was found to be 48.7% (95% C.I. 43.3-54.1%). All sera were then run with the LFA, which returned an apparent prevalence of 37.9% (95% C.I. 32.7-43.3%). The mean corrected OD from the Ag-ELISA for the Kenya samples was 0.46 (95% C.I. 0.39-0.53).

Lake Kyoga Region, central Uganda

390 pigs were sampled from 6 districts in the Lake Kyoga region. Sufficient sera were available to run the HP10 Ag-ELISA on 378 samples, with the point estimate of prevalence for circulating T. solium antigens found to be 25.3% (95% C.I. 21.0-30.0%). Sufficient sera was available to run 298 of these samples with the LFA, returning an apparent prevalence of 38.9% (95% C.I. 33.4-44.7%). The mean corrected OD from the Ag-ELISA for the Uganda samples was 0.021 (95% C.I. 0.017-0.025) which was significantly lower ($p < 0.001$) than the mean of the Kenyan samples, due to the different chromogen used.

The corrected OD values from each pig population were plotted against the LFA result as shown in Figure 5.4 on the next page. The mean corrected OD values were significantly ($p < 0.001$) lower in those samples classified as “negative” by the LFA than those classified as “positive”. This difference was consistent across both populations tested.
5.4. RESULTS

(a) Kenyan Samples

(b) Ugandan Samples

(c) Samples from Both Populations Combined

Figure 5.4: Boxplot of Corrected OD Values from HP10 Ag-ELISA against LFA Result
5.4.1 Sensitivity and Specificity with HP10 ELSIA as “Pseudo” gold standard

The cross-tabulation of results for the two populations with the two tests (ELISA/LFA) is shown in Table 5.1. Using the HP10 ELISA as the “pseudo” gold standard measures of diagnostic performance of the LFA were calculated as shown in Table 5.2.

<table>
<thead>
<tr>
<th>Population</th>
<th>LFA</th>
<th>HP10 ELISA</th>
<th>Total (Test)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Kenya</td>
<td>+</td>
<td>96</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>71</td>
<td>142</td>
</tr>
<tr>
<td>Uganda</td>
<td>+</td>
<td>55</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>35</td>
<td>147</td>
</tr>
<tr>
<td>Total (“True”)</td>
<td>257</td>
<td>384</td>
<td>n = 641</td>
</tr>
</tbody>
</table>

Table 5.1: Cross Tabulation of ELISA and LFA Results for Kenya and Uganda Porcine Populations

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Result</th>
<th>95% C.I.</th>
<th>Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity (Se)</td>
<td>58.75%</td>
<td>52.47-64.84</td>
<td>(5.1)</td>
</tr>
<tr>
<td>Specificity (Sp)</td>
<td>75.26%</td>
<td>70.63-79.50</td>
<td>(5.2)</td>
</tr>
<tr>
<td>Positive Likelihood Ratio (PLR)</td>
<td>2.37</td>
<td>1.94-2.91</td>
<td>(5.3)</td>
</tr>
<tr>
<td>Negative Likelihood Ratio (NLR)</td>
<td>0.55</td>
<td>0.47-0.64</td>
<td>(5.4)</td>
</tr>
<tr>
<td>Positive Predictive Value (PPV)</td>
<td>61.38%</td>
<td>54.99-67.50</td>
<td>(5.5)</td>
</tr>
<tr>
<td>Negative Predictive Value (NPV)</td>
<td>73.16%</td>
<td>68.5-77.47</td>
<td>(5.6)</td>
</tr>
</tbody>
</table>

Table 5.2: Diagnostic Test Parameters for LFA using HP10 ELISA Results as a “Pseudo” Gold Standard

5.4.2 Bayesian Index of Agreement

A Bayesian approach to establishing agreement between tests was used with uninformed priors and returned the following levels of agreement between the LFA and the HP10 ELISA. Positive agreement = 60% (95% B.C.I. 55-65%) and negative agreement = 74.2% (95% B.C.I. 71-77%). Showing that the agreement between tests was better in the negative rather than positive direction.

5.4.3 Latent Class Model

The model performed well as indicated by good mixing of the chains shown in the trace plot in Figure 5.5 on the facing page and a Gelman-Rubin statistic of 1 for all parameters (where a value substantially over 1 indicates lack of convergence).
The posterior estimates of population prevalence and diagnostic parameters can be found in Table 5.3 on the next page and the distributions can be seen in Figure 5.6 on the following page.

The positive likelihood ratio for the HP10 ELISA was found to be 2.9 and 6.4 for the LFA, the negative likelihood ratios were 0.448 and 0.198 respectively.

5.4.4 Stability testing data for the Lateral Flow Assay

The LFA kept at 4°C provided consistent results for 10 months of testing. The LFA stored at 4°C returned identical results to those stored at 20°C. The assays stored at 37°C, however, begun to show inconsistent results from three months onwards, with a weakening of the positive indicator line and from month 6 onwards the control line became so weak as to be un-readable.

![MCMC History Plots of Hui-Walter Model](image)

Figure 5.5: MCMC History Plots of Hui-Walter Model
The plots record every 10th sample from 500,000 iterations and the x axis is the sequence of iterations and the y axis the parameter value from that iteration.
CHAPTER 5. EVALUATING A NOVEL DIAGNOSTIC FOR T. SOLIUM

Parameter | Posterior Estimate % (95% B.C.I.)
--- | ---
Se ELISA  | 65.2 (59.7-70.7)
Se LFA    | 82.7 (72.5-91.9)
Sp ELISA  | 77.6 (73.9-81.0)
Sp LFA    | 87.0 (80.2-93.4)
Prev western Kenya | 48.3 (39-57.8)
Prev central Uganda | 28.7 (19.7-38.5)

Table 5.3: Posterior Estimations of Diagnostic Test Parameters and Population Prevalence with 95% Bayesian Credibility Intervals

5.5 Discussion

The global “Road Map” for *Taenia solium* cysticercosis control requires that by 2015 validated control strategies for control will be available and by 2020 these strategy will be scaled up in selected countries (World Health Organization, 2012a). In order for these milestones to be reached, the international community must firstly be armed with the epidemiological data to inform control policies and to be “tool ready” with the relevant technologies to carry out and monitor the validated strategies.

I believe that the user-friendly rapid, LFA discussed in this chapter is one of the tools which will greatly assist the realisation of this road map. A diagnostic assay which can be used under field conditions will enable researchers and the veterinary services to quickly identify endemic areas, monitor the effectiveness of vaccination programs, assist in the exclusion of infective pigs from the food chain and enable strategic administration of anthelmintic treatments. In Chapter 3 on page 51 the use of such an assay was evaluated and was found to have the potential to reduce the exposure of pork consumers to viable *T. solium* cysts by 72.6% (95% C.I. 62.1-80.9%) and to be one of the most cost-effective strategies evaluated at an ICER of $0.25/infective meal avoided.

A great benefit of using such an assay to exclude infected pigs from the food chain is that infected animals can treated with oxfendendazole and represented for slaughter when cysts are non-viable. This provides an important safe-guard to public health, whilst allowing farmers to retain an important source of income.

It is important that care is taken that a situation does not arise where infected pigs are consequently sold into the informal “black” market. A similar situation occurred in Tanzania where infected pigs were unable to be sold into the local market due to an ongoing health education campaign. This led to them being sold into a neighboring area where education had not been given (Ngowi et al., 2008). Potential ways to mitigate such risks are discussed in Section 3.5 on page 81.

This assay may also be used to detect cysticercosis in cattle and humans, as the HP10 Ag-ELISA has been successfully used in these species as well as in pigs (Fleury et al., 2007; Harrison et al., 1989; Sciutto et al., 1998). As well as utility within the epidemiological setting, making this diagnostic tool available to the human health services would assist clinicians in the diagnosis of neurocysticercosis in resource poor areas.

In order to be of utility in this role the LFA needs to perform to a similar or better diagnostic standard as the HP10 ELISA on which it is based. The work presented here
shows that there is a discrepancy between the performance of the prototype LFA and the HP10 ELISA. The Bayesian Index of Agreement both suggest that the LFA has better agreement with the HP10 ELISA in the negative direction. These data suggest that the prototype requires further development to optimise the detection of circulating antigen.

A latent class model was used to evaluate the two diagnostic tests in the absence of a gold-standard. This model indicates that the HP10 ELISA is performing with a Se of 65.2% (95% B.C.I. 59.7 -70.66) and Sp of 77.6 (95% B.C.I.73.9-81). The credibility intervals overlap with those obtained by a previous latent class model, which estimated a Se of 70.4% (95% B.C.I. 52.7-84.7%) and Sp of 66.1% (95% B.C.I. 44.6-85.1%) (Krecek et al., 2011). The narrowing of the Bayesian credibility intervals in the current study indicates a more informed estimate of performance, potentially bringing the estimate closer to that of the true performance of the assay.

The prototype LFA appears to perform promisingly, with a Se of 82.7% (95% B.C.I.72.5-91.9) and Sp of 87% (95% B.C.I. 80.2-93.4). One hypothesis for the improved specificity of the monoclonal antibodies in the lateral flow format compared to the ELISA format may be a lack of non-specific binding which may occur in an ELISA format, and that utilising a detection method which does not rely on an essentially arbitrary cut-off value may have improved the sensitivity of the assay.

The LFA performing with sensitivity and specificity as estimated here is not yet adequate for use in a clinical setting, as the positive likelihood ratio of 6.3 and negative likelihood ratio of 0.198 for the LFA are not strong enough for the test to act as a stand alone tool to rule in or rule out a diagnosis (Deeks and Altman, 2004).

Latent class models are a tool to understand the performance of diagnostic assays, but cannot replace assessment against a “gold standard” (Albert and Dodd, 2004). There are several biases which may have been present in the analysis presented. The assumption of conditional independence may have been violated as the same MAb has been used for both diagnostic tests (Branscum et al., 2005). The modelling of conditional dependence between tests, can however, bring its own bias into an analysis if the covariances are mis-specified (Albert and Dodd, 2004). The decision was made therefore not to model such dependence as I felt inadequately informed to do so in a robust way.

Further development of this assay will require formal validation against the gold standard. The next stage of test development is already underway, supported by ASARECA
and a large scale field trial is planned where pigs will be tested, slaughtered and finely dissected for the detection of cysts. Cysts will then be tested for viability according to the protocol described by Sikasunge et al. (2008).

The current prototype assay has two further limitations for use under field conditions: instability at high ambient temperatures and the requirement to use sera as opposed to whole blood.

A successful assay for field use must be stable at high ambient temperatures, to reduce the reliance on refrigeration. The results of the stability trial conducted here indicate a high degree of instability in the prototype at temperatures over 20°C with the assay performing well when stored at 4°C or 20°C for up to 10 months, but only remaining stable for 3 months when stored at 37°C.

Addition of a blood filter would dramatically increase the utility of the assay. The present formulation of the assay requires the ability to separate sera from whole blood. This can be achieved using a centrifuge or by allowing the blood cells to settle out of solution. These techniques, however, require either equipment or time and therefore prevent the assay from being performed at the “pen side”. A blood filter would enable a venous sample to be taken from the ear vein of a pig using a blood lancet and capillary tube and used directly on the LFA, with a result being returned within 15 minutes.

The latent class model estimated a prevalence of porcine cysticercosis of 48.3% (95% B.C.I 39-57.8%) in western Kenya and in central Uganda of 28.7% (95% B.C.I 19.7-38.5%). The wide credibility intervals on these estimates indicate the large degree of uncertainty arising from the use of imperfect diagnostic tests. The prevalence estimate for Uganda is significantly ($p < 0.001$) higher than a previous estimate using the B60/B158 Ag-ELISA in southeast Uganda (8.5%, 95% C.I. 6-11%) (Waiswa et al., 2009). The estimate for western Kenya is also significantly ($p = 0.02$) higher than that estimated using the HP10 Ag-ELISA for a pig population in neighbouring Nyanga province in Kenya (32.8%, 95% C.I. 26.8-39.2%) (Eshitera et al., 2012). The previous studies do not make any adjustments in their estimates for the parameters of the diagnostic tests used which may account for some of the disparity observed here.

Control of this parasite cannot wait for the development of a “perfect” diagnostic test. We may need to accept, therefore, that obtaining and presenting accurate point estimates of prevalence, when using the imperfect diagnostic tests available, may be an unrealistic goal.

Despite the uncertainty surrounding these prevalence estimates, I believe they provide
strong evidence that both regions are endemic for *T. solium* and require urgent intervention to control this important zoonotic parasite.

Potential control strategies are discussed further in Chapter 7 on page 165 but interventions should include tackling issues such as the presence of free-range pigs, open human defecation and poor meat inspection that have been identified in the two study areas (Eshitera *et al.*, 2012; Kagira *et al.*, 2010; Nsadha *et al.*, 2010; Thomas *et al.*, 2013; Waiswa *et al.*, 2009).

5.6 Conclusion

This chapter describes progress towards the development of a suitable diagnostic assay for the detection of *T. solium* cysticercosis under field conditions. The initial evaluation of the prototype LFA indicates that continued development of this assay is justified, but that several improvements are required before it could be utilised in the field and the assay must then be evaluated against a gold standard.

Evidence is also presented for the endemic status of *T. solium* cysticercosis in the pig populations of both study areas. These regions are therefore strong candidates for the implementation of control programs, such as the deployment of a rapid diagnostic assay to aid the exclusion of infected pigs from the food chain as described in Chapter 3 on page 51.

Development of this assay will continue to advance alongside the design and validation of sustainable control strategies for *T. solium*. Implementation of these strategies are likely to require the involvement of an inter-disciplinary team, including experts in socio-economics and behavioural change, to ensure that any effort that is made to exclude infected pigs from the food chain does not encourage people to use informal markets to sell their pigs. The development of this diagnostic assay will be a not therefore be a “magic bullet” but could certainly be a useful addition to the arsenal in the battle against cysticercosis.
Chapter 6

The Spatial Ecology of Free-range Domestic Pigs (Sus scrofa) in Western Kenya

This chapter has been published as:
6.1 Abstract

6.1.1 Background

In many parts of the developing world, pigs are kept under low-input systems where they roam freely to scavenge food. These systems allow poor farmers the opportunity to enter into livestock keeping without large capital investments. This, combined with a growing demand for pork, especially in urban areas, has led to an increase in the number of small-holder farmers keeping free range pigs as a commercial enterprise. Despite the benefits which pig production can bring to a household, keeping pigs under a free range system increases the risk of the pig acquiring diseases, either production-limiting or zoonotic in nature. This study used Global Positioning System (GPS) technology to track free range domestic pigs in rural western Kenya, in order to understand their movement patterns and interactions with elements of the peri-domestic environment.

6.1.2 Results

We found that these pigs travel an average of 4,340 m in a 12 hr period and had a mean home range of 10,343 m² (range 2,937-32,759 m²) within which the core utilisation distribution was found to be 964 m² (range 2463,289 m²) with pigs spending on average 47% of their time outside their homestead of origin.

6.1.3 Conclusion

These are the first data available on the home range of domestic pigs kept under a free range system: the data show that pigs in these systems spend much of their time scavenging outside their homesteads, suggesting that these pigs may be exposed to infectious agents over a wide area. Control policies for diseases such as *T. solium* cysticercosis, Trypanosomiasis, Trichinellosis, Toxoplasmosis or African Swine Fever therefore require a community-wide focus and pig farmers require education on the inherent risks of keeping pigs under a free range system. The work presented here will enable future research to incorporate movement data into studies of disease transmission, for example for the understanding of transmission of African Swine Fever between individuals, or in relation to the life-cycle of parasites including *T. solium*. 
6.2 Background

Throughout the developing world the demand for meat products has been increasing by 4% per annum since the 1980s (Delgado et al., 1999), and with continuing population growth this trend is unlikely to abate. The need for fast-maturing sources of animal protein, which require low cereal inputs places the non-ruminant animals in prime position for fulfilling this growing demand. To this end pig production is becoming increasingly popular, with pork and poultry contributing 76% of the increased meat consumption in the developing world between 1982-1998 (Delgado et al., 2001). Pigs, *Sus scrofa*, have lower social prestige than cattle, but they are cheap to purchase and to raise and are therefore a popular option for resource-poor farmers, particularly women (Dewey et al., 2011). Taking advantage of the pigs natural ability as a scavenger, many of these resource poor farmers opt for an extensive, low input form of production, whereby the pigs roam freely. These systems allow an animal to be kept without the need for expensive supplementary feedstuffs (Lekule and Kyvsgaard, 2003). Pig production under these free range systems has been documented in many African countries, including: Kenya (Githgia et al., 2006), Uganda (Waiswa et al., 2009), Tanzania (Ngowi et al., 2004), Cameroon (Assana et al., 2010) and Zambia (Phiri et al., 2002). Within our study area of western Kenya there is abundant evidence of this production system, as illustrated in Figure 6.1 on the next page.
6.2.1 Diseases of Free Ranging Pigs

Pigs kept under all production systems can be the host of a variety of zoonotic and non-zoonotic pathogens, but allowing pigs to roam freely increases the disease transmission risk to the pig itself, to other wild and domestic animals, and to humans. Some diseases of particular relevance when considering free-roaming pigs are discussed below.

*Porcine cysticercosis*

The zoonotic tapeworm, *T. solium*, is one of the leading causes of acquired epilepsy in the developing world (Commission on Tropical Diseases, 1994). The parasite has a two-host life cycle, with humans as the definitive host, who become infected after consumption of viable cysticerci in under-cooked pork. The adult tapeworm inhabits the small intestine, causing an infection known as taeniasis, and gravid proglottids, containing thousands of infective eggs, detach from the adult worm and are excreted in faeces in an intermittent fashion (Garcia and Del Brutto, 2003). Ingestion of these...
eggs, by either pigs or humans, results in the larval stage penetrating the intestinal wall and moving through the lymph and blood vessels to encyst in muscle, eyes or the CNS as cysticerci (Flisser, 2006).

As contact with infective human faecal material by pigs is a requisite for the successful propagation of the parasite life cycle, it stands to reason that keeping pigs under a free-ranging system would increase the risk of the pigs acquiring this infection; this has been corroborated in several epidemiological studies (Assana et al., 2010; Pouedet et al., 2002; Sikasunge et al., 2007; Widdowson et al., 2000).

**Trichinelllosis**

Trichinella spp. are tissue dwelling nematodes, which are transmitted to humans by the ingestion of undercooked meat containing infective larvae. The parasite has a wide range of mammalian hosts, but the majority of human infections are acquired through the consumption of pork, with European cases almost exclusively from outdoor or back-yard production systems (Alban et al., 2008). Pigs acquire the infection through ingestion of infected wildlife carcasses, kitchen or slaughter waste. The ability of pigs to scavenge such material increases vastly when they are allowed to free range, heightening the relative risk of infection in comparison to confined pigs. The relative risk for Trichinella infection was estimated to increase by a factor of 25100 times for free range pigs in comparison to pigs kept in indoor units (Schuppers et al., 2010).

