PREVALENCE OF PORCINE CYSTICERCOSIS AND ASSOCIATED RISK FACTORS IN FREE RANGE PIGS IN HOMA-BAY DISTRICT, KENYA.

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University or for any other award.

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DEDICATION

This thesis is dedicated to my loving parents Dr. and Mrs. Eshitera whose advice and support always gave me great inspiration and motivation, my brothers Evans and Allan, and sister Lucy for their prayers, encouragement and all manner of support. To my aunt Regina Muhanji for her continued support and trust in my capability. To my fiancée Evalyn for her valuable support and constant encouragement.

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LIST OF ABBREVIATIONS

^0C -   Degrees Celsius

Ab-ELISA   -   Antibody Enzyme Linked Immunosorbent Assay

Ag-ELISA   -   Antigen Enzyme Linked Immunosorbent Assay

BSA   -   Bovine Serum Albumin

C.I   -   Confidence Interval

CAT   -   Computerized Axial Tomography

CNS   -   Central Nervous System

CSF   -   Cerebrospinal fluid

d.f.   -   Degrees of freedom

D.V.O   -   District Veterinary Officer

F.A.O   -   Food and Agricultural Organisation

M   -   Meters

McAb   -   Monoclonal Antibodies

Mg   -   Micrograms

N.C.C	-	Neurocysticercosis
O.R.	-	Odds ratio
OD	-	Optical density
P	-	Probability value
PAF	-	Population attributable fraction
PBS	-	Phosphate Buffered Saline
pH	-	Acidity/Basicity
RR	-	Relative risk
TMB	-	Tetramethylbenzidine
μl	-	Microlitres
WHO	-	World Health Organization
χ^2	-	Chi square

ABSTRACT

The present study was conducted in the year 2010 to determine the prevalence of porcine cysticercosis and the potential risk factors for taeniosis in Homa Bay District. Presence of porcine cysticercosis infection was determined using both ante-mortem lingual palpation of pigs and serological assay through HP10 Ag- ELISA, while the risk factors for taeniosis were determined through administration of a standard questionnaire at household level to respondents by face to face interviews. One member of the household familiar with the day to day management of the pigs was interviewed on the risk factors.

A total of three hundred and ninety two pigs from two hundred and ninety nine households were sampled.

Porcine cysticercosis was found to be prevalent amongst the free range pigs in the district with individual pig prevalence of 5.6% (3.3%, 7.9%; 95% C.I.) by lingual palpation and seroprevalence of 43.3% (37%, 49.7%; 95% C.I.). The household prevalence by ante-mortem lingual palpation was 7.4% (4.4%, 10.3%; 95% C.I.), while the household sero-prevalence was 46.9% (39.6%, 54.2%; 95% C.I.).

The computed kappa statistic value of 0.02 indicated a very poor agreement between the ante-mortem lingual palpation prevalence and the HP10 Ag-ELISA sero-prevalence.

The only risk factor that was significantly associated to the occurrence of porcine cysticercosis at farm level was lack of evidence of latrine use with a P value of 0.032.

It was concluded that porcine cysticercosis is prevalent in free range pigs in Homa Bay District. Lack of latrine use was the only risk factor found to be significantly associated with the presence of *T. solium* cysticercosis.

The presence of homesteads lacking latrines and failure of people to use the available latrines alongside presence of free roaming pigs were found to be contributing to the maintenance and spread of the parasite.

Mass deworming of the human population together with education on the need for strict use of latrines together with housing of the pigs would go a long way in disrupting the life cycle of *T. solium* in Homa Bay District.

INTRODUCTION

Taenia solium taeniosis-cysticercosis remains a major public health concern in many developing countries of Latin America, Africa and Asia (Sarti *et al.,* 1992). The occurrence and prevalence of the infection is associated with cultural practices of eating raw or undercooked pork as well as poor socio-economic and sanitary conditions. Studies have demonstrated that in endemic areas, *T. solium* porcine infections have been associated with poverty, absence of latrines and free access by scavenging pigs to human faeces (Diaz *et al.,* 1992; Schantz *et al.,* 1992; Sarti *et al.,* 1997).

In Kenya, very little epidemiological work has been done on *T. solium* taeniosis-cysticercosis, and it is assumed to be rare (Githigia *et al.,* 2002; Githigia *et al.,* 2006; Mutua *et al.,* 2007). Few cases have been reported in pigs during routine meat inspection.

In humans 2 cases of neurocysticercosis have been reported in Kenya in the recent past, both cases had cysts in the brain while one had an additional cyst in the eye (Phiri *et al.,* 2003). One of the cases was reported at Nairobi Hospital and the other at Kakamega District Hospital.

The lingual examination method has been used to detect palpable cysts, which may indicate porcine cysticercosis. However, the use of this method to detect the disease in pigs has been met with much reluctance owing to its low sensitivity, only capable of detecting *C. cellulosae* cysts in heavily infected pigs and its requirements for technical expertise (Sciutto *et al.,* 1998). Nonetheless, it has been found to be more readily available and less costly than Ag-ELISA testing. Githigia *et al.* (2002) examined a total of three hundred pigs in South Nyanza and one

hundred and seven pigs in Busia, Kenya, using the lingual palpation method and reported prevalence rates of 10% and 14%, respectively.