**Toxoplasmosis**

*Toxoplasma gondii* is a zoonotic protozoan parasite with a wide range of intermediate hosts, including pigs and humans, who acquire infection through the ingestion of infective oocysts excreted by cats, tachyzoites in raw milk, or encysted bradyzoites in infected meat (van der Giessen et al., 2007). The majority of human infections are thought to come from the ingestion of meat, in particular pork (Dubey, 2009; Tenter et al., 2000). The risk of infection for a pig is again related to its ability to scavenge in areas contaminated with either cat faecal material containing oocysts, or carcasses containing infective bradyzoites; therefore, it is strongly associated with free-roaming behaviours. Two studies from the Netherlands have found a significantly higher risk of sero positivity for toxoplasma antibodies in free range pigs than for those on an intensive pig unit (Kijlstra et al., 2004; Tenter et al., 2000). Exposure to infective cat faeces or to infected carcasses in pigs raised outdoors are risks for disease transmission,
which are likely to be exacerbated in the free range systems of the developing world.

African swine fever

African Swine Fever (ASF) is a hemorrhagic virus of the Asfarviridae family, which has major epizootic potential (Bengis et al., 2002). This infection is characterised by high mortality in domestic swine. It is transmitted either by direct or in-direct contact between domestic pigs or wild suids with or without an arthropod vector and is maintained by three distinct cycles: 1) a sylvatic cycle between the Argasid tick and warthogs, and possibly bush pigs or giant forest hogs (Anderson et al., 1998); 2) a cycle between domestic pigs and the Argasid tick; and 3) a domestic pig cycle not requiring ticks (Lubisi et al., 2005). There is also evidence that recently infected bush pigs and warthogs may be able to directly infect domestic pigs without need for the tick vector (Anderson et al., 1998). Wild boars have been implicated in virus transmission when they come into contact with infected free range domestic pigs, as was thought to be involved with the 2007 spread of ASF through Georgia (Jori and Bastos, 2009). Domestic pigs kept under free range systems are therefore at higher risk of contracting and transmitting ASF through contact with infected tick vectors or infected wild and domestic suids. Our study site in western Kenya has seen several ASF outbreaks over the last few years, most recently in 2011 (OIE, 2011).

Trypanosomiasis

Trypanosoma spp., transmitted by the tsetse fly (Glossina spp.), cause a reduction in productivity in pigs and pose a high risk to human health, with T. brucei gambiense and T. brucei rhodesiense causing Human African Trypanosomiasis (HAT). The pig is a significant source of blood meals for the tsetse fly (Simo et al., 2006; Waiswa et al., 2006) and has been implicated in the epidemiology of both human and animal trypanosomiasis, with outdoor, free-roaming pigs being at particular risk of contact with tsetse flies. In particular, pigs have been identified as a significant reservoir of T.b. rhodesiense in our study site (von Wissmann et al., 2011).

Non-zoonotic helminths

Helminths, such as Ascaris suum and Trichuris suis, are responsible for substantial economic losses for pig producers throughout the world, through reduced weight gain,
higher feed:gain ratio, condemnation of carcasses or organs and expenditure on prophylaxis or treatment (Stewart and Hale, 1988). Ascaris suum and Trichuris suis both require temperatures over 1537°C for embryonation and larval development, and the prevalence of these parasites have been found to be higher in outdoor pig units than intensive, indoor units (Nansen and Roepstorff, 1999). In a previous survey of free range pigs in the current study area pigs have been found to carry a substantial parasite burden, with an overall nematode prevalence of 84.2% and mean egg per gram (EPG) of 2,355 (Kagira et al., 2012), which is likely leading to detrimental economic burden for their (often already poor) keepers.

6.2.2 Spatial Ecology

To gain an understanding of the dynamics of disease within populations of free range pigs, the ecology of these animals must first be established. The behaviour of the domestic pig has been studied extensively within the context of intensive farming methods or through experiments to understand their social dynamics or learning ability (Held et al., 2005; Laughlin and Mendl, 2000). Knowledge of domestic pig behaviour under free range conditions, specifically the size of the home ranges and habitat preferences is, however, very limited with only one published paper from Mexico specifically looking to understand pig ecology under these systems (Copado et al., 2004). The authors of this paper identified some interesting aspects of free-ranging pig behaviour, specifically in relation to coprophagia. What was lacking, however, was the quantification of home range size and of habitat preferences of the pigs within this free range system, important elements of an understanding of the disease risks to which free range pigs are exposed.

The home range of an animal is “that area traversed by the individual in its normal activities of food gathering, mating, and caring for young. Occasional sallies outside the area, perhaps exploratory in nature, should not be considered as in part of the home range” (Burt, 1943). There are many different techniques available for determining the home range of animals and these have been extensively reviewed (Harris et al., 1990; Worton, 1987). We utilise two such methods: minimum convex polygon (MCP) and local convex hull (LoCoH). The MCP is the simplest of the convex hull methods, which represents the smallest polygon with no inside angle greater than 180° that can be drawn to encompass all locations at which the animal was recorded. This is a simple measure to calculate and is used by the International Union for Conservation of Nature as the standard measure of a species home range (Burgman and Fox, 2003). The MCP
method, however, is very sensitive to outlying points, which may reflect exploratory animal movement or measurement errors, providing an estimate of home range far beyond that utilised in the animals normal activities.

The k-1 nearest neighbours local convex hull technique was devised to improve on the MCP: it combines small MCPs which contain k-1 nearest neighbours, until all data points are included (Getz and Wilmers, 2004). This technique has been shown to perform well to reduce type I (exclusion of utilised areas) and type II (inclusion of un-utilised areas) errors and is particularly useful in locations where geographical features provide hard boundaries to a home range. This method also allows the isopleths containing any percentiles of the data points to be identified, providing us with the ability to determine utilisation distributions for various percentiles of use, for example the 50% isopleth, which corresponds to the core utilisation distribution and the 90% isopleth, corresponding to the true home range (van Beest et al., 2011). There are several studies which investigate the home range of truly wild living pigs (Mayer and Brisbin Jr, 2008), these being feral pigs of either domestic, European wild boar or hybrid origin. These studies have found a large variability in the home range (all based upon MCP determination) of these feral swine, from 0.52 km\(^2\) (Baber and Coblentz, 1986) to 20.3 km\(^2\) (Nugent et al., 2002) for wild caught and released feral pigs. The large variability in roaming behaviours in these studies makes it difficult to extrapolate the findings outside of these particular study environments, potentially due to the impact of environmental features on the home range (e.g. proximity to human habitats, sharp ravines or cliff faces, forest cover, etc.). The environment that wild pig studies have encompassed are mainly forested or conservation areas, where the ability to move freely over large distances is greater and human interference is negligible. An extrapolation to the roaming behaviour of domestically bred and raised, albeit free-roaming, pigs would be highly inadvisable. Here, we determine the geographical range of free-ranging domestic pigs in western Kenya, how far they travel during a day and night, and with which environmental features they spend time interacting.

6.3 Methods

6.3.1 Study area

The study area, shown in Figure 6.2 on page 150, is representative of the Lake Victoria Crescent ecosystem. It falls within a 45 km radius of Busia town in western Kenya,
bordered by Uganda to the west, Lake Victoria to the south, Mount Elgon to the north and Rift Valley Province to the east. The area is occupied predominately by members of the Luo, Luhya and Teso tribes. The area has bi-annual rains, occurring in March-May and August-October and supports a predominantly mixed crop-livestock production system with an average farm size of 0.5 ha (Kristjanson et al., 2004). Within this area, ten 3rd level administrative units, called divisions were selected based upon the popularity of pig production in these districts. Together these 10 divisions, Amagoro, Amukura, Budalangi, Butula, Chakol, Matayos, Funyula, Nambale Ujunga and Ukwala contain over 67% of the total pig population of the study area, which is estimated to be 66,307 by the district office of livestock and production: One sublocation (the smallest, 1st level administrative unit) from each of these Divisions, was selected at random using the Hawths tools extension (Beyer, 2004) for ArcMap 9.1 (ESRI, Redlands, USA). The ten selected study sub-locations, Bulemia, Anyiko, Asango, Sigalame, Nasewa, Bulwani, Malanga, Chakol, Amakuru and Kumuria can be seen in Figure 6.2 on the next page.
Figure 6.2: Map of Study Area Showing Selected Divisions: Geographical Data Sourced from the ILRI GIS Unit (International Livestock Reserch Institute, 2009) with Locator Map Showing Location of Study Site in Kenya and of Kenya in Africa
6.3.2 Animals

Between March 2011 and February 2012, one free range pig was randomly selected from each selected sublocation. The sample frame consisted of all pig keeping households within the sublocation, as provided by the relevant sublocation chief, a random number generator was used to pick the farmer from this list (farmers numbered first to last). On the selected farms pigs were excluded from the study if they were in the last trimester of pregnancy, were currently nursing piglets, were below 2 months of age or were due to be slaughtered in the next week (7 days from the day of selection). If more than one pig remained after exclusion they were allocated a number in age order and a random number generator was used to select the pig to be recruited, this was easy to achieve without any specific identifying procedure as the average pig herd size in the study site is only 2.6 (See Chapter 4 on page 87). The study was explained to the farmer and their consent obtained before the animal was recruited into the study. The pigs were selected across the course of the year as only one GPS collar was available; the data were therefore obtained across different seasons.

6.3.3 Data collection

A Garmin eTrex hand held GPS unit was used to obtain the coordinates of the homestead to which the pig belonged. The perimeter of the homestead, being that area utilised by the house for domestic activity (therefore excluding cropped fields), was tracked by walking along the boundary and if there was no discernible boundary the homestead members were asked for their best approximation of where their homestead perimeter lay. Features of the homestead (latrine, human dwelling, cooking point, rubbish disposal) were also mapped. A short questionnaire on pig husbandry was completed with the member of the homestead with the greatest involvement in the management of the pig.

The pig was restrained using a pig snare behind the upper canines and a lingual palpation to check for cysticercosis was performed (Mutua et al., 2007). Blood was collected from the external jugular or anterior vena cava into a 10 ml plain BD Vacutainer® tube using an 18 gauge 11/2” needle. A peripheral ear vein blood sample was collected using a blood lancet and micro-haematocrit tube and thick and thin blood smears were made immediately in the field. The pig was observed for the presence of ectoparasites and a note was made of the presence or absence of lice, mites or adult ticks, although ectoparasite species were not recorded. A faecal sample was taken by digital extraction
from each pig and all biological samples were transported on ice to the Busia laboratory facility. A webbing collar fitted with a GPS unit and General Packet Radio Service (GPRS) data transmission system (Savannah Tracking Ltd, Nairobi, Kenya) was then fitted to the pig, as shown in Figure 6.3, and the pig released. The collar weighed 350 g and operated using a 5400 mAmp/H rechargeable battery. Data were regularly uploaded to a server through the GPRS transmission system. The collar was set to record coordinates every 3 minutes for a one week (7 day) period from the day of recruitment.

Faecal samples were analysed for intestinal parasites using the McMasters (Nolan, 2006) and Kato-Katz (Ash et al., 1994) methods. Thick and thin blood smears were stained with Giemsa and these smears were examined by microscopy for haemoparasites. Serum samples were analysed by HP10 Antigen ELISA (Harrison et al., 1989) for the presence of viable *T. solium* infections.
6.3. METHODS

6.3.4 Analysis

Pig movement data from the GPS server were downloaded as a .csv file into Microsoft Excel and imported into ArcMap 9.1 and projected into UTM WGS 36 N. The LoCoh extension (Fortmann-Roe and Gettz, 2005) for ArcGIS (Getz and Wilmers, 2004) was used to produce a utilization distribution of these data using the k-1 nearest neighbour local convex hull technique with 10 percentile isopleths. The value of K was determined by taking the square root of the number of GPS positions available as suggested by the software developers.

ArcMap 9.1 was then used to select the density isopleths representing both 50% (core utilisation distribution) and 90% (home range) of the points. A minimum convex polygon (MCP) was calculated using the Hawths Tools extension for ArcGIS. The Hawths Tools extension was then used to calculate the area of the layer files created from these selections, to create a track from the GPS movement data and to determine the length of that track. Homestead points of interest and the perimeter boundary recorded using the hand held unit in the field were also imported into ArcMap 9.1. Individual points for each feature of a homestead and the perimeter boundary of each homestead (habitable area, as determined by the head of the household), were projected into UTM WGS 36 N and combined with the collar data to create informative data layers.

The area of the perimeter boundary polygon was calculated using Hawths Tools. The homestead features and the homestead itself were given a 5 m buffer using Hawths Tools, 5 m being chosen to represent the accuracy of the GPS units used. All pig movement data points which fell within these buffer areas were selected and the time spent within the areas were calculated as a percentage of the total number of positions recorded for each pig. All statistical analysis was performed using the R language and environment for statistical computing (R Development Core Team, 2005). The variables of interest were tested for violation of the assumption of normality using the Shapiro-Wilks test of normality, and due to the rejection of the null hypothesis (sample originating from a normally distributed population) for several of the variables it was decided to use non-parametric statistical methods, namely the Kruskal-Wallis rank sum test, Spearmans Rank Correlation and the Wilcoxon signed rank test.
6.4 Results

Ten pigs were selected and tracked during the time of this study, comprising 4 females, 2 male castrates and 4 male intact pigs with an average age of 6.7 months. All 10 pigs were kept under a free range system during the time of study. All pigs were fed supplementary food, being a combination of crop and household waste, with the household waste being fed uncooked to 8 of the 10 pigs. No farmer reported any previous clinical episodes for any of the sampled pigs. Only 3 pigs had received any prophylactic treatments, which included Levamisole (1 pig), Deltamethrin (1 pig) and an unknown anthelmintic (1 pig). Anthelmintic treatment appeared to make no significant effect on the total nematode EPG (Kruskal-Wallis chi-squared = 2.7, \( p = 0.260 \)). All pigs in this study were found to be infected by at least one parasite, with all pigs suffering from ectoparasites (adult ticks and lice in all cases) and 8 out of 10 also being infected with gastrointestinal parasites (Strongyloides spp., Strongyles, Trichuris spp., Coccidia and Ascaris spp. all being found). Three pigs were found to be infected with T. solium cysticercosis using the HP10 antigen ELISA (Harrison and Parkhouse, 1989; Harrison et al., 1989). A summary of the parasite burden for each pig is shown in Table 6.1 on the facing page. No haemoparasites were observed in any of the pigs.

The minimum convex polygon, home range and core utilisation distribution were determined for each pig and are illustrated in Figure 6.4 on page 156. The movement parameters calculated for each pig are also summarised in Table 6.2 on page 158. The mean distance moved by a free range pig in our study site over a 12 hr period was 4,340 m, with pigs moving 4,169 m (range 1,401-6,383 m) during daylight hours and 4,511 m (range 1,293-7,809 m) at night, with no significant difference between these periods (Wilcoxon signed rank test \( w = 1 \) \( p = 1 \)). The mean core utilisation distribution was found to be 947 m\(^2\) (range 1333,353 m\(^2\)) and the mean home range was found to be 15,085 m\(^2\) (range 2,937-74,887 m\(^2\)).
<table>
<thead>
<tr>
<th>Pig ID</th>
<th>Lice and adult ticks</th>
<th>T. Solium Cysticercosis</th>
<th>Gastrointestinal Parasites</th>
<th>EPG</th>
<th>TPS*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Yes</td>
<td>No</td>
<td>Strongyles</td>
<td>3,600</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Coccidia</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>Ascaris</em> spp.</td>
<td>13,900</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Yes</td>
<td>No</td>
<td><em>Strongyloides</em> spp.</td>
<td>24</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Strongyles</td>
<td>2,400</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>Ascaris</em> spp.</td>
<td>700</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Yes</td>
<td>Yes</td>
<td>None</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Yes</td>
<td>Yes</td>
<td>Strongyles</td>
<td>1,600</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>Ascaris</em> spp.</td>
<td>2,050</td>
<td></td>
</tr>
<tr>
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<td>No</td>
<td><em>Strongyloides</em> spp.</td>
<td>50</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Strongyles</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>Ascaris</em> spp.</td>
<td>3,300</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Yes</td>
<td>No</td>
<td>None</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Yes</td>
<td>No</td>
<td>Strongyles</td>
<td>100</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Coccidia</td>
<td>250</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Yes</td>
<td>No</td>
<td><em>Trichuris</em> spp.</td>
<td>100</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>Ascaris</em> spp.</td>
<td>5,650</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Yes</td>
<td>No</td>
<td>Strongyles</td>
<td>750</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>Trichuris</em> spp.</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>Ascaris</em> spp.</td>
<td>400</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>Strongyloides</em> spp.</td>
<td>750</td>
<td>6</td>
</tr>
<tr>
<td>10</td>
<td>Yes</td>
<td>Yes</td>
<td>Strongyles</td>
<td>350</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Coccidia</td>
<td>9,200</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>Ascaris</em> spp.</td>
<td>1,100</td>
<td></td>
</tr>
</tbody>
</table>

% pigs infected | 100% | 30% | 80%  
(95% C.I.)      | (59-100) | (7-65) | (44-97) |

Table 6.1: Summary of Parasitic Infections in Study Pigs

*TPS = Total parasite spectrum, the total number of parasite species infesting each pig.*
Figure 6.4: Illustration of Movement Parameters for Each Pig
In this small study, neither sex of pig or season were found to influence movement parameters as shown in Tables 6.3 on the next page & 6.4 on the following page and no correlation was found between any movement parameter and the total parasite burden, calculated as sum of the eggs per gram (EPG) for all nematode species identified. No correlation was found either between movement parameters and the EPG of Strongyloides spp, Strongyles, Trichuris spp. and Ascaris spp, though a moderate correlation (Spearmans rank correlation rho = 0.75, \( p = 0.010 \)) was found between the home range area and the Coccidia EPG, though this was heavily influenced by an outlier as shown in Figure 6.5.

![Box Plot of Home Range Area and Coccidia EPG (rho = 0.75, \( p = 0.010 \))](image)

Figure 6.5: Box Plot of Home Range Area and Coccidia EPG (rho = 0.75, \( p = 0.010 \))

Pigs spent on average half (53%) of their time within the perimeter boundary of the households, or, otherwise stated, almost half their time outside the homestead. These homestead boundaries were often ill-defined, and all were porous. The time spent interacting within a 5 m radius of certain homestead features is shown in Table 6.5 on page 160. The pigs in this study were shown to only spend on average 1.3% of their time interacting with the latrine area in their homestead of origin, 1.6% in the vicinity of the rubbish disposal area, 2.7% in the vicinity of the human dwellings and 4.3% in the
<table>
<thead>
<tr>
<th>Pig ID</th>
<th>Ave. Daily Distance Moved (m)</th>
<th>Ave. Nightly Distance Moved (m)</th>
<th>Core utilisation distribution (m²)</th>
<th>Home Range (m²)</th>
<th>MCP Area (m²)</th>
<th>Homestead area (m²)</th>
<th>% time spent within homestead perimeter</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1,401</td>
<td>1,293</td>
<td>612</td>
<td>9,315</td>
<td>108,617</td>
<td>224</td>
<td>54.1</td>
</tr>
<tr>
<td>2</td>
<td>3,707</td>
<td>4,067</td>
<td>409</td>
<td>12,685</td>
<td>346,585</td>
<td>2,143</td>
<td>70.7</td>
</tr>
<tr>
<td>3</td>
<td>3,824</td>
<td>3,479</td>
<td>424</td>
<td>5,380</td>
<td>709,809</td>
<td>1,048</td>
<td>61.6</td>
</tr>
<tr>
<td>4</td>
<td>3,463</td>
<td>3,387</td>
<td>133</td>
<td>5,805</td>
<td>123,189</td>
<td>1,707</td>
<td>51.1</td>
</tr>
<tr>
<td>5</td>
<td>2,992</td>
<td>2,815</td>
<td>410</td>
<td>2,937</td>
<td>101,650</td>
<td>1,666</td>
<td>65.7</td>
</tr>
<tr>
<td>6</td>
<td>4,557</td>
<td>4,812</td>
<td>701</td>
<td>4,993</td>
<td>197,420</td>
<td>775</td>
<td>34.7</td>
</tr>
<tr>
<td>7</td>
<td>5,933</td>
<td>7,809</td>
<td>1,582</td>
<td>19,554</td>
<td>267,869</td>
<td>4,328</td>
<td>61.7</td>
</tr>
<tr>
<td>8</td>
<td>6,383</td>
<td>6,927</td>
<td>967</td>
<td>7,540</td>
<td>429,339</td>
<td>1,646</td>
<td>66.7</td>
</tr>
<tr>
<td>9</td>
<td>4,608</td>
<td>4,293</td>
<td>873</td>
<td>7,749</td>
<td>81,218</td>
<td>802</td>
<td>8.55</td>
</tr>
<tr>
<td>10</td>
<td>4,825</td>
<td>6,219</td>
<td>3,353</td>
<td>74,887</td>
<td>289,990</td>
<td>2,834</td>
<td>53.8</td>
</tr>
<tr>
<td>Ave.</td>
<td>4,169</td>
<td>4,511</td>
<td>947</td>
<td>15,085</td>
<td>265,569</td>
<td>1,717</td>
<td>52.9</td>
</tr>
</tbody>
</table>

Table 6.2: Movement Parameters of Spatial Ecology Study Pigs

<table>
<thead>
<tr>
<th>Season</th>
<th>Daily Distance Moved (m)</th>
<th>Home Range (m²)</th>
<th>Core Utilisation Distribution (m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet (pigs 3,4,5,6,7)</td>
<td>4,154</td>
<td>7,734</td>
<td>650</td>
</tr>
<tr>
<td>Dry (pigs 1,2,8,9,10)</td>
<td>4,185</td>
<td>22,435</td>
<td>1,243</td>
</tr>
<tr>
<td>Kruskal-Wallis rank sum test</td>
<td>H = 0.2727, p = 0.602</td>
<td>H = 3.153, p = 0.076</td>
<td>H = 0.8836, p = 0.347</td>
</tr>
</tbody>
</table>

Table 6.3: Pig Movement in Wet and Dry Seasons

<table>
<thead>
<tr>
<th>Sex of Pig</th>
<th>Daily Distance Moved (m)</th>
<th>Home Range (m²)</th>
<th>Core Utilisation Distribution (m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female (n = 4)</td>
<td>4,756</td>
<td>28,030</td>
<td>1,511</td>
</tr>
<tr>
<td>Male Castrate (n = 2)</td>
<td>2,613</td>
<td>7,348</td>
<td>518</td>
</tr>
<tr>
<td>Male Intact (n = 4)</td>
<td>4,362</td>
<td>12,015</td>
<td>596</td>
</tr>
<tr>
<td>Kruskal-Wallis rank sum test</td>
<td>H = 2.046, p = 0.360</td>
<td>H = 2.3727, p = 0.310</td>
<td>H = 1.146, p = 0.56</td>
</tr>
</tbody>
</table>

Table 6.4: Movement Parameters According to Sex of Pig
vicinity of the cooking point: it is important to note that these interactions were only determined within the homestead of origin. Time spent interacting with homestead features was not found to influence parasite burden apart from in the case of Ascaris spp., where time spent interacting with latrines was found to be positively correlated with the EPG count (Spearman’s Rank Correlation rho = 0.81, p = 0.005).
### Table 6.5: Interactions Between Pigs and Homestead Features

<table>
<thead>
<tr>
<th>Pig ID</th>
<th>Homestead area (m²)</th>
<th>% time within homestead perimeter</th>
<th>% time interacting with latrine</th>
<th>% time interacting with rubbish disposal</th>
<th>% time interacting with cooking point</th>
<th>% time interacting with human dwellings</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>224</td>
<td>54.1</td>
<td>4.9**</td>
<td>0.2</td>
<td>2.5***</td>
<td>1.03</td>
</tr>
<tr>
<td>2</td>
<td>2143</td>
<td>70.7</td>
<td>0.1**</td>
<td>5.2</td>
<td>11.5*</td>
<td>6.8</td>
</tr>
<tr>
<td>3</td>
<td>1048</td>
<td>61.6</td>
<td>0.7**</td>
<td>Not observed</td>
<td>11.6**</td>
<td>5.9</td>
</tr>
<tr>
<td>4</td>
<td>1707</td>
<td>51.1</td>
<td>0.2**</td>
<td>4.1</td>
<td>0.9**</td>
<td>4.0</td>
</tr>
<tr>
<td>5</td>
<td>1666</td>
<td>65.7</td>
<td>0.8**</td>
<td>0.6</td>
<td>1.6*</td>
<td>2.0</td>
</tr>
<tr>
<td>6</td>
<td>775</td>
<td>34.7</td>
<td>0**</td>
<td>0.2</td>
<td>5.9*</td>
<td>2.8</td>
</tr>
<tr>
<td>7</td>
<td>4328</td>
<td>61.7</td>
<td>2.1**</td>
<td>0.1</td>
<td>0.1*</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>1646</td>
<td>66.7</td>
<td>3.7**</td>
<td>3.7</td>
<td>Not observed</td>
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</tr>
<tr>
<td>9</td>
<td>802</td>
<td>8.55</td>
<td>0.03*</td>
<td>0.2</td>
<td>Not observed</td>
<td>0.1</td>
</tr>
<tr>
<td>10</td>
<td>2834</td>
<td>53.8</td>
<td>0.3*</td>
<td>0</td>
<td>0*</td>
<td>1</td>
</tr>
<tr>
<td>Ave.</td>
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<td>52.9</td>
<td>1.3%</td>
<td>1.6%</td>
<td>4.3%</td>
<td>2.7%</td>
</tr>
</tbody>
</table>

* Homestead feature fully enclosed. ** Homestead feature partially enclosed, *** Homestead feature not enclosed.
6.5 Discussion

This is the first study to have investigated the ecology of domestic pigs kept under a free range system, utilising GPS technology. We found that these pigs travel an average of 4,340 m in a 12 hr period and had a mean home range of 10,343 m², within which the core utilisation distribution was found to be 964 m². The lack of significant difference ($p = 0.824$) between day and night time movement indicates that the pigs are benefiting from a foraging strategy which involves both night and day scavenging. Nocturnal behaviour has been observed in wild pigs (Robert et al., 1987) who seem to be able to adjust their activity patterns based upon food availability (Fraser, 1984).

Although this study was not designed to investigate population level influences on the movement parameters, it is interesting to note that no influence of season or sex of pig was found on any of the movement parameters. The pigs in this study were not influenced by management imposed restrictions on their movements during certain times of the year as selection criteria for the study animals was that they were kept on a free range basis. Another study in western Kenya that this team has conducted found only a 1.4% change in confinement in pigs between the wet and dry seasons (see Chapter 4 on page 87).

No influence was found in this small study on parasite burden from movement parameters or interaction with homestead features apart from a positive correlation between Ascaris spp. EPG and the time spent interacting with latrines (Spearmans Rank Correlation rho = 0.81, $p = 0.005$) and a moderate positive correlation between Coccidia EPG and home range area (Spearmans rank correlation rho = 0.75, $p = 0.010$), the second of which appears to be highly influenced by one outlier. We could hypothesis that there may be a higher number of earthworms and dung beetles around a latrine area, which could be acting as paratelic hosts.

Despite the lack of association between the parameters measured and the health status of the pigs in this study, these findings do, however, have major implications for our understanding of pig husbandry and disease control within resource poor settings. For example, a domestic, free-ranging pig spends only 50% of time within the homestead that owns it, indicating a high likelihood of exposure to environmental features, contaminants and pathogens outside the home area. Thus, when considering control policies for reducing infectious diseases in pigs, interventions targeting only pig owning households may be less effective than expected, and a community approach is clearly required.
Three out of the ten pigs recruited into this study were found to be positive for \textit{T. solium} circulating antigen, which is a high prevalence compared to previous studies in the area which have found between 4\% (Kagira \textit{et al.}, 2012) and 10.5\% (Githgia \textit{et al.}, 2006). However, a survey of 343 pigs at slaughter facilities in the study area immediately prior to the onset of the current study has found a prevalence of circulating antigen, using the same HP10 ELSIA of 48.7\% (See Chapter 5 on page 121). This indicated that the area is, in fact, hyper-endemic for \textit{T. solium} and we are therefore unsurprised that pigs selected on the basis of a known risk factor for cysticercosis infection were found to be infected.

In the case of \textit{T. solium} cysticercosis, the porcine infection is acquired by the ingestion of infective eggs or proglottids in human faecal material that contaminates the pigs environment. Many studies have looked at the presence or absence of a latrine in a homestead as being a risk factor for cysticercosis infection in pigs; however, there has been no consensus between these studies. Some authors have found that the presence of a latrine is a risk factor for porcine cysticercosis (Pouedet \textit{et al.}, 2002; Sarti \textit{et al.}, 1992) and others that latrines are protective (Kagira \textit{et al.}, 2010; Mutua \textit{et al.}, 2007; Ngowi \textit{et al.}, 2004). In this study we found no association between the time spent interacting with a latrine on the homestead of origin and the \textit{T. solium} status of the pig, which we believe suggests that the presence or absence of a latrine in an individual home is of less relevance to parasite transmission than overall provision of sanitation for the wider community in which the pig roams.

Although the observations made during this study suggest that pigs spend only a small amount of time interacting with the latrine area in their own homesteads (1.3\%), we cannot discount the potential for pigs to come into contact with human faecal material elsewhere on the homestead or in neighboring homesteads. We also note that any degree of access to human faecal material in or around a latrine, however short in time, is enough for transmission of the parasite to occur. Furthermore, 25\% of homesteads in our study area do not have access to a latrine (see Section 4.4.1 on page 95, Chapter 4 on page 87), meaning that many people have no choice but to engage in open defecation, raising a very real possibility for pigs to contact human faecal material, and therefore potentially infective \textit{T. solium} eggs. Finally, not all latrines are of the same quality, such that pigs may be able to access latrine buildings that are not properly enclosed: in this study area only 29\% (see Section 4.4.1 on page 95, Chapter 4 on page 87) of latrine buildings were completely enclosed, and therefore not accessible to scavenging animals.
One method for the improvement of sanitation, which uses the whole community approach is the so called “community led total sanitation” (Kamal and Chambers, 2008). This method attempts to trigger a community’s engagement with its own sanitation issues to reduce open defecation. Using this approach, communities take control of producing locally appropriate latrines and ensure that all community members use them. Such blanket coverage is likely to be far more effective than piecemeal individual adoption of latrines with respect to the exposure of free range pigs to faecal material.

Gastro-intestinal and ectoparasite infections are another important, production limiting issue for pig producers, as shown in Table 6.1 on page 155. Heavy infestation with these parasites can lead to reduced weight gain in pigs (Stewart and Hale, 1988), reducing the economic potential of these livestock. We found that only 2 of the 10 pigs recruited into this study were said to have had any anthelmintic treatment (2 pigs) in the 6 months prior to the study, and this was not found to have any influence on parasite load (in EPG for any nematode species). A lack of influence of levamisole treatment on EPG was also found in another study in western Kenya (Stewart and Hale, 1988), suggesting either anthelmintic resistance, or incorrect usage of the drugs.

Improved husbandry practices, including the use of effective anthelmintics at correct dosages, would enhance pig health and production in this study area. Importantly, we also find that the distances that free range pigs move on a daily basis (mean of 4.1 km during daylight and 4.5 km at night) are likely to entail high energy expenditure. Mature pigs 6-10 months old presenting at slaughter in this region have been found to have mean live weights at the abattoir of 30 kg, giving a dressed weight of only 22.5 kg and earning the farmer only 2,000-2,500 KES (Levy et al., 2009), equivalent to US$24-29 per animal. Encouraging the confinement of pigs is likely to improve feed conversion and weight gain, by both reducing un-necessary energy expenditure as well as limiting parasite burden through environmental exposure.

Confinement of pigs would also reduce the risk of contact with other domestic or wild pigs: pig to pig contact is a driver of ASF virus transmission. ASF regularly causes outbreaks in this region, with two reported outbreaks at the end of 2010, both of which were reported as being resolved by early 2012 (OIE, 2011). Confining pigs within correctly constructed pig stys would also reduce the chances of contact between pigs and tsetse flies (Peter et al., 2005) the vectors of Trypanosoma spp. Western Kenya is a trypanosomiasis endemic area and pigs are known to be important hosts and reservoirs (von Wissmann et al., 2011; Waiswa et al., 2006).

Both Trichinellosis and Toxoplasmosis are very real threats to these free-ranging pigs,
with access to kitchen waste, in particular meat products, being a risk factor for infection. Such swill is also implicated in ASF transmission. Pigs in this study were observed spending an average of 5.9% of their time in the vicinity of the cooking and waste disposal areas of their homestead of origin, illustrating the potential for ingestion of meat, which may contain infective tissue cysts of \textit{Toxoplasma gondii} or \textit{Trichinella spirallis}. Porcine toxoplasmosis can also be acquired through the ingestion of sporulated oocysts in cat faecal material: given that 49% of households in this region (See Section 4.4.1 on page 95, Chapter 4 on page 87) report owning cats, combined with the scavenging behaviour of free range pigs, it is easy to infer from this the degree of contact with feline faecal material which takes place that may propagate this parasite.

While confinement would clearly be advantageous, there are practical and societal difficulties to overcome in encouraging the practice, not least because free range pig keeping is attractive to farmers due to the low input nature of the production system and the ease of implementation. Local extension services in areas where free ranging is practiced across East Africa should work to convince farmers that investing in improving pig production can reap important economic benefits in terms of weight at slaughter, as well as improve bio-security and herd health on small-holdings.

6.6 Conclusion

These data provide new insights into the behaviour of pigs kept under a free range system in a resource-poor setting. We believe that the data presented here can be used in conjunction with information on pig population densities to build contact network models and to better understand transmission of several pathogenic organisms. For example, understanding transmission of African swine fever between individual pigs or between domestic and wild pigs. The movement data can also be combined with information on ration formulation and daily weight gain to provide evidence-driven advice to farmers on how to change their animal husbandry practices to improve the profitability of pig production. The key messages are: 1) pigs kept under these systems spend almost half their time outside their homestead boundaries, such that the village environment beyond the farm matters just as much as the environment on the farm itself to pathogen transmission, and 2) free range domestic pigs expend tremendous energy foraging in the village environment, thus reducing their potential for weight gain and economic benefit to their owners.
Chapter 7

Discussion: *Taenia solium* in Western Kenya: The Current Situation and the Way Forward
Poverty is both a cause and consequence of the high rates of mortality and morbidity due to infectious diseases suffered by many of the world’s population. A number of the pathogens which afflict these marginalised communities have historically been “neglected”, by the global community, in terms of research (Vanderelst and Speybroeck, 2010) and investment in new pharmaceutical products for treatment or prevention (Trouiller et al., 2002; Yamey, 2002). There is now growing acknowledgment of the link between health and poverty and a growing voice calling for control of these endemic, neglected tropical diseases (NTDs) for the benefit of the world’s poorest communities (Molyneux et al., 2005).

The World Health Organization and partners have been instrumental in highlighting the NTDs, and their possibly even more neglected “cousins” the neglected zoonotic diseases, through a series of international meetings and reports (World Health Organization, 2003, 2007, 2010; World Health Organization/DFID-AHP, 2005). Many successes have been achieved within the last decade (Hotez et al., 2007; Molyneux et al., 2005) and a recent inclusion of a group of the NTDs in the Global Burden of Disease Survey 2010 (Murray et al., 2013) shows a reduction in the DALY burden (from a retrospectively estimated 1990 figure) of all bar Schistosomiasis and Trachoma (Murray et al., 2013).

Much more must still be done, however, and to this end a “Road Map” for the control of the NTDs has been produced (World Health Organization, 2012a), and a declaration to this effect signed in London by many public and private sector stakeholders (World Health Organization, 2012b). This road map was recently endorsed by the World Health Assembly, who adopted resolution WHOA66.12 on the 27th May 2013 (World Health Organization, 2013a).

Most recent reports suggest that approximately 30% of the global population (excluding India and Bangladesh) are currently receiving preventative prophylaxis for at least one of the neglected tropical diseases. To meet the goals of the road map, however the speed that interventions are rolled out needs to be increased dramatically (World Health Organization, 2013b).

One of the neglected tropical diseases for which the road map requires the global community to prepare control strategies is the zoonotic helminth *Taenia solium*, the focus of this thesis. It is expected that validated control strategies should be available by 2015, to be rolled out on a larger geographical scale by 2020 (World Health Organization, 2012a). In order to do so, however, baseline epidemiological data must be provided, both for the parasite itself, and to identify areas of co-endemicity, for which integrated control strategies for the NTDs can be devised.
This thesis has provided evidence for the endemic status of *Taenia solium* in western Kenya, an area in which soil transmitted helminths, Schistosomiasis and Amoebiasis are also prevalent (see Section 4.4.1 on page 95). We have demonstrated how the current levels of infection in pigs, combined with poor meat hygiene practices are causing a high level of infection risk associated with consumption of pork in the region (see Section 3.4 on page 72) and how a common husbandry practice of free-range pig production, combined with poor latrine provision may result in transmission of the parasite back to the porcine host (see Section 6.5 on page 161). The performance of a novel, user-friendly diagnostic assay for cysticercosis has also been evaluated (see Chapter 5 on page 121), moving us closer to being “tool ready” for proposed intervention strategies.

A key strength of the studies presented here is the integrated nature of the data collection, utilising a multi-disciplinary team to collect data on multiple pathogens in multiple (human and animal) hosts. This approach is to be strongly encouraged in the investigation of the neglected tropical diseases, as co-endemicity is incredibly common (Singer and Ryff, 2007) and is particularly important when dealing with zoonotic diseases, which have a tendency to “fall between the stools” of veterinary and public health remits (Lembo et al., 2011). This approach has allowed us to complete one of only few studies which have investigated the epidemiology of *T. solium* simultaneously in the porcine and human host in a community setting.

All stages of the zoonotic parasite *T. solium* have been demonstrated in the current study population which is also co-endemic for schistosomiasis and soil transmitted helminths. This is strong evidence for the need for suitable control strategies to be implemented in this region for the integrated control of these neglected tropical diseases.

As described in Chapter 4 on page 87 there may be over 4000 people suffering from epileptic seizures due to neurocysticercosis (NCC) within this population of approximately 3 million people. The seizures can lead to physical impairment and injury, cognitive disorders and, potentially most damagingly, social stigmatisation, with the associated depression, anxiety and other mental disorders which may arise as a consequence (de Boer et al., 2008).

A taeniasis prevalence of 13.9% (95% C.I. 11.3-16.6%) was estimated in this population, although it appears that the majority of these infections are likely to be *T. saginata*, with the prevalence of *T. solium* estimated to be under 1% (0%, 95% C.I. 0-0.9%) in line with studies in other endemic communities (DeGiorgio et al., 2005; Praet et al., 2013).
Pork consumption was found to be a significant risk factor for taeniasis at the homestead level (OR = 1.1, 95% C.I. 1.0-1.24, \( p =0.04 \)), which corroborates the findings in Chapter 3 on page 51 where pork consumed in western Kenya was shown to be a high risk product. The stochastic risk model indicated that there are approximately 810,000 potentially infective pork meals consumed in the study area in any one year. Preventing consumption of infective pork would be an effective way to reduce taeniasis cases in the population with the use of a pre-slaughter diagnostic test, described in Chapter 5 on page 121, having the potential to avoid 72.6\% (95\% C.I. 62.1-80.9\%) of potentially infective meals consumed in one year (See Table 3.3 on page 75).

The data presented in this thesis leaves some uncertainty surrounding the prevalence of porcine cysticercosis in this community and this uncertainty is in part linked to limitations arising from the study design and diagnostic technologies used.

The cross-sectional study which comprises a major part of the work presented here was designed to obtain valid epidemiological data on a range of zoonotic and non-zoonotic pathogens in the community of western Kenya. We wished to obtain a representative sample of the whole community, both livestock owners and non-livestock owning homesteads, which was achieved, with the population described in detail in Section 4.4.1 on page 95. This study design did not, therefore, specifically target pig owning homes which are, as shown in Figure 2.6 on page 43, clustered in certain districts within the study area. This, in combination with a lower than expected prevalence of pig ownership and pig herd size (as discussed in Section 4.5 on page 113) led to a very small sample of pigs in this study.

The difficulty in recruiting sufficient numbers of pigs at the homestead level was acknowledged early in the trialling phase of the homestead study, and the design of the slaughter facility study grew out of this issue. I also realised that it was the pigs entering the food chain that were important in the life cycle of this parasite and that this study would be the most important in understanding the current risk attributable to pork consumed in western Kenya.

The prevalence of viable *T. solium* cysticercosis in the pigs sampled at slaughter was estimated by the latent class model in Chapter 5 on page 121 to be 48.3\% (95\% B.C.I. 39-57.8\%). Similar prevalence levels have been estimated using Bayesian models in Burkino Faso (32.7\%, (95\% BCI: 25.4-68.3\%) and 48.2\% (95\% BCI: 35.4-82.6\%) in two separate villages) (Ganaba *et al.*, 2011) and South Africa (56.7\% (95\% B.C.I. 40.6-76.3\%) (Krecek *et al.*, 2011). Putting western Kenya amongst the regions with the highest reported prevalences.
Bayesian frameworks allow for the inclusion of uncertainty surrounding diagnostic test parameters. This is important here as there are considerable variations in sensitivity and specificity estimates for the HP10 Ag-ELISA (as shown in Table 1.1 on page 15). The diagnostic parameters varying depending on species, type of infection (natural vs experimental), weight of infection and location of cysts (eg. intra-parenchymal vs extraparenchymal) (Ferrer et al., 2005; Fleury et al., 2007, 2003; Krecek et al., 2011; Sciutto et al., 1998).

The adjustments made on point estimate figures in Chapter 4 on page 87 were made using the latest, species specific estimates of sensitivity and specificity, these adjustments do not incorporate the uncertainty that surround these estimates and so do not reflect reality as closely as perhaps a Bayesian approach would. These adjustments also make the assumption that the tests work within consistent parameters across individuals. This assumption may have been violated due to the infection heterogeneity which is likely to be found within the population (Greiner and Gardner, 2000a). This heterogeneity of infection was taken into account within the stochastic risk model presented in Chapter 3 on page 51, but is more difficult to account for in estimations of prevalence.

In the porcine host, there is also a potential, though un-quantified cross-reaction of the HP10 antigen ELISA with *T. hydategina* (Leslie Harrison, Per. comms.). Although this parasite is not thought to be an important parasite of pigs in East Africa (Dorny et al., 2004), it has been detected in pigs at low levels in Zambia (Dorny et al., 2004) and Tanzania (Ngowi et al., 2004). It may be, therefore, leading to inflated estimates of the parasite in the porcine host. This is not an issue in human testing, however, as *T. solium* is the only *Taenia* spp. which uses humans as an intermediate host.

I feel it is important, therefore, that the prevalence estimates presented in this thesis and those provided from many other studies, require careful interpretation. We must take into account what is known about the diagnostic tests used and combine this with a “holistic” assessment of the likely risk within the community under investigation; utilising our knowledge about the pig husbandry systems, sanitation levels and provision of veterinary and meat hygiene services the in the area.

Despite the uncertainty surrounding the prevalence estimates reported here, the detection of *T. solium* infections in the porcine and human hosts, in combination with the presence of key risk factors (See Table 4.3 on page 100) provides substantial evidence to justify intervention programs in this region.
The control of *T. solium* at a global level, cannot afford to wait for the development of a “perfect” test. What are lacking, however, as is the case with many of the NTDs, are diagnostic tools which can be used in resource poor settings where well equipped laboratories and trained laboratory technicians may not be available (Petti *et al.*, 2005). This is the case for both porcine and human cysticercosis (Fleury *et al.*, 2007; Pal, 2000).

Presented in Chapter 5 on page 121 is an evaluation of an LFA which is currently being developed to address this need. The assay appears to show promising performance, but the caveats expressed here about the diagnostic assays against which it has currently been compared, and the inherent uncertainty within Bayesian evaluation techniques require future validation of this test to be carried out against a “gold standard” such as the fine dissection of pigs.

During the current study I was unable to obtain and experimentally infect pigs. Had I been able to do so post-mortem inspections could have been carried out to quantitative infection levels and calibrate the new assay accordingly. It is strongly hoped that continued funding for this project will enable these activities, as well as the addition of a blood filter to enable “pen-side” use.

The utility of such a diagnostic was demonstrated in Chapter 3 on page 51 and has the potential to be part of an integrated control strategy for *T. solium* and other NTDs within this study area. Considering the evidence provided in this thesis and the vast commentary surrounding this area, I would suggest that the following strategy could be trialed in our study area, for which extensive baseline data is now available.

A proposed integrated control strategy for *T. solium*, schistosomiasis, ascariasis, hookworm and trichuriasis in this study area would consist of the following activities:

- Regular mass drug administration in humans
- Targeted screening and treatment of at-risk groups of humans
- Vaccination and anthelmintic administration in pigs
- Improvement in pig husbandry practices
- Provision of a pre-slaughter diagnostic to pig traders

Elimination of human taeniasis carriers through chemotherapy is a priority, both to break the life cycle of the parasite and to reduce the infection risk to humans of neurocysticercosis (Pawlowski, 2008). The co-endemicity of other NTDs, (see Table 4.4 on
would justify human treatment with at least a combined package of albendazole and Praziquantel, from the “rapid impact” drug package for NTDs (Hotez, 2007; Molyneux et al., 2005). Preventative chemotherapy provided as mass-drug administration on a yearly basis (Hotez, 2007), should be supplemented by targeted identification and treatment of any new taeniasis cases, either through self-reporting or from targeted screening of at-risk groups (i.e. slaughter-workers) (Pawlowski, 2008).

Such mass drug administration must take place respecting several potential issues:

- Effective community sensitisation must take place for these programs to be successful (Parker et al., 2008). The recipients of treatment must understand for what reason they are being provided with the drugs, the potential side-effects and also be provided with suitable preventative health education messages (Amarillo et al., 2008).

- Acknowledgements must be made to the possibility of anthelmintic resistance development (Geerts and Gryseels, 2001). Although very few cases of Praziquantel resistance have been documented for Schistosomiasis (Fenwick and Webster, 2006), correct dosages must be adhered.

- Praziquantel and albendazole are cysticidal drugs, and have the ability to trigger epileptic seizures in otherwise asymptomatic individuals, through no neurological signs have been routinely reported to date in the national Schistosomiasis control programmes (Winkler, 2012). It is incredibly important, however, given the likely presence of latent NCC cases in this population that the onset of neurological signs are intensively monitored and investigated throughout such a control program.

- Suitable provision must be made for the disposal of faeces after drug administration so that people and pigs may not come into contact with potentially infective faecal material. The collection of this material for incineration would also provide an opportunity for samples to be taken for monitoring of the programs’ effectiveness.

Preventative chemotherapy programs (PCP) for control of T. solium, Schistosomiasis and soil transmitted helminths are relatively inexpensive (approximately $0.2/dose of praziquantel and $0.02/dose of albendazole (Brady et al., 2006) to purchase, although many PCPs have anthelmintics donated by pharmaceutical companies (Brockarie et al., 2013)), but success depends on addressing the issues listed above. PCP has been carried out successfully by a multitude of parties involved in the control of the NTDs
with over 700 million individual treatments made annually (Brockarie et al., 2013). If the experience of ongoing programs is utilised correctly the feasibility of successfully implementing a PCP in western Kenya is high.

Targeted treatments of taeniasis cases are less problematic in terms of acceptibility to patients, potential for adverse reactions and possibility of anthelmintic resistance development. Detecting cases for treatment is, however, difficult due to problems inherent in the health systems in endemic communities. These problems relate to the health-seeking behaviour of the community members, poor diagnostic ability at health centres and poor reporting systems within the health service.

For patients to report to the health service for treatment they need to both understand the need to treatment, requiring effective public health education. The cultural norms of the society need to permit the patient to report to official health care services (Hildenwall et al., 2008; Nsungwa-Sabiiti et al., 2004) and other barriers to treatment need addressing, such as the high costs of treatment which often keep those suffering from the NTD’s from accessing health services (Abel-smith and Rawal, 1992).

If patients are encouraged to self-report to health services for treatment of T. solium there needs to be adequate diagnostic capacity at the health centres for reliable identification of carriers. Due to the poor sensitivity of microscopy (Allan et al., 1996; Praet et al., 2013) provision of copro-Ag ELISA for diagnoses could help identify the majority of cases. I do not belive that the lack of species specificity is an issue in this case as all carriers of Taenia spp. will benefit from anthelmintic treatment.

It is important to follow-up the contacts of identified cases, both to monitor for NCC and to treat for Taeniasis. Cases identified and treated should be recorded in order to assist in the monitoring and evaluation of any program. In order to do this robust reporting structures are required, which is not the case in many of the developing nations where the NTDs are prevalent (Coulibaly and Yameogo, 2000).

If specific monitoring of at-risk groups were to be adopted in order to identify and treat cases, this would circumvent the problems inherent with relying on established health care systems but would require substantial funding and infrastructure support to undertake.

One-off treatment interventions targeting solely the porcine or human host have been modeled elsewhere (Kyvsgaard et al., 2007) and in each case after initial decline, prevalence levels returned to pre-intervention levels due to sustained infection pressure from the other host. In contrast, mass-drug administration in humans combined with a vac-
cination campaign in pigs leads to a sustained fall in prevalence in both human and porcine hosts (Kyvsgaard et al., 2007). This is a great example of the “one health” concept in action, where control of a disease requires both animal and human level interventions (World Health Organization/DFID-AHP, 2005).

Therefore, as well as interventions targeted at humans it is important that control of zoonotic diseases incorporate the animal host as well. Using the example of Brucellosis, the human health benefit of animal based interventions has been demonstrated (Roth et al., 2003) and similar benefits have been shown for other zoonotic diseases (Zinsstag et al., 2007). The acceptability and feasibility of animal based interventions may also be better than encountered in interventions which are purely human based.

There are three interventions targeted at the porcine host which should be combined. Firstly a combination of TSOL18 vaccination, utilising the protocol used in Cameroon (Assana et al., 2010), modified slightly, by the addition of anthelmintic treatment to the pig at each vaccination visit. The addition of the anthelmintic treatment would help enhance the productivity of the pig. Currently there is a high prevalence of parasitism in the pigs in our study site (see Sections 4.4.1 on page 95 and 6.4 on page 154) and this is likely to contribute to the low weight gain in typical village pigs (Mutua et al., 2012).

Providing prophylactic drugs to pigs is not the norm, but is not uncommon either, with almost half of farmers (47.8%, 95% C.I. 34.8-59.6) reporting the use of prophylactic worm control. There is not, therefore, a huge behavioural change to overcome in the idea of providing a vaccination and anthelmintic regime, especially if there are tangible economic benefits which can be demonstrated to the farmers.

The second, related aspect to any control program should be the education of farmers about correct feeding and confinement of their pigs. In Chapter 6 on page 141 it has been shown that free range pigs travel a large distance during their scavenging activities and this movement increases their risk of contracting infectious diseases. African Swine Fever is a very real threat to pig production in the region (OIE, 2011) and poor productivity and low slaughter weights of pigs is also a reality under such production systems (Levy et al., 2009; Mutua et al., 2011, 2012). Assisting farmers to change their husbandry practices by expanding on the “trainer of trainers” approach (Wohlgemut et al., 2010), will not only enhance the T. solium control program, but would provide the knowledge with which to increase their herd productivity and therefore economic gain.
The third aspect of this strategy would be the provision of the LFA to pork traders. If traders could test pigs prior to purchase it would reduce their economic risk should an infective pig be condemned at slaughter. As discussed in Chapters 5 on page 121 and 3 on page 51 infected pigs identified this way can be treated with oxfendazole and be ready for slaughter within 2 months of treatment (Gonzalez et al., 1998), with a 3 month period in which the animal is refractory to re-infection (Gonzalez et al., 2001).

As discussed in Section 3.5 on page 81 animal based interventions for a disease which is currently not appreciated by farmers as production limiting may be difficult to engage farmers in. Although there is currently legislation in place requiring the condemnation of infected meat (of Kenya, 1972), it is currently not being enforced in western Kenya to any great degree.

One key aspect of the successful implementation of any of the porcine based measures outlined here is the full cooperation of the veterinary services ensuring that legislation is enforced. Strengthening capacity of this government service and enforcing current legislation regarding the condemnation of unfit meat would provide an incentive for pig producers to take steps to control this parasite. Adopting a porcine vaccination under a situation where legislation is enforced provides a saving to farmers compared to the alternative of no action (see Section 3.4.6 on page 79).

Successful adoption of an integrated control program such as outlined here will also require close cooperation between the health and veterinary services. This cooperation has historically been lacking and is one of the key reasons for the neglect of many endemic zoonotic diseases.

The false dichotomy of veterinary and human medicine begins during the education process. Graduates from both disciplines have only a limited experience in cross-disciplinary thinking and the ability to consider zoonotic diseases in the wider context of public health (Cripps, 2000). Poor communication between health and veterinary services has been widely reported (Coulibaly and Yameogo, 2000; Wastling et al., 1999) and must be urgently addressed.

Kenya is, however, currently leading the way for improvements in the communication between these services, with the Zoonotic Disease Unit (ZDU) having been set up to specifically facilitate integrated control and surveillance of zoonotic disease in a 'one health' framework http://zdukenya.org.

The evidence presented in this thesis suggests that the communities residing in our study area are currently exposed to an unacceptable burden of *T. solium* and other
parasitic diseases and therefore NTD control must be of highest priority to improve quality of life. It is my hope that with the support of the ZDU, an integrated control program as outlined above could assist in the attainment of the goals set out in the “road-map for control” across a spectrum of the NTDs, providing health benefits to the community as a whole, whilst also having economic benefits for the pork producers of western Kenya. It is also my belief that due to the global increase in demand for pork, given the correct advice and support, pig production can become an important part of a farmers strategy to lift their families out of poverty.
Appendices
.1 Informed Consent Form

Each participant was required to sign that they understood the information included on the following two pages and were happy to participate in the PAZ study.
Study title: Epidemiology of zoonotic infections amongst livestock and their keepers in Western Kenya

Informed Consent Document

Instructions

- Enumerator to distribute read and explain to participant. Use English, Swahili or local language, as appropriate.
- One signed copy for hardcopy file, one signed copy for participant.

We are visiting you to invite you to participate in a research project which aims to understand the importance of zoonotic diseases in your community. Zoonotic diseases are diseases that you or your family may get from direct or indirect contact with animals. Our objective is ultimately to learn to control these diseases better, and in particular, understand how controlling such diseases in animals may prevent them from infecting people. This is a research project jointly run by the Kenya Medical Research Institute (KEMRI), the International Livestock Research Institute (ILRI) in Nairobi and the University of Edinburgh (UK). It is funded by the Wellcome Trust in the UK.

To carry out this research, we would like to ask you some questions about the animals you keep, the way in which you farm and live, your health and health problems, and also collect some samples for further detailed analysis. We are visiting your home with two teams, one which, with your permission, will collect samples from you, and one which will collect samples from your livestock. The outcome of this research will be a better understanding of zoonotic diseases. Findings from this investigation will help us advise both human and animal health authorities in your region and the rest of Kenya and beyond about improving health.

What is involved

Your participation will take approximately 30 minutes of your time. You have been selected randomly for this project (meaning that everyone in this region had an equal chance of being invited to participate), but you are free to decline participation if you would prefer not to take part. Taking part in this will involve

1) answering some general questions about your health and your home;
2) allowing us to take some measurements like your height and weight;
3) providing us with a sample of your faeces to look for parasites like worms;
4) allowing our qualified technician/nurse to take a 25ml sample of blood from your arm – equivalent of 2 tablespoons, so that we can take these samples to the laboratory (in Busia) and examine the blood for infections that you may have or have had in the past and that can be detected in your blood.

Measurements and samples will be taken by a qualified clinician or technician. There will be some discomfort associated with sampling blood, which will use a needle to collect blood from your arm. This discomfort is transient.

Benefits to participants

We will offer you a general health check as part of this study - by taking measurements like your height and weight and conducting an examination, we can advise you if you appear in good or bad health and suggest whether we think you should attend a clinic for further tests. We will advise you of the most appropriate facility for further consultation if required. If you would like us to, we can also prepare a report which we will send to you to inform you of what parasites we find in your faecal sample and blood sample – eg worms, malaria. This health check and parasitology report that we are offering is free of charge to you, and if you choose not to participate in the sampling for the project, we will none-the-less carry out the health check if you wish: participation is thus entirely voluntary and there is no consequence to you for not participating should you choose not to.

Anonymity/secondary use of material

Beyond the health check and parasitology tests, your participation will be totally anonymous. We will conduct further tests for a range of diseases on your sample, but it will no longer be...
possible for us to identify you individually with your test results – the link between your identity and your test results can therefore not be shared with anyone, and your names will never appear in any reports. These anonymous samples will be stored and analysed at KEMRI or ILRI or an appropriate international laboratory, and, while remaining anonymous, may be used for further studies here in Kenya or at an international laboratory if necessary to do further work on them in the future. Afterwards, samples will be stored and there may be further examination of your samples, but again, these analyses will be anonymous and cannot be linked to you individually. As we will be unable to locate a specific individual’s samples from our storage (because the storage is anonymous), agreement to participate makes implicit your agreement for the material to be used in future studies. Your answers to our questions, our measurements and results will remain completely confidential to all involved at all stages of the project – even other members of the project team will not be able to link specific samples to you or even to know the name of the village the samples came from. In the case of the parasitology results, if you choose to receive the results of these tests, we will indicate on the report what steps you might follow for medical follow-up – eg visiting your local District Hospital.

The project has been reviewed and approved by KEMRI/Kenya National Ethical Review Committee. For further questions, please contact Prof Njeri Wamae, KEMRI CMR (020 2720 409) or Dr Eric Fèvre, Busia PAZ/IDEAL laboratory, PO Box 261, Busia, for detailed questions or worries after the team has left (tel Busia 05 522 233), or the Secretary, KEMRI Ethical Review Committee (020 272 2541) if you have any concerns.

**Participant/authorised guardian statement**

I confirm that I have understood the above description of the study and that I have had the opportunity to ask any questions about this study that I wish to ask. I confirm that I am happy to provide answers to the questions that will be asked of me and that I am happy to allow the project team to take the necessary samples for this project. I confirm that my samples may be stored and shipped as is necessary for the completion of this project, and may be stored beyond the project for further medical research. I am aware that from the point of collection, I will not be personally identifiable; I understand that the project will not routinely report back the specific results of the tests to be carried out on my samples.

<table>
<thead>
<tr>
<th>Date</th>
<th>Sublocation name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participant name</td>
<td>Signature or thumb print</td>
</tr>
</tbody>
</table>

** Enumerator statement**

I confirm that I have fully explained to the subject the nature and purpose of the procedures described above, explained any risks and described the system of anonymous data gathering. I have asked the subject if he or she has any further questions, and answered these questions to the best of my ability.

<table>
<thead>
<tr>
<th>Name</th>
<th>Signature</th>
</tr>
</thead>
</table>

Barcode
The Costs of intervention strategies were calculated as below:

**Vaccination campaign**

Cost = Pig population × % vaccine coverage × (cost of vaccine + oxfenbendazole per pig)

\[(1)\]

**Meat inspection**

Cost = (no. Pigs slaughtered formally/year × cost of inspection per pig)

+ (no. Pigs slaughtered formally/year × P(infected))

× P(detected on inspection) × (cost condemnation + transport/condemned pig))

+ (no. Pigs slaughtered formally/year × P(uninfected))

× P(detected on inspection (false +ve)) × (cost condemnation + transport/condemned pig))

\[(2)\]

**Pre-slaughter diagnostic**

Cost = (no. Pigs slaughtered formally/year × cost of test/pig)

+ (no. Pigs slaughtered formally/year × P(pig infected))

× P(detected on test) × (cost of oxfenbendazole & transport/ detected pig))

+ (no. Pigs slaughtered formally/year × P(pig uninfected)

× P(detected on test (false +ve)) × (cost oxfenbendazole + transport/detected pig))

\[(3)\]

**Health Education**

Cost = (4hr opportunity cost) × (total population / 7.33)

\[(4)\]

Where 7.33 is the average size of a homestead in Western Kenya (field data) and assuming only one person per homestead receives education intervention

**Combination Interventions** Were costed by applying the interventions in the sequence:
Vaccination
→ Pre-slaughter diagnostic
→ improved meat inspection
→ effect of health education on cooking practice

Therefore; the calculations would be as follows;

**Vaccination & pre-slaughter diagnostic**

\[
\text{Cost} = (\text{Pig population} \times \% \text{vaccine coverage} \times (\text{cost of vaccine} + \text{oxfenbendazole per pig}))
\]

\[
+ (\text{no. Pigs slaughtered formally/year} \times \text{cost of test/pig})
\]

\[
+ (\text{no. Pigs formally slaughtered/year} \times P(\text{vaccinated})
\]

\[
\times P(\text{infected | vaccinated}) \times P(\text{detected on test})
\]

\[
\times (\text{cost of oxfenbendazole + transport/detected pig}))
\]

\[
+ (\text{no. Pigs formally slaughtered/year} \times P(\text{vaccinated})
\]

\[
\times P(\text{un-infected | vaccinated}) \times P(\text{detected on test (false +ve)})
\]

\[
\times (\text{cost of oxfenbendazole + transport/detected pig}))
\]

\[
+ (\text{no. Pigs formally slaughtered/year} \times P(\text{unvaccinated})
\]

\[
\times P(\text{infected | unvaccinated}) \times P(\text{detected on test})
\]

\[
\times (\text{cost of oxfenbendazole + transport/detected pig}))
\]

\[
+ (\text{no. Pigs formally slaughtered/year} \times P(\text{unvaccinated})
\]

\[
\times P(\text{un-infected | unvaccinated}) \times P(\text{detected on test (false +ve)})
\]

\[
\times (\text{cost of oxfenbendazole + transport/detected pig}))
\]

(5)
Vaccination + improved meat inspection

\[
\text{Cost} = (\text{Pig population} \times \%\text{vaccine coverage} \times (\text{cost of vaccine} + \text{oxfenbendazole per pig}))
\]
\[
+ (\text{no. Pigs slaughtered formally/year} \times \text{cost of inspection/pig})
\]
\[
+ (\text{no. Pigs formally slaughtered/year} \times P(\text{vaccinated})
\]
\[
\times P(\text{infected} | \text{vaccinated}) \times P(\text{detected on inspection})
\]
\[
\times (\text{cost of condemnation + transport/condemned pig})
\]
\[
+ (\text{no. Pigs formally slaughtered/year} \times P(\text{vaccinated})
\]
\[
\times P(\text{un-infected} | \text{vaccinated}) \times P(\text{detected on inspection (false +ve)})
\]
\[
\times (\text{cost of condemnation + transport/condemned pig})
\]
\[
+ (\text{no. Pigs formally slaughtered/year} \times P(\text{unvaccinated})
\]
\[
\times P(\text{infected} | \text{unvaccinated}) \times P(\text{detected on inspection})
\]
\[
\times (\text{cost of condemnation + transport/condemned pig})
\]
\[
+ (\text{no. Pigs formally slaughtered/year} \times P(\text{unvaccinated})
\]
\[
\times P(\text{un-infected} | \text{unvaccinated}) \times P(\text{detected on inspection (false +ve)})
\]
\[
\times (\text{cost of condemnation + transport/condemned pig})
\]
Vaccination, pre-slaughter diagnostic & improved meat inspection

Cost = (Pig population \times \% vaccine coverage \times (cost of vaccine + oxfenbendazole per pig))
+ (no. Pigs slaughtered formally/year \times cost of test/pig)
+ (no. Pigs slaughtered formally/year \times cost of inspection/pig)
+ (no. Pigs formally slaughtered/year \times P(vaccinated)
\times P(\text{infected} | \text{vaccinated}) \times P(\text{detected on test})
\times (\text{cost of oxfenbendazole + transport/detected pig}))
+ (no. Pigs formally slaughtered/year \times P(\text{vaccinated})
\times P(\text{un-infected} | \text{vaccinated}) \times P(\text{detected on test (false +ve)})
\times (\text{cost of oxfenbendazolene + transport/detected pig}))
+ (no. Pigs formally slaughtered/year \times P(\text{unvaccinated})
\times P(\text{infected} | \text{unvaccinated}) \times P(\text{detected on test})
\times (\text{cost of oxfenbendazole + transport/detected pig}))
+ (no. Pigs formally slaughtered/year \times P(\text{vaccinated})
\times P(\text{un-infected} | \text{vaccinated}) \times P(\text{detected on test (false +ve)})
\times (\text{cost of oxfenbendazole + transport/detected pig}))
+ (no. Pigs formally slaughtered/year \times P(\text{unvaccinated})
\times P(\text{infected} | \text{unvaccinated}) \times P(\text{detected on test (false -ve)})
\times P(\text{detected on inspection})
\times (\text{cost of condemnation + transport/condemned pig}))
+ (no. Pigs formally slaughtered/year \times P(\text{vaccinated})
\times P(\text{un-infected} | \text{vaccinated}) \times P(\text{detected on test (false +ve)})
\times P(\text{detected on inspection (false +ve)})
\times (\text{cost of condemnation + transport/condemned pig}))
+ (no. Pigs formally slaughtered/year \times P(\text{unvaccinated})
\times P(\text{infected} | \text{unvaccinated}) \times P(\text{detected on test (false -ve)})
\times P(\text{detected on inspection (false +ve)})
\times (\text{cost of condemnation + transport/condemned pig}))
+ (no. Pigs formally slaughtered/year \times P(\text{unvaccinated})
\times P(\text{un-infected} | \text{unvaccinated}) \times P(\text{detected on test (false +ve)})
\times P(\text{detected on inspection (false +ve)})
\times (\text{cost of condemnation + transport/condemned pig}))
### Sensitivity Analysis, Baseline Model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>-100% Probability</th>
<th>+100% Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1. Pig was slaughtered informally</td>
<td>0.0620 (0.061-0.063)</td>
<td>0.0621 (0.061-0.063)</td>
</tr>
<tr>
<td>P2. Pig is infected (formal slaughter)</td>
<td>0.0038 (0.0037-0.0039)</td>
<td>0.141 (0.139-0.142)</td>
</tr>
<tr>
<td>P3. Pig is infected (informal slaughter)</td>
<td>0.0582 (0.0577-0.0588)</td>
<td>0.067 (0.066-0.068)</td>
</tr>
<tr>
<td>P4. Pig is lightly infected</td>
<td>0.073 (0.072-0.074)</td>
<td>0.051 (0.0507-0.0515)</td>
</tr>
<tr>
<td>P5. Infected pig detected at meat inspection (as currently performed in the study area)</td>
<td>0.062 (0.0614-0.0627)</td>
<td>0.061 (0.0609-0.0621)</td>
</tr>
<tr>
<td>P6. Uninfected pig is detected at meat inspection (false positive)</td>
<td>0.062 (0.0615-0.0627)</td>
<td>0.062 (0.0615-0.0627)</td>
</tr>
<tr>
<td>P7. Any one meal is infected (lightly infected pig)</td>
<td>0.050 (0.0493-0.0507)</td>
<td>0.077 (0.07612-0.0775)</td>
</tr>
<tr>
<td>P8. Any one meal is infected (heavily infected pig)</td>
<td>0.012 (0.0117-0.0123)</td>
<td>0.111 (0.110-0.113)</td>
</tr>
<tr>
<td>P11. Pork is eaten undercooked</td>
<td>0.0</td>
<td>0.124 (0.123-0.125)</td>
</tr>
</tbody>
</table>

Table 1: **Influence of 100% Change in Baseline Parameters on Probability that any one Pork Meal is Infective at Consumption**
## Sensitivity Analysis, Intervention Options

<table>
<thead>
<tr>
<th>Parameter, Probability that:</th>
<th>-100% change from baseline</th>
<th>+100% change from baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1. Pig was slaughtered informally</td>
<td>0.002 (0.0019-0.0023)</td>
<td>0.0048 (0.0046-0.0049)</td>
</tr>
<tr>
<td>P15. Pig is vaccinated</td>
<td>0.0079 (0.0078-0.008)</td>
<td>-0.002 (-0.002-0.0019)</td>
</tr>
<tr>
<td>P16. Vaccinated pig being infected (formal slaughter)</td>
<td>0.0009 (0.001-0.0009)</td>
<td>0.0055 (0.0053-0.0056)</td>
</tr>
<tr>
<td>P17. Vaccinated pig being infected (informal slaughter)</td>
<td>0.0019 (0.0018-0.00205)</td>
<td>0.004 (0.0039-0.0043)</td>
</tr>
<tr>
<td>P18. Infected pig being detected improved meat inspection</td>
<td>0.0043 (0.00041-0.0044)</td>
<td>0.0015 (0.0014-0.00152)</td>
</tr>
<tr>
<td>P19. Uninfected pig is detected at meat inspection (false positive)</td>
<td>0.0028 (0.0027-0.003)</td>
<td>0.0028 (0.0027-0.003)</td>
</tr>
<tr>
<td>P20. Detected using pre-slaughter diagnostic (lightly infected pig)</td>
<td>0.0037 (0.0035-0.0038)</td>
<td>0.002 (0.0019-0.0021)</td>
</tr>
<tr>
<td>P21. Detected using pre-slaughter diagnostic (heavily infected pig)</td>
<td>0.009 (0.0086-0.0092)</td>
<td>-0.0031 (-0.0032-0.0029)</td>
</tr>
<tr>
<td>P22. Detected using pre-slaughter diagnostic (un-infected pig)</td>
<td>0.0029 (0.0027-0.003)</td>
<td>0.0029 (0.0027-0.003)</td>
</tr>
<tr>
<td>P23. Pork is eaten undercooked under health education strategy</td>
<td>0.0</td>
<td>0.0057 (0.0055-0.0059)</td>
</tr>
</tbody>
</table>

Table 2: Influence of 100% Change in Baseline Intervention Parameters on Probability that any one Pork Meal is Infective at Consumption
.5 Logistic Regression for Human HP10 Positivity

Table 3: Comparison of mixed-effects logistic regression risk factor models for human HP10 positivity (cysti)

<table>
<thead>
<tr>
<th>Model</th>
<th>AIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>cysti $\sim$ stud + (1 $</td>
<td>$ homestead_id)</td>
</tr>
<tr>
<td>cysti $\sim$ keep_cattle + (1 $</td>
<td>$ homestead_id)</td>
</tr>
<tr>
<td>cysti $\sim$ water_ping + (1 $</td>
<td>$ homestead_id)</td>
</tr>
<tr>
<td>cysti $\sim$ keep_chicken + (1 $</td>
<td>$ homestead_id)</td>
</tr>
<tr>
<td>cysti $\sim$ charger + (1 $</td>
<td>$ homestead_id)</td>
</tr>
<tr>
<td>cysti $\sim$ water_well + (1 $</td>
<td>$ homestead_id)</td>
</tr>
<tr>
<td>cysti $\sim$ keep_pigs + (1 $</td>
<td>$ homestead_id)</td>
</tr>
<tr>
<td>cysti $\sim$ keep_dogs + (1 $</td>
<td>$ homestead_id)</td>
</tr>
<tr>
<td>cysti $\sim$ TV + (1 $</td>
<td>$ homestead_id)</td>
</tr>
<tr>
<td>cysti $\sim$ cupboard + (1 $</td>
<td>$ homestead_id)</td>
</tr>
<tr>
<td>cysti $\sim$ sex + (1 $</td>
<td>$ homestead_id)</td>
</tr>
<tr>
<td>cysti $\sim$ sex + electric_one + (1 $</td>
<td>$ homestead_id)</td>
</tr>
<tr>
<td>cysti $\sim$ sex + wildlife + (1 $</td>
<td>$ homestead_id)</td>
</tr>
<tr>
<td>cysti $\sim$ sex + hookworm + (1 $</td>
<td>$ homestead_id)</td>
</tr>
<tr>
<td>cysti $\sim$ sex + pigs_ap_knowledge + (1 $</td>
<td>$ homestead_id)</td>
</tr>
<tr>
<td>cysti $\sim$ sex + num_people + (1 $</td>
<td>$ homestead_id)</td>
</tr>
<tr>
<td>cysti $\sim$ sex + had_apeworm + (1 $</td>
<td>$ homestead_id)</td>
</tr>
<tr>
<td>cysti $\sim$ sex + watch + (1 $</td>
<td>$ homestead_id)</td>
</tr>
<tr>
<td>cysti $\sim$ sex + latrine + (1 $</td>
<td>$ homestead_id)</td>
</tr>
<tr>
<td>cysti $\sim$ sex + HIV + (1 $</td>
<td>$ homestead_id)</td>
</tr>
<tr>
<td>cysti $\sim$ sex + weakness + (1 $</td>
<td>$ homestead_id)</td>
</tr>
<tr>
<td>cysti $\sim$ sex + medication + (1 $</td>
<td>$ homestead_id)</td>
</tr>
<tr>
<td>cysti $\sim$ sex + pork_homestead + (1 $</td>
<td>$ homestead_id)</td>
</tr>
<tr>
<td>cysti $\sim$ sex + Hemocue + (1 $</td>
<td>$ homestead_id)</td>
</tr>
<tr>
<td>cysti $\sim$ sex + bike + (1 $</td>
<td>$ homestead_id)</td>
</tr>
</tbody>
</table>

Continued on next page
Table 3 – continued from previous page

<table>
<thead>
<tr>
<th>Model</th>
<th>AIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>cysti $\sim sex + slaughter + (1 \mid homestead_id)$</td>
<td>825.3</td>
</tr>
<tr>
<td>cysti $\sim sex + religion + (1 \mid homestead_id)$</td>
<td>823.5</td>
</tr>
<tr>
<td>cysti $\sim sex + tribe + (1 \mid homestead_id)$</td>
<td>827</td>
</tr>
<tr>
<td>cysti $\sim sex + vets_used + (1 \mid homestead_id)$</td>
<td>775.3</td>
</tr>
<tr>
<td>cysti $\sim sex + open_def + (1 \mid homestead_id)$</td>
<td>824.7</td>
</tr>
<tr>
<td>cysti $\sim sex + eat_meat_other + (1 \mid homestead_id)*$</td>
<td>650.8</td>
</tr>
</tbody>
</table>

$^2$Cysti = Individual positive on HP10 ELISA, stud = homestead engaged in other studies, water spring = homestead source of water is spring, charger = homestead owns a charger, water well = homestead source of water is well, TV = homestead owns a TV, cupboard = homestead owns a cupboard, electric.none = homestead owns no electric goods, wildlife = wildlife seen around homestead, hookworm = hookworm found on faecal smear, pig.tapes.knowledge = respondent knows that tapeworm can be carried by pigs, num.people = number of people in homestead, had.tapeworm = self-reported tapeworm infection, watch = homestead owns a watch, latrine = presence and type of latrine on homestead, HIV = result of HIV test, sl.freq= frequency of cattle slaughter on homestead, weakness = recent weakness experienced, medication = currently taking medication, pork.homsetead = pork is consumed on the homestead, hemocue = Hemocue reading g/dl, bicycle = homestead owns a bike, slaughter = involved in animal slaughter, vets.used = any animals and if so was a vet used in last 12 months, open.def = open defecation practised, eat.meat.other = eat meat at another location outside the homestead, (1 \mid homestead\_id) = random effect, *Final Model
.6 Logistic Regression for Copro-Ag ELISA Positivity

Table 4: Comparison of mixed-effects logistic regression risk factor models for human copro-ag ELISA positivity (Taenia)

<table>
<thead>
<tr>
<th>Model</th>
<th>AIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taenia $\sim$ tribe(1</td>
<td>homestead_id)</td>
</tr>
<tr>
<td>Taenia $\sim$ tribe + consistency + (1</td>
<td>homestead_id)</td>
</tr>
<tr>
<td>Taenia $\sim$ tribe + strong + (1</td>
<td>homestead_id)</td>
</tr>
<tr>
<td>Taenia $\sim$ tribe + strong + iodamoeba + (1</td>
<td>homestead_id)</td>
</tr>
<tr>
<td>Taenia $\sim$ tribe + strong + iodamoeba + dwellings + (1</td>
<td>homestead_id)</td>
</tr>
<tr>
<td>Taenia $\sim$ tribe + strong + iodamoeba + dwellings + dogs + (1</td>
<td>homestead_id)</td>
</tr>
<tr>
<td>Taenia $\sim$ tribe + strong + iodamoeba + dwellings + hunt + (1</td>
<td>homestead_id)</td>
</tr>
<tr>
<td>Taenia $\sim$ tribe + strong + iodamoeba + dwellings + pork + + (1</td>
<td>homestead_id)</td>
</tr>
<tr>
<td>Taenia $\sim$ tribe + strong + iodamoeba + dwellings + skin + (1</td>
<td>homestead_id)</td>
</tr>
<tr>
<td>Taenia $\sim$ tribe + strong + iodamoeba + dwellings + skin + ascaris + (1</td>
<td>homestead_id)</td>
</tr>
<tr>
<td>Taenia $\sim$ tribe + strong + iodamoeba + dwellings + skin + distance + (1</td>
<td>homestead_id)</td>
</tr>
<tr>
<td>Taenia $\sim$ tribe + strong + iodamoeba + dwellings + skin + pump + (1</td>
<td>homestead_id)</td>
</tr>
<tr>
<td>Taenia $\sim$ tribe + strong + iodamoeba + dwellings + skin + tap + (1</td>
<td>homestead_id)</td>
</tr>
<tr>
<td>Taenia $\sim$ tribe + strong + iodamoeba + dwellings + skin + electric.none + (1</td>
<td>homestead_id)</td>
</tr>
<tr>
<td>Taenia $\sim$ tribe + strong + iodamoeba + dwellings + skin + drought + (1</td>
<td>homestead_id)</td>
</tr>
<tr>
<td>Taenia $\sim$ tribe + strong + iodamoeba + dwellings + skin + Ent + (1</td>
<td>homestead_id)</td>
</tr>
</tbody>
</table>

Continued on next page
<table>
<thead>
<tr>
<th>Model</th>
<th>AIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taenia (\sim) tribe + strong + iodamoeba + dwellings + skin + trich + (1</td>
<td>homestead_id)</td>
</tr>
<tr>
<td>Taenia (\sim) tribe + strong + iodamoeba + dwellings + skin + flood + (1</td>
<td>homestead_id)</td>
</tr>
<tr>
<td>Taenia (\sim) tribe + strong + iodamoeba + dwellings + skin + slaught + (1</td>
<td>homestead_id)</td>
</tr>
<tr>
<td>Taenia (\sim) tribe + strong + iodamoeba + dwellings + skin + goats + (1</td>
<td>homestead_id)</td>
</tr>
<tr>
<td>Taenia (\sim) tribe + strong + iodamoeba + dwellings + skin + religion + (1</td>
<td>homestead_id)</td>
</tr>
<tr>
<td>Taenia (\sim) tribe + strong + iodamoeba + dwellings + skin + religion + sex + (1</td>
<td>homestead_id)</td>
</tr>
<tr>
<td>Taenia (\sim) tribe + strong + iodamoeba + dwellings + skin + religion + cysti + (1</td>
<td>homestead_id)</td>
</tr>
<tr>
<td>Taenia (\sim) tribe + strong + iodamoeba + dwellings + skin + religion + lat + (1</td>
<td>homestead_id)*</td>
</tr>
<tr>
<td>Taenia (\sim) tribe + strong + iodamoeba + dwellings + skin + religion + tran.none + (1</td>
<td>homestead_id)</td>
</tr>
</tbody>
</table>

\(^2\)Taenia = Individual positive on copro-ag ELISA, tribe = tribal affiliation, consistency = fecal consistency, religion = religious affiliation, sex = male or female, skin = individual engaged in skinning animals, iodamoeba = individual positive for Iodamoeba spp., strong. = individual positive for Strongoloidies spp., dwellings = no. dwellings on homestead, drought = village has experience drought, dogs = keep dogs, goats = keep goats, pork = individual eats pork, ascaris = Individual positive for Ascaris spp., elec.none = homestead owns no electrical goods, tran.none = homestead owns no transport, lat = use latrine, trich = positive for Trichuris spp., Ent = positive for Entemoba histolytica , slaught = at least one person on homestead engaged in slaughtering animals, pump = pumped source of water, tap = water from tap, flood = village suffered from flooding in last 12 months, distance = distance to healthcare, cysti = individual positive on HP10, (1 | homestead_id) = random effect, *Final Model
## Logistic Regression for Porcine HP10 Positivity

Table 5: Comparison of mixed-effects logistic regression risk factor models for porcine HP10 positivity (pcysti)

<table>
<thead>
<tr>
<th>Model</th>
<th>AIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>$pycsti \sim sheep + (1 \mid homestead_id)$</td>
<td>87.3</td>
</tr>
<tr>
<td>$pycsti \sim sheep + borehole + (1 \mid homestead_id)$</td>
<td>88.7</td>
</tr>
<tr>
<td>$pycsti \sim sheep + piped + (1 \mid homestead_id)$</td>
<td>89.3</td>
</tr>
<tr>
<td>$pycsti \sim sheep + vet + (1 \mid homestead_id)$</td>
<td>85.8</td>
</tr>
<tr>
<td>$pycsti \sim sheep + TP + (1 \mid homestead_id)$*</td>
<td>83.2</td>
</tr>
<tr>
<td>$pycsti \sim sheep + TP + hunt + (1 \mid homestead_id)$</td>
<td>84.9</td>
</tr>
<tr>
<td>$pycsti \sim sheep + TP + open.def(1 \mid homestead_id)$</td>
<td>84.1</td>
</tr>
</tbody>
</table>

---

1TP = total protein g/l, hunt = at least 1 person on homestead hunts, sheep = keep sheep on homestead, open.def = at least 1 person reports open defecation, borehole = homestead source of water is borehole, piped = homestead source of water is piped, vet = vet used in last 12 months, (1 | homestead_id) = random effect, *Final Model
.8 Bayesian Index of Agreement in “R”

```r
# R code for Bayesian indices of agreement as provided by Nicholas Praet
# 26.04.2012

# Insert the fixed alpha's
alpha1 <- 0.25  # non-informative prior: all alpha's = 0.25
alpha2 <- 0.25
alpha3 <- 0.25
alpha4 <- 0.25

# Insert the cell-values - kenya only
a <- 106
b <- 24
c <- 92
d <- 121

# kenya and uganda combined (crude)
a <- 151
b <- 95
c <- 106
d <- 289

K <- 400

# A few calculations following the algorithm of Graham and Bull (1998)
pi <- rbeta(K, (a+alpha1), (b+c+d+alpha2+alpha3+alpha4))
be <- rbeta(K, (d+alpha4), (b+c+alpha2+alpha3))
pi4 <- (1-pi)*be
pos <- (2*pi)/(pi + 1 - pi4)
neg <- (2*pi4)/(pi4 + 1 - pi)
diff <- pos - neg
quapos <- quantile(pos, c(.025, .975))
quaneg <- quantile(neg, c(.025, .975))
quadiff <- quantile(diff, c(.025, .975))

p1 <- a/(a+b+c+d)
p2 <- b/(a+b+c+d)
p3 <- c/(a+b+c+d)
p4 <- d/(a+b+c+d)

positivagre <- 2*p1/(2*p1+p2+p3)
negativagre <- 2*p4/(2*p4+p2+p3)

# Results
print(p1, digits=2)
print(p2, digits=2)
```
.9 Latent Class Model in “R”

```r
# Latent Class Model in “R”

# = 2 test 2 population Hu-Walter model by Mark Bronsvoort March 2011, Modified by Lian Thomas May 2012=

# NOTE uses JAG - just another gibbs sampler 3.1.0 from http://mcmc-jags.sourceforge.net/ ####
# #### this analysis assumes same se/sp of tests in each of 2 populations with different prevalences####

rm(list=ls()) ## this removes all previous vector assignments etc

# = load packages =
library(runjags) # see http://cran.r-project.org/web/packages/runjags/
library(rjags) # sourceforge.net /

# = get the data =

x <- read.csv(file = "C:\Documents and Settings\Lian\My Documents\Liams Docs \DATABASE\NGS_new.csv", header = TRUE, stringsAsFactors = FALSE)

### format data for model ###

```
cc <- as.vector(table(z$HP10Result, z$LFA_result, z$Population, dnn=c("ELISA", "LFA", "Pop")))

pop <- t(matrix(cc, 4, 2))

pop

dd <- list(n.pop=2, n = apply(pop, 1, sum), pop=pop)

dd

### arguments for runjags: data (1) a vector or list of the names of the data objects used by the model,
#(2) a (named) list of the data objects themselves, or
#(3) the name of a 'dump' format file containing the data objects, which must end in '.txt' in this instance using a dump.format file which just formats
#the data as it's needed for JAGS

modelData <- dump.format(dd)

# defining inits; = initialization = a list with n.chains elements; each
# element of the list is itself a list of starting values for the BUGS model,
#or a function creating (possibly random) initial values. If inits is NULL, JAGS
# will generate initial values for parameters.
#
# i.e. the default JAGS n.chains = 3 so each init will have a list of three
# starting points, one for each chain then number of inits = number of tests

# disperse start of chains so you don't over-inform your model#
modelInit1 <- dump.format(list(se=c(0.8, 0.5), sp=c(0.8, 0.5), prev=c(0.1, 0.4)))

modelInit2 <- dump.format(list(se=c(0.6, 0.8), sp=c(0.6, 0.8), prev=c(0.4, 0.2)))

modelInit3 <- dump.format(list(se=c(0.5, 0.6), sp=c(0.5, 0.6), prev=c(0.1, 0.5)))

modelInits <- c(modelInit1, modelInit2, modelInit3)

# set.seed(1)
bw = 0.3
n.adapt = 500000
n_burnin = 500000
n.iter = 100000
thin = 10
n.chains = 3

# probability of observing each test population in each subpopulation
modelsString <- 

model {

  for (w in 1:n.pop )
  {

    prev[w] ~ dbeta(1,1);  
    pop[w,1:4] ~ dmulti(par[w,1:4],n[w]);

    par[w,1] <- ((1-se[1]) * (1-se[2]) * prev[w]) + (sp[1] * sp[2] * (1-prev[w]));

  }

  # ===============================
  # = priors for diagnostic tests =
  # ===============================

  se[2] ~ dbeta(128, 71)
  sp[2] ~ dbeta(227, 58)

  se[1] ~ dbeta(12, 6)
  sp[1] ~ dbeta(12, 6)

  # =============
  # = Run model =
  # =============

  print(system.time(
    x <- run.jags( model = modelsString, 
    monitor = c("se", "sp", "prev"),
    data = modelData, 
    burnin = n_burnin,
    sample = n_iter, 
    thin = thin,
    n.chains = n.chains,
    inits = modelInits )
  ))

  mcmc <- list( result = as.mcmc.list(x[[1]]),
```r
model = modelString,
data = modelData)

# store results
dput(mcmc, file=paste("model1",gsub(":\", ",", date())))

# = some exploratory plots =

print(densityplot(mcmc[[1]], bw=0.005, layout=c(2,3)))
print(summary(mcmc[[1]]))
par(mfrow=c(6,1))
par(mai=c(0,0,0,0))
traceplot(mcmc[[1]])

# crossplot
quartz()
par(las=2)
crosscorr.plot(mcmc[[1]], las=2)

# Gelman Rubin statistic
gelman.diag(mcmc[[1]])
```

NGS_code.R
.10 Publications Arising from this Thesis
The spatial ecology of free-ranging domestic pigs (Sus scrofa) in western Kenya

Lian F Thomas1,2, William A de Glanville1,2, Elizabeth A Cook1,2 and Eric M Fèvre1,2*

Abstract

Background: In many parts of the developing world, pigs are kept under low-input systems where they roam freely to scavenge food. These systems allow poor farmers the opportunity to enter into livestock keeping without large capital investments. This, combined with a growing demand for pork, especially in urban areas, has led to an increase in the number of small-holder farmers keeping free range pigs as a commercial enterprise. Despite the benefits which pig production can bring to a household, keeping pigs under a free range system increases the risk of the pig acquiring diseases, either production-limiting or zoonotic in nature. This study used Global Positioning System (GPS) technology to track free range domestic pigs in rural western Kenya, in order to understand their movement patterns and interactions with elements of the peri-domestic environment.

Results: We found that these pigs travel an average of 4,340 m in a 12 hr period and had a mean home range of 10,343 m2 (range 2,937–32,759 m2) within which the core utilisation distribution was found to be 964 m2 (range 246–3,289 m2) with pigs spending on average 47% of their time outside their homestead of origin.

Conclusion: These are the first data available on the home range of domestic pigs kept under a free range system: the data show that pigs in these systems spend much of their time scavenging outside their homesteads, suggesting that these pigs may be exposed to infectious agents over a wide area. Control policies for diseases such as Taenia solium, Trypanosomiasis, Trichinelllosis, Toxoplasmosis or African Swine Fever therefore require a community-wide focus and pig farmers require education on the inherent risks of keeping pigs under a free range system. The work presented here will enable future research to incorporate movement data into studies of disease transmission, for example for the understanding of transmission of African Swine Fever between individuals, or in relation to the life-cycle of parasites including Taenia solium.

Keywords: Pig, GPS, Taenia, Trichinella, African swine fever, Toxoplasma, Trypanosoma, Free range

Background

Throughout the developing world the demand for meat products has been increasing by 4% per annum since the 1980s [1], and with continuing population growth this trend is unlikely to abate. The need for fast-maturing sources of animal protein, which require low cereal inputs places the non-ruminant animals in prime position for fulfilling this growing demand. To this end pig production is becoming increasingly popular, with pork and poultry contributing 76% of the increased meat consumption in the developing world between 1982–1998 [2].

Pigs, Sus scrofa, have lower social prestige than cattle, but they are cheap to purchase and to raise and are therefore a popular option for resource-poor farmers, particularly women [3]. Taking advantage of the pig’s natural ability as a scavenger, many of these resource poor farmers opt for an extensive, low input form of production, whereby the pigs roam freely. These systems allow an animal to be kept without the need for expensive supplementary feedstuffs [4]. Pig production under these free range systems has been documented in many African countries, including: Kenya [5], Uganda [6], Tanzania [7], Cameroon [8] and Zambia [9]. Within our study area of western Kenya there is abundant evidence of this production system, as illustrated in Figure 1.
Pigs kept under all production systems can be the host of a variety of zoonotic and non-zoonotic pathogens, but allowing pigs to roam freely increases the disease transmission risk to the pig itself, to other wild and domestic animals, and to humans. Some diseases of particular relevance when considering free-roaming pigs are discussed below.

Porcine cysticercosis
The zoonotic tapeworm, *Taenia solium*, is one of the leading causes of acquired epilepsy in the developing world [10]. The parasite has a two host life cycle, with humans as the definitive host, who become infected after consumption of viable cysticerci in under-cooked pork. The adult tapeworm inhabits the small intestine, causing an infection known as taeniasis, and gravid proglottids, containing thousands of infective eggs, detach from the adult worm and are excreted in faeces in an intermittent fashion [11]. Ingestion of these eggs, by either pigs or humans, results in the larval stage penetrating the intestinal wall and moving through the lymph and blood vessels to encyst in muscle, eyes or the central nervous system (CNS) as cysticerci [12].

As contact with infective human faecal material by pigs is a requisite for the successful propagation of the parasite lifecycle, it stands to reason that keeping pigs under a free-ranging system would increase the risk of the pigs acquiring this infection; this has been corroborated in several epidemiological studies [8,13-15].

Trichinella spp.
Trichinella spp. are tissue dwelling nematodes, which are transmitted to humans by the ingestion of undercooked meat containing infective larvae. The parasite has a wide range of mammalian hosts, but the majority of human infections are acquired through the consumption of pork, with European cases almost exclusively from outdoor or back-yard production systems [16]. Pigs acquire the infection through ingestion of infected wildlife carcasses, kitchen or slaughter waste. The ability of pigs to scavenge such material increases vastly when they are allowed to free range, heightening the relative risk of infection in comparison to confined pigs. The relative risk for *Trichinella* infection was estimated to increase by a factor of 25–100 times for free range pigs in comparison to pigs kept in indoor units [17].

Toxoplasmosis
*Toxoplasma gondii* is a zoonotic protozoan parasite with a wide range of intermediate hosts, including pigs and humans, who acquire infection through the ingestion of infective oocysts excreted by cats, tachyzoites in raw milk, or encysted bradyzoites in infected meat [18]. The majority of human infections are thought to come from the ingestion of meat, in particular pork [19,20]. The risk of infection for a pig is again related to its ability to scavenge in areas contaminated with either cat faecal material containing oocysts, or carcasses containing infective bradyzoites; therefore, it is strongly associated with free-roaming behaviours.

Two studies from the Netherlands have found a significantly higher risk of seropositivity for toxoplasma antibodies in free range pigs than for those on an intensive pig unit [18,21]. Exposure to infective cat faeces or to infected carcasses in pigs raised outdoors are risks for disease transmission, which are likely to be exacerbated in the free range systems of the developing world.

African swine fever (ASF)
ASF is a hemorrhagic virus of the Asfarviridae family, which has major epizootic potential [22]. This infection is characterised by high mortality in domestic swine. It is transmitted either by direct or in-direct contact between domestic pigs or wild suids with or without an arthropod vector and is maintained by three distinct cycles: 1) a sylvatic cycle between the Argasid tick and warthogs, and possibly bush pigs or giant forest hogs [23]; 2) a cycle between domestic pigs and the Argasid tick; and 3) a domestic pig cycle not requiring ticks [24]. There is also evidence that recently infected bushpigs and warthogs may be able to directly infect domestic pigs without need for the tick vector [23]. Wild boars have been implicated in virus transmission when they come into contact with infected free range domestic pigs, as was thought to be involved with the 2007 spread of ASF through Georgia [25]. Domestic pigs kept under free range systems are therefore at higher risk of contracting and transmitting ASF through contact with infected tick vectors or infected wild and domestic suids. Our study site in western Kenya has seen several ASF outbreaks over the last few years, most recently in 2011 [26].
Trypanosomiasis

*Trypanosoma* spp., transmitted by the tsetse fly (*Glossina* spp.), cause a reduction in productivity in pigs and pose a high risk to human health, with *T. brucei gambiense* and *T. brucei rhodesiense* causing Human African Trypanosomiasis (HAT). The pig is a significant source of blood meals for the tsetse fly [27,28] and has been implicated in the epidemiology of both human and animal trypanosomiasis, with outdoor, free-roaming pigs being at particular risk of contact with tsetse flies. In particular, pigs have been identified as a significant reservoir of *T. b. rhodesiense* in our study site [29].

Non-zoonotic helminths

Helminths, such as *Ascaris suum* and *Trichuris suis*, are responsible for substantial economic losses for pig producers throughout the world, through reduced weight gain, higher feed:gain ratio, condemnation of carcasses or organs and expenditure on prophylaxis or treatment [30]. *Ascaris suum* and *Trichuris suis* both require temperatures over 15°C for embryonation and larval development, and the prevalence of these parasites have been found to be higher in outdoor pig units than intensive, indoor units [31]. In a previous survey of free range pigs in the current study area pigs have been found to carry a substantial parasite burden, with an overall nematode prevalence of 84.2% and mean egg per gram (EPG) of 2,355 [32], which is likely leading to detrimental economic burden for their (often already poor) keepers.

To gain an understanding of the dynamics of disease within populations of free range pigs, the ecology of these animals must first be established. The behaviour of the domestic pig has been studied extensively within the context of intensive farming methods or through experiments to understand their social dynamics or learning ability [33,34]. Knowledge of domestic pig behaviour under free range conditions, specifically the size of the ‘home ranges’ and habitat preferences is, however, very limited with only one published paper from Mexico specifically looking to understand pig ecology under these systems [35]. The authors of this paper identified some interesting aspects of free-ranging pig behaviour, specifically in relation to coprophagia. What was lacking, however, was the quantification of ‘home range’ size and of habitat preferences of the pigs within this free range system, important elements of understanding the disease risks to which free range pigs are exposed.

The home range of an animal is “…that area traversed by the individual in its normal activities of food gathering, mating, and caring for young. Occasional sallies outside the area, perhaps exploratory in nature, should not be considered as in part of the home range” [36]. There are many different techniques available for determining the home range of animals and these have been extensively reviewed [37,38]. We utilise two such methods: minimum convex polygon (MCP) and local convex hull (LoCoH). The MCP is the simplest of the convex hull methods, which represents the smallest polygon with no inside angle greater than 180° that can be drawn to encompass all locations at which the animal was recorded. This is a simple measure to calculate and is used by the International Union for Conservation of Nature as the standard measure of a species home range [39]. The MCP method, however, is very sensitive to outlying points, which may reflect exploratory animal movement or measurement errors, providing an estimate of home range far beyond that utilised in the animal’s normal activities.

The k-1 nearest neighbours local convex hull technique was devised to improve on the MCP: it combines small MCPs which contain k-1 nearest neighbours, until all data points are included [40]. This technique has been shown to perform well to reduce type I (exclusion of utilised areas) and type II (inclusion of un-utilised areas) errors and is particularly useful in locations where geographical features provide hard boundaries to a home range. This method also allows the isopleths containing any percentiles of the data points to be identified, providing us with the ability to determine utilisation distributions for various percentiles of use, for example the 50% isopleth, which corresponds to the ‘core utilisation distribution’ and the 90% isopleth, corresponding to the true ‘home range’ [41].

There are several studies which investigate the home range of truly ‘wild living pigs’ [42], these being feral pigs of either domestic, European wild boar or hybrid origin. These studies have found a large variability in the home range (all based upon MCP determination) of these feral swine, from 0.52 km² [43] to 20.3 km² [44] for wild caught and released feral pigs. The large variability in roaming behaviours in these studies makes it difficult to extrapolate the findings outside of these particular study environments, potentially due to the impact of environmental features on the home range (e.g. proximity to human habitats, sharp ravines or cliff faces, forest cover, etc.). The environment that wild pig studies have encompassed are mainly forested or conservation areas, where the ability to move freely over large distances is greater and human interference is negligible. An extrapolation to the roaming behaviour of domestically bred and raised, albeit free-roaming, pigs would be highly inadvisable.

Here, we determine the geographical range of free-ranging domestic pigs in western Kenya, how far they travel during a day and night, and with which environmental features they spend time interacting.

**Methods**

**Study area**

The study area, shown in Figure 2, is representative of the Lake Victoria Crescent ecosystem. It falls within a
45 km radius of Busia town in western Kenya, bordered by Uganda to the west, Lake Victoria to the south, Mount Elgon to the north and Rift Valley Province to the east. The area is occupied predominately by members of the Luo, Luhya and Teso tribes. The area has bi-annual rains, occurring in March-May and August-October and supports a predominantly mixed crop-livestock production system with an average farm size of 0.5 ha [45]. Within this area, ten 3rd level administrative units, called divisions, were selected based upon the popularity of pig production in these districts. Together these 10 divisions, Amagoro, Amukura, Budalangi, Butula, Chakol, Matayos, Funyula, Nambale Ujunga and Ukwala contain over 67% of the total pig population of the study area, which is estimated to be 66,307 by the district office of livestock and production. One sublocation (the smallest, 1st level administrative unit) from each of these Divisions, was selected at random using the Hawths tools extension [46] for ArcMap 9.1 (ESRI, Redlands, USA). The ten selected study sub-locations, Bulemia, Anyiko, Asango, Sigalame, Nasewa, Bulwani, Malanga, Chakol, Amakuru and Kumuria can be seen in Figure 2.

**Animals**

Between March 2011 and February 2012, one free range pig was randomly selected from each selected sublocation. The sample frame consisted of all pig keeping households within the sublocation, as provided by the relevant sublocation chief, a random number generator was used to pick the farmer from this list (farmers numbered first to last). On the selected farms pigs were excluded from the study if they were in the last trimester of pregnancy, were currently nursing piglets, were below 2 months of age or were due to be slaughtered in the next week (7 days from the day of selection). If more than one pig remained after exclusion they were allocated a number in age order and a random number generator was used to select the pig to be recruited, this was easy to achieve without any specific identifying procedure as the average pig herd size in the study site is only 2.6 (Unpublished Obs. EMF, LFD, EAC, WAdG).
The study was explained to the farmer and their consent obtained before the animal was recruited into the study. The pigs were selected across the course of the year as only one GPS collar was available; the data were therefore obtained across different seasons.

Data collection
A Garmin eTrex handheld GPS unit was used to obtain the coordinates of the homestead to which the pig belonged. The perimeter of the homestead, being that area utilised by the house for domestic activity (therefore excluding cropped fields), was tracked by walking along the boundary and if there was no discernible boundary the homestead members were asked for their best approximation of where their homestead perimeter lay. Features of the homestead (latrine, human dwelling, cooking point, rubbish disposal) were also mapped. A short questionnaire on pig husbandry was completed with the member of the homestead with the greatest involvement in the management of the pig.

The pig was restrained using a pig snare behind the upper canines and a lingual palpation to check for cysticercosis was performed [48]. Blood was collected from the external jugular or anterior vena cava into a 10 ml plain BD vacutainer® tube using an 18 gauge 1 ½ ” needle. A peripheral ear vein blood sample was collected using a blood lancet and micro-haematocrit tube and thick and thin blood smears were made immediately in the field. The pig was observed for the presence of ectoparasites and a note was made of the presence or absence of lice, mites or adult ticks, although ectoparasite species were not recorded. A faecal sample was taken from each pig and all biological samples were transported on ice to the Busia laboratory facility. A webbing collar fitted with a GPS unit and General Packet Radio Service (GPRS) data transmission system (Savannah Tracking Ltd, Nairobi, Kenya) was then fitted to the pig, as shown in Figure 3, and the pig released. The collar weighed ~350 g and operated using a 5400 mAmp/H rechargeable battery. Data were regularly uploaded to a server through the GPRS transmission system. The collar was set to record coordinates every 3 minutes for a one week (7 day) period from the day of recruitment.

Faecal samples were analysed for intestinal parasites using the McMasters [49] and Kato-Katz [50] methods. Thick and thin blood smears were stained with Giemsa and these smears were examined by microscopy for haemoparasites. Serum samples were analysed by HP10 Antigen ELISA [51] for the presence of viable $T. solium$ infections.

Analysis
Pig movement data from the GPS server were downloaded as a .csv file into Microsoft Excel and imported into ArcMap 9.1 and projected into UTM WGS 36 N. The LoCoh extension [52] for ArcGIS [40] was used to produce a utilization distribution of these data using the k-1 nearest neighbour local convex hull technique with 10 percentile isopleths. The value of K was determined by taking the square root of the number of GPS positions available as suggested by the software developers.

ArcMap 9.1 was then used to select the density isopleths representing both 50% (core utilisation distribution) and 90% (home range) of the points. A minimum convex polygon (MCP) was calculated using the Hawths Tools extension for ArcGIS. The Hawths Tools extension was then used to calculate the area of the layer files created from these selections, to create a track from the GPS movement data and to determine the length of that track.

Homestead points of interest and the perimeter boundary recorded using the handheld unit in the field were also imported into ArcMap 9.1. Individual points for each feature of a homestead and the perimeter boundary of each homestead (habitable area, as determined by the head of the household), were projected into UTM WGS 36 N and combined with the collar data to create informative data layers.

The area of the perimeter boundary polygon was calculated using Hawths Tools. The homestead features and the homestead itself were given a 5 m ‘buffer’ using Hawths Tools, 5 m being chosen to represent the accuracy of the GPS units used. All pig movement data points which fell within these buffer areas were selected and the time spent within the areas were calculated as a percentage of the total number of positions recorded for each pig.

All statistical analysis was performed using the ‘R’ language and environment for statistical computing [53]. The variables of interest were tested for violation of the assumption of normality using the Shapiro-Wilks test of normality, and due to the rejection of the null hypothesis
(sample originating from a normally distributed population) for several of the variables it was decided to use non-parametric statistical methods, namely the Kruskal-Wallis rank sum test, Spearman's Rank Correlation and the Wilcoxon signed rank test.

**Results**

Ten pigs were selected and tracked during the time of this study, comprising 4 females, 2 male castrates and 4 male intact pigs with an average age of 6.7 months. All 10 pigs were kept under a free range system during the time of study. All pigs were fed supplementary food, being a combination of crop and household waste, with the household waste being fed uncooked to 8 of the 10 pigs.

No farmer reported any previous clinical episodes for any of the sampled pigs. Only 3 pigs had received any prophylactic treatments, which included Levamisole (1 pig), Deltamethrin (1 pig) and an unknown anthelminthic (1 pig). Reported anthelminthic treatment appeared to make no significant effect on the total nematode EPG (Kruskal-Wallis chi-squared = 2.7, p = 0.26). All pigs in this study were found to be infected by at least one parasite, with all pigs suffering from ectoparasites (adult ticks and lice in all cases) and 8 out of 10 also being infected with gastrointestinal parasites (Strongyloides spp., Strongylus spp., Trichuris spp., Coccidia and Ascaris spp. all being found). Three pigs were found to be infected with Taenia solium cysticercosis using the HP10 antigen ELISA [51,54]. A summary of the parasite burden for each pig is shown in Table 1. No haemoparasites were observed in any of the pigs.

The minimum convex polygon, home range and core utilisation distribution were determined for each pig and are illustrated in Figure 4. The movement parameters calculated for each pig are also summarised in Table 2. The mean distance moved by a free range pig in our study site over a 12 hr period was 4,340 m, with pigs moving 4,169 m (range 1,401–6,383 m) during daylight hours and 4,511 m (range 1,293–7,809 m) at night,

<table>
<thead>
<tr>
<th>Pig ID</th>
<th>Ectoparasite infection (lice and adult ticks in all)</th>
<th>Taenia Solium Cysticercosis</th>
<th>Gastrointestinal parasites</th>
<th>EPG</th>
<th>Total parasite spectrum*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>√</td>
<td>Strongyles, 3,600</td>
<td>Coccidia, 50</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ascaris spp. 13,900</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>√</td>
<td>Strongyloides spp. 24</td>
<td>Strongyles, 2,400</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ascaris spp. 700</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>√</td>
<td>Strongyles, 1,600</td>
<td>Ascaris spp. 2,050</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>√</td>
<td>Strongyloides spp. 50</td>
<td>Strongyles, 48</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ascaris spp. 3,300</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>√</td>
<td>Strongyles, 100</td>
<td>Coccidia, 250</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>Trichuris spp. 100</td>
<td>Ascaris spp. 5,650</td>
<td>3</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Ascaris spp. 200</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>Strongyles 750</td>
<td>Trichuris spp. 400</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>Strongyles, 350</td>
<td>Coccidia, 9,200</td>
<td>80%</td>
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<td>8</td>
<td></td>
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<td>9</td>
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<td>Strongyloides spp., 750</td>
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<td></td>
<td>Strongyloides spp. 750</td>
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</table>

% pigs infected: 100% 30% 80%

*Total parasite spectrum is defined as the total number of parasite species infesting each pig.
**Figure 4** Illustration of movement parameters for each pig.

**Table 2 Pig movement data**

<table>
<thead>
<tr>
<th>Pig ID</th>
<th>Ave. daily distance moved (m)</th>
<th>Ave. nightly distance moved (m)</th>
<th>Core utilisation distribution (m²)</th>
<th>Home range (m²)</th>
<th>MCP Area (m²)</th>
<th>Homestead area (m²)</th>
<th>% time spent within homestead perimeter</th>
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<td>612</td>
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<td>3,387</td>
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<td>123,189</td>
<td>1,707</td>
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<td>4,511</td>
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<td>265,569</td>
<td>1,717</td>
<td>52.9</td>
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with no significant difference between these periods (Wilcoxon signed rank test $w = 1$, $p = 1$). The mean core utilisation distribution was found to be $947 \text{ m}^2$ (range $133–3,353 \text{ m}^2$) and the mean home range was found to be $15,085 \text{ m}^2$ (range $2,937–74,887 \text{ m}^2$).

In this small study, neither sex of pig or season were found to influence movement parameters as shown in Tables 3 & 4 and no correlation was found between any movement parameter and the total parasite burden, calculated as sum of the eggs per gram (EPG) for all nematode species identified. No correlation was found either between movement parameters and the EPG of *Strongyloides* spp., *Strongyles*, *Trichuris* spp. and *Ascaris* spp., though a moderate correlation (Spearman’s rank correlation $\rho = 0.75$, $p = 0.01$) was found between the home range area and the Coccidia EPG, though this was heavily influenced by an outlier as shown in Figure 5.

Pigs spent on average half (53%) of their time within the perimeter boundary of the households, or, otherwise stated, almost half their time outside the homestead. These homestead boundaries were often ill-defined, and all were porous. The time spent interacting within a 5 m radius of certain homestead features is shown in Table 5. The pigs in this study were shown to only spend on average 1.3% of their time interacting with the latrine area in their homestead of origin, 1.6% in the vicinity of the rubbish disposal area, 2.7% in the vicinity of the human dwellings and 4.3% in the vicinity of the cooking point: it is important to note that these interactions were only determined within the homestead of origin. Time spent interacting with homestead features was not found to influence parasite burden apart from in the case of *Ascaris* spp., where time spent interacting with latrines was found to be positively correlated with the EPG count (Spearman’s Rank Correlation $\rho = 0.81$, $p = 0.005$).

**Discussion**

This is the first study to have investigated the ecology of domestic pigs kept under a free range system, utilising GPS technology. We found that these pigs travel an average of 4,340 m in a 12 hr period and had a mean home range of 10,343 m², within which the core utilisation distribution was found to be 964 m². The lack of significant difference ($p = 0.824$) between day and night time movement indicates that the pigs are benefiting from a foraging strategy which involves both night and day scavenging. Nocturnal behaviour has been observed in wild pigs [55] who seem to be able to adjust their activity patterns based upon food availability [56].

Although this study was not designed to investigate population level influences on the movement parameters, it is interesting to note that no influence of season or sex of pig was found on any of the movement parameters. The pigs in this study were not influenced by management imposed restrictions on their movements during certain times of the year as selection criteria for the study animals was that they were kept on a free range basis. Another study in western Kenya that this team has conducted found only a 1.4% change in confinement in pigs between the wet and dry seasons (Unpublished Obs. LFT, EMF, EAC, WA d G).

No influence was found in this small study on parasite burden from movement parameters or interaction with homestead features apart from a positive correlation between *Ascaris* spp. EPG and the time spent interacting with latrines (Spearman’s Rank Correlation $\rho = 0.81$, $p = 0.005$) and a moderate positive correlation between Coccidia EPG and home range area (Spearman’s rank correlation $\rho = 0.75$, $p = 0.01$), the second of which appears to be highly influenced by one outlier. We could hypothesise that there may be a higher number of earthworms and dung beetles around a latrine area, which could be acting as paratenic hosts.

Despite the lack of association between the parameters measured and the health status of the pigs in this study, these findings do, however, have major implications for our understanding of pig husbandry and disease control within resource poor settings. For example, a domestic,
free-ranging pig spends only ~50% of time within the homestead that owns it, indicating a high likelihood of exposure to environmental features, contaminants and pathogens outside the home area. Thus, when considering control policies for reducing infectious diseases in pigs, interventions targeting only pig owning households may be less effective than expected, and a community approach is clearly required.

Three out of the ten pigs recruited into this study were found to be positive for *T. solium* circulating antigen, which is a high prevalence compared to previous studies in the area which have found between 4% [32] and 10.5% [5]. However, a survey of 343 pigs at slaughter facilities in the study area immediately prior to the onset of the current study has found a prevalence of circulating antigen, using the same HP10 ELSIA of 55% (In prep. LFT, EMF, EAC, WAdG). This indicated that the area is, in fact, hyper-endemic for *T. solium* and we are therefore unsurprised that pigs selected on the basis of a known risk factor for cysticercosis infection were found to be infected.

In the case of *Taenia solium* cysticercosis, the porcine infection is acquired by the ingestion of infective eggs or proglottids in human faecal material that contaminates the pigs’ environment. Many studies have looked at the presence or absence of a latrine in a homestead as being a risk factor for cysticercosis infection in pigs; however, there has been no consensus between these studies. Some authors have found that the presence of a latrine is a risk factor for porcine cysticercosis [13,57] and others that latrines are protective [7,48,58]. In this study we found no association between the time spent interacting with a latrine on the homestead of origin and the *T. solium* status of the pig, which we believe suggests that the presence or absence of a latrine in an individual home is of less relevance to parasite transmission than overall provision of sanitation for the wider community in which the pig roams.

Although the observations made during this study suggest that pigs spend only a small amount of time interacting with the latrine area in their own homesteads (1.3%), we cannot discount the potential for pigs to come into contact with human faecal material elsewhere on the homestead or in neighbouring homesteads. We also note that any degree of access to human faecal material in or around a latrine, however short in time, is enough for transmission of the parasite to occur. Furthermore, 25% of homesteads in our study area do not

### Table 5 Interactions between pigs and homestead features

<table>
<thead>
<tr>
<th>Pig ID</th>
<th>Homestead area (m²)</th>
<th>% time spent within homestead perimeter</th>
<th>% time spent interacting with latrine</th>
<th>% time spent interacting with rubbish disposal</th>
<th>% time spent interacting with cooking point</th>
<th>% time spent interacting with human dwelling</th>
</tr>
</thead>
<tbody>
<tr>
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<td>224</td>
<td>54.1</td>
<td>4.5**</td>
<td>0.2</td>
<td>2.5***</td>
<td>1.03</td>
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<tr>
<td>2</td>
<td>2143</td>
<td>70.7</td>
<td>0.1**</td>
<td>5.2</td>
<td>11.5*</td>
<td>6.8</td>
</tr>
<tr>
<td>3</td>
<td>1048</td>
<td>61.6</td>
<td>0.7**</td>
<td>Not observed</td>
<td>11.6*</td>
<td>5.9</td>
</tr>
<tr>
<td>4</td>
<td>1707</td>
<td>51.1</td>
<td>0.2**</td>
<td>4.1</td>
<td>0.9*</td>
<td>4.0</td>
</tr>
<tr>
<td>5</td>
<td>1666</td>
<td>65.7</td>
<td>0.8**</td>
<td>0.6</td>
<td>1.6*</td>
<td>2.0</td>
</tr>
<tr>
<td>6</td>
<td>775</td>
<td>34.7</td>
<td>0**</td>
<td>0.2</td>
<td>5.9*</td>
<td>2.8</td>
</tr>
<tr>
<td>7</td>
<td>4328</td>
<td>61.7</td>
<td>2.1**</td>
<td>0.1</td>
<td>0.1*</td>
<td>0</td>
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<tr>
<td>8</td>
<td>1646</td>
<td>66.7</td>
<td>3.7**</td>
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<td>Not observed</td>
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<tr>
<td>9</td>
<td>802</td>
<td>855</td>
<td>0.03*</td>
<td>0.2</td>
<td>Not observed</td>
<td>0.1</td>
</tr>
<tr>
<td>10</td>
<td>2834</td>
<td>53.8</td>
<td>0.3*</td>
<td>0</td>
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<tr>
<td>Ave.</td>
<td>1717</td>
<td>52.9</td>
<td>1.3%</td>
<td>1.6%</td>
<td>4.3%</td>
<td>2.7%</td>
</tr>
</tbody>
</table>

* Homestead feature fully enclosed.
** Homestead feature partially enclosed.
*** Homestead feature not enclosed.
have access to a latrine (In prep. EMF, LFD, EAC, WAdG), meaning that many people have no choice but to engage in open defecation, raising a very real possibility for pigs to contact human faecal material, and therefore potentially infective _T. solium_ eggs. Finally, not all latrines are of the same quality, such that pigs may be able to access latrine buildings that are not properly enclosed: in this study area only 29% (in prep. LFT, EMF, EAC, WAdG) of latrine buildings were completely enclosed, and therefore not accessible to scavenging animals.

One method for the improvement of sanitation, which uses the whole community approach is the so called “community led total sanitation” [59]. This method attempts to trigger a community’s engagement with its own sanitation issues to reduce open defecation. Using this approach, communities take control of producing locally appropriate latrines and ensure that all community members use them. Such blanket coverage is likely to be far more effective than piecemeal individual adoption of latrines with respect to the exposure of free range pigs to faecal material.

Gastro-intestinal and ectoparasite infections are another important, production limiting issue for pig producers, as shown in Table 1. Heavy infestation with these parasites can lead to reduced weight gain in pigs [30], reducing the economic potential of these livestock. We found that only 2 of the 10 pigs recruited into this study were said to have had any anthelmintic in the 6 months prior to the study, and this was not found to have any influence on parasite load (in EPG for any nematode species). A lack of influence of levamisole treatment on EPG was also found in another study in western Kenya [32], suggesting either anthelmintic resistance, or incorrect usage of the drugs. Improved husbandry practices, including the use of effective anthelmintics at correct dosages, would enhance pig health and production in this study area. Importantly, we also find that the distances that free range pigs move on a daily basis (mean of 4.1 km during daylight and 4.5 km at night) are likely to entail high energy expenditure. Mature pigs 6–10 months old presenting at slaughter in this region have been found to have mean live weights at the abattoir of 30 kg, giving a dressed weight of only 22.5 kg and earning the farmer only 2,000–2,500 KES [60], equivalent to US$24–29 per animal. Encouraging the confinement of pigs is likely to improve feed conversion and weight gain, by both reducing un-necessary energy expenditure as well as limiting parasite burden through environmental exposure.

Confinement of pigs would also reduce the risk of contact with other domestic or wild pigs: pig to pig contact is a driver of African Swine Fever (ASF) virus transmission. ASF regularly causes outbreaks in this region, with two reported outbreaks at the end of 2010, both of which were reported as being resolved by early 2012 [26]. Confining pigs within correctly constructed pig stys would also reduce the chances of contact between pigs and tsetse flies [61] the vectors of _Trypanosoma spp_. Western Kenya is a trypanosomiasis endemic area and pigs are known to be important hosts and reservoirs [28,29].

Both Trichinellosis and Toxoplasmosis are very real threats to these free-ranging pigs, with access to kitchen waste, in particular meat products, being a risk factor for infection. Such swill is also implicated in ASF transmission. Pigs in this study were observed spending an average of 5.9% of their time in the vicinity of the cooking and waste disposal areas of their homestead of origin, illustrating the potential for ingestion of meat, which may contain infective tissue cysts of _Toxoplasma gondii_ or _Trichinella spiralis_. Porcine toxoplasmosis can also be acquired through the ingestion of sporulated oocysts in cat faecal material: given that 49% of households in this region (unpublished obs.EMF, LFT, WAdG, EAC) report owning cats, combined with the scavenging behaviour of free range pigs, it is easy to infer from this the degree of contact with feline faecal material which takes place that may propagate this parasite.

While confinement would clearly be advantageous, there are practical and societal difficulties to overcome in encouraging the practice, not least because free range pig keeping is attractive to farmers due to the low input nature of the production system and the ease of implementation. Local extension services in areas where free ranging is practiced across East Africa should work to convince farmers that investing in improving pig production can reap important economic benefits in terms of weight at slaughter, as well as improve biosecurity and herd health on small-holdings.

**Conclusion**

These data provide new insights into the behaviour of pigs kept under a free range system in a resource-poor setting. We believe that the data presented here can be used in conjunction with information on pig population densities to build contact network models and to better understand transmission of several pathogenic organisms. For example, understanding transmission of African swine fever between individual pigs or between domestic and wild pigs. The movement data can also be combined with information on ration formulation and daily weight gain to provide evidence-driven advice to farmers on how to change their animal husbandry practices to improve the profitability of pig production. The key messages are: 1) pigs kept under these systems spend almost half their time outside their homestead boundaries, such that the village environment beyond the farm matters just as much as the environment on the farm itself to
pathogen transmission, and 2) free range domestic pigs expend tremendous energy foraging in the village environment, thus reducing their potential for weight gain and economic benefit to their owners.

Abbreviations
ASF: African swine fever; ASFV: African swine fever virus; CNS: Central nervous system; EPG: Eggs per gram; GIS: Geographic information systems; GPS: Global positioning system; GPSRS: General packet radio system; ILRI: International livestock research institute; LoCoH: Local convex hull; MCP: Minimum convex polygon.

Competing interests
No author has any competing interest to declare.

Authors’ contributions
LFT and EMF designed and implemented the study, and undertook data analysis. EMF obtained funding for the study, WAdG and EAC both assisted in the implementation of the study. All authors made contributions to conception, design, and revision of the manuscript. All authors read and approved the final manuscript.

Acknowledgements
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References
Focusing on neglected zoonoses

Two tenets at the core of the One Health concept are the belief that human and animal health are irrevocably entwined and that the improvement of both requires close collaboration between the medical and veterinary professions with support from allied disciplines. An ongoing Wellcome Trust-supported project in Western Kenya – the ‘People, Animals and their Zoonoses’ (PAZ) project – holds the One Health theme at its centre as it endeavours to study neglected zoonoses and provide policy-relevant information about their epidemiology. Veterinarian Lian Doble and the project’s leader Eric Fèvre explain why this study is so important.

Neglected diseases

In these marginalised communities, zoonotic diseases exert a heavy burden. Often, the burden is not due to the headline-grabbing zoonoses about which much is said on the international stage. Rather, the important zoonoses are pathogens that cause a variety of endemic diseases including (but not confined to) brucellosis, bovine TB, Q fever, endemic Rift Valley fever, cysticercosis and zoonotic trypanosomiasis.

The likelihood of acquiring these zoonotic diseases within a shamba is high, due to the abundance of risk factors. A natural environment conducive to transmission, regular close contact between people and their animals, access of those animals to human waste, little preventative health provision for domestic stock, inconsistent meat inspection, and poor quality food and forage for both humans and animals all contribute. In addition, other non-zoonotic infections, such as HIV/AIDS, are often present, which may impact on the probability of individuals acquiring a zoonotic infection in the first place.

Unfortunately, many of these zoonoses will remain undiagnosed or misdiagnosed in both livestock and people. Many factors are involved – a lack of health-seeking behaviour, the prohibitive cost of medical services, lack of veterinary service delivery, poor diagnostic
test availability, and/or lack of awareness among the population itself, or indeed within the medical and veterinary services.

This is clearly demonstrated by the ubiquitous diagnosis of malaria for any case of fever. While malaria is undoubtedly a very serious health issue, its overdiagnosis hides many other problems. To compound this, people in marginalised communities can easily fall off the policy radar – many may be born, live and die without official record being made of them and, as such, they have a weak, or nonexistent, political voice. Thus, while the diseases are grouped as ‘neglected zoonotic diseases’, it would be equally correct to identify them as ‘diseases of neglected populations’.

A joint expert consultation cosponsored by the World Health Organization (WHO) and the UK’s Department for International Development (DFID) Animal Health Programme in 2006 highlighted the problem posed by neglected zoonotic diseases in the developing world. It outlined a number of research areas where substantial efforts are needed to address the paucity of information about these important diseases at national and local levels. This research agenda included assessment of the true burden of neglected zoonoses on individuals and society, the costs of these diseases to livestock production, an in-depth understanding of risk factors in animals and humans, and the application and validation of appropriate diagnostic tools for use in the affected communities.

One health in practice
The PAZ study involves a multidisciplinary team of scientists from the School of Biological Sciences at the University of Edinburgh, the International Livestock Research Institute (ILRI) in Kenya and the Kenya Medical Research Institute (KEMRI). It brings together epidemiologists, veterinarians, medical health professionals and laboratory technologists working as a single team in a study area covering a large proportion of the Western and Nyanza Provinces of Kenya, stretching from Lake Victoria in the south along the Ugandan border towards Mount Elgon in the north. The human and animal health teams will visit over 500 homesteads during the four-year project, collecting data and samples from people, cattle and pigs, while offering health checks and advice or referral to those who require it. Bringing basic healthcare facilities directly to the people is one of the ways in which such activities can have immediate benefit, although it is the future outcomes of this research on which the greatest value is placed.

The research focuses on quantifying the importance of the zoonoses in the context of other infectious diseases, understanding in detail the factors that put livestock and people at risk, characterising the diversity in the natural environment that affects disease risk, trialling novel field-appropriate diagnostic tests and designing livestock-targeted interventions that are reasonably cheap and easy to implement and that may have an impact on human public health.

The Wellcome Trust has funded the establishment of a well-equipped field laboratory (shared with other Wellcome-funded projects operating here, with the support of the Kenya Department of Veterinary Services) in the town of Busia, the project’s centre of field operations, although interactions with other local, regional and international labs, as well as representatives of national veterinary and medical institutions, will be crucial for processing material, generating some of the data required and disseminating the results appropriately.

The very process of investigating disease simultaneously in human and animal populations involves putting the idea of one health into daily practice and the results will add weight to the argument for more integrated health surveillance and control for zoonotic diseases.

More information can be found at www.zoonotic-diseases.org
doi: 10.1136/vr.c2337

Diseases of interest to the PAZ project

**Bovine tuberculosis**

To what degree *Mycobacterium bovis* contributes to the current human epidemic of TB is unknown, although evidence so far implicates it in a large number of cases across the developing nations. A higher proportion of extrapulmonary cases than occurs in *M tuberculosis* infections, and resistance to many of the first-line TB drugs, make it a difficult pathogen to treat effectively. Productivity losses in cattle add to the dual burden of this disease on communities. Concurrent HIV infection is known to greatly enhance the pathogenic progress of TB, making this disease of particular concern in populations where HIV infection is rife.

**Cysticercosis**

Infection by the intermediate stage of the porcine/human tapeworm *Taenia solium* is almost unknown in the developed world, but is becoming recognised as a major problem in Africa, Latin America and Asia. The beef tapeworm, *Taenia saginata*, is also thought to be endemic here. These cestodes affect many millions worldwide, and it is estimated by the WHO that 7 *solium* is responsible in endemic areas for 50,000 deaths each year. Infection of the CNS by the parasite (neurocysticercosis) is thought to be the leading cause of acquired epilepsy worldwide. The parasite life cycle is perpetuated through a combination of factors, including free-range pig production, inadequate latrine provision, lack of meat inspection, and little education on food hygiene – a package of factors ubiquitous throughout the developing world.

**Brucellosis**

Infection by the Gram-negative bacterium *Brucella abortus* is acquired through either direct contact with infective abortion material or from unpasteurised dairy products. In bovids the infection causes abortions, stillbirths and reduced milk yield. In people, it causes fever, possibly accompanied by nausea and joint or muscle pain. It can also be a cause of human infertility. It is often undiagnosed due to its similarity with malarial fevers.

**Q fever**

Another organism responsible for many undiagnosed fevers is *Coxiella burnettii*. Although generally non-pathogenic in cattle, this infection can cause abortion in sheep and goats and a severe and sometimes chronic fever in people, which can result in endocarditis. Infection is generally contracted via inhalation of the organism, which is shed in large quantities in aborted materials and is also found in milk, urine and faeces of infected animals.

**Rift Valley fever**

Caused by a virus of the family Bunyaviridae, Rift Valley fever is responsible for severe disease in both people and animals with high rates of morbidity and mortality. Confined to the African continent until an outbreak in Saudi Arabia in 2000, there is the potential for this virus to become a serious concern outside of Africa. Infection in humans is generally the result of mosquito-borne transmission or contact with blood or organs of infected animals.

**Trypanosomiasis**

Limited in distribution to certain areas of southern and eastern Africa, the zoonotic form of sleeping sickness (human African trypanosomiasis) caused by *Trypanosoma brucei rhodesiense* is maintained in the animal reservoir, and control programmes require efforts to either eliminate the tsetse fly vector or to treat the cattle reservoir. Production losses in cattle are caused by a variety of other pathogenic trypanosomes which would also be addressed by sustained control of the zoonotic forms.
Bibliography


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<td>1943</td>
<td>Territoriality and home range concepts as applied to mammals</td>
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Garcia H. H. et al. (2001). Transient antibody response in Taenia solium infection in field conditions; A major contributor to high seroprevalence. Tropical Medicine 65(1), 31–32.


Huerta M. et al. (2002). Synthetic peptide vaccine against Taenia solium pig cysticercosis: successful vaccination in a controlled field trial in rural Mexico. Vaccine 20, 262–266.


Ogunsanmi A., Taiwo V., Ohore G., et al. (2000). Application of antigen-detection en-
zyme immunoassay for the diagnosis of porcine Trypanosoma brucei infection. Veter-
inarski Arhiv 70(5), 231–238.

Ohrt C. et al. (2007). Establishing a malaria diagnostics centre of excellence in Kisumu,

wahtid.php/Reviewreport/Review?page_refer=MapEventSummary&reportid=
10307.

Okome-Nkoumou M. et al. (2010). Epileptiform seizures revealing neurocysticercosis:

Olsen A. et al. (2009). Strongyloidiasis—the most neglected of the neglected tropical dis-
eesases? Transactions of the Royal Society of Tropical Medicine and Hygiene 103(10),
967–972.

ONeal S. et al. (2012). Seroprevalence of antibodies against Taenia solium cysticerci
among refugees resettled in United States. Emerging Infectious Diseases 18, 431–
438.

O’Neal S. E. et al. (2012). Geographic correlation between tapeworm carriers and

Neurology, Neurosurgery & Psychiatry 68(2), 137–143.

Parker M., Allen T., Hastings J. (2008). Resisting control of neglected tropical diseases:
dilemmas in the mass treatment of schistosomiasis and soil-transmitted helminths in

Pawlowski Z., Allan J., Sarti E. (2005). Control of Taenia solium taeniasis/cysticerc-
cosis: from research towards implementation. International Journal for Parasitology
35(11-12), 1221–1232.

Pawlowski Z. S. (2008). Control of neurocysticercosis by routine medical and veterinary
services. Transactions of the Royal Society of Tropical Medicine and Hygiene 102(3),
228–232.


Pract N. et al. (2013). Bayesian modelling to estimate the test characteristics of coprology, coproantigen ELISA and a novel real-time PCR for the diagnosis of taeniasis. Tropical Medicine & International Health 18, 608–614.