Most pigs in Kenya are raised under the intensive system with the extensive management system being predominant in some parts of Nyanza and Western Kenya where pigs are let free to scavenge. Surprisingly, this free-range system is slowly emerging in certain urban and slum areas of the country. The 1970s ban by the Kenya Government of the free-range pig keeping system led to a dramatic decline in the prevalence of porcine cysticercosis (Githigia *et al.,* 2001). However, the problem may still exist in certain rural areas where pigs are kept under free-range system with poor sanitation. Githigia *et al.* (2006) reported prevalence rates of 9%, 15% and 3% in field investigations conducted in Township, Funyula and Budalang'i divisions of Busia District, respectively. In Teso District, Mutua *et al.* (2007) have reported a prevalence of 6.5%. There have been no studies to establish the prevalence of porcine cysticercosis and its risk factors in Homa Bay District, where pigs are reared under the free-range system.

This study investigated the prevalence of porcine cysticercosis and the potential risk factors of *Taenia solium* taeniosis and cysticercosis in Homa Bay District using the lingual palpation method and the more sensitive Ag-ELISA method.

1.1   Objectives

1.1.1   Main objective

To determine the prevalence of porcine cysticercosis and the risk factors of *Taenia solium*-taeniosis and cysticercosis in Homa Bay District.

1.1.2   Specific objectives

1) To estimate the prevalence of porcine cysticercosis using the lingual palpation and the Ag-ELISA methods in Homa Bay District.

2) To determine the risk factors for porcine cysticercosis and human taeniosis and cysticercosis in Homa Bay District.

1.2   Justification of the study

Taenia solium Cysticercosis is an emerging zoonosis in the developing world and recently has been diagnosed in non endemic regions following migration of carriers. These infections are an important constraint to pig production which is emerging as an important livestock enterprise in Kenya lower space requirement and higher returns compared to species such as cattle and also a threat to human health. The control of these infections is therefore necessary. The epidemiology of T. solium infections is generally lacking in Kenya and no studies have been carried to determine the prevalence of porcine cysticercosis at the associated risk factors in Homa Bay District.

LITERATURE REVIEW

2.1 Pig population and production in Kenya

In Kenya, the pig population is estimated at 334,689 (KNBS, 2009b) of which, 80% are reared under commercial enterprises mainly in Central, Rift Valley and Nairobi provinces (Ng'ang'a *et al.*, 2008). The District has an estimated 36,400 pigs which are reared under small scale enterprises (DVO, personal communication 2010). The small scale enterprises comprise the free range systems popular in Nyanza and Western provinces of Kenya.

2.2 Porcine cysticercosis

Porcine cysticercosis is caused by the presence and development of the larva (*Cysticercus cellulosae)* of *T. solium* in the striated muscles of pigs. The cysticerci are whitish vesicles measuring 8-10 mm with invaginated scolexes appearing as white spots with double rows of hooks similar to those of the adult worms (Pedro and Boris, 1980). *Taenia solium* is a two-host zoonotic cestode. The adult stage, usually up to 10 metres in length, lives in the small intestines of humans, the only known definitive host. The gravid proglottid at the terminal end of the worm contains eggs that are infective to both pigs and humans leading to the development of the larval stage. The natural intermediate host is the pig; no other intermediate host of *T. solium* has been reported in nature (Garcia *et al.*, 2002). Humans become infected with the larvae by accidental ingestion of *T. solium* infective eggs through the fecal-oral route.

In the normal cycle of transmission, humans acquire intestinal infection (taeniosis) by ingesting undercooked pork infected with *T. solium* cysticerci. Infection in pigs follows ingestion of

human feces containing either the ova or gravid proglottids of the tapeworm. These then hatch to oncospheres in the gastrointestinal tract of the pig. The oncospheres travel via the bloodstream and lodge in various body organs to develop into cysticercii called *Cysticercus cellulosae* (Schantz *et al.*, 1998).

It has been shown that humans can develop cysticercosis and serve as dead end intermediate hosts if they consume *T. solium* eggs. The *T. solium* eggs develop into cysticerci in various organs of man including the eyes, spinal cord and central nervous system and cause minimal or no tissue reaction, but death of the cysts in the central nervous system can elicit an intense tissue response. This leads to Neurocysticercosis (NCC). Thus, symptoms often do not appear for years after infection. There are wide variations of clinical manifestations of neurocysticercosis. These are a consequence of the inflammation around a cyst(s), space occupation and impedance to the flow of cerebrospinal fluid (CSF), less commonly meningeal or vascular inflammation, and non-CNS disease. Seizures are the most common symptom in 70%-90% of patients (Del Brutto, 1997; White, 1997). These may occur both when a cyst is degenerating (Rajshekar *et al.*, 1995), and around a chronic, calcified lesion (Del Brutto *et al.*, 1992).

2.3   Life cycle of *Taenia solium*

Taenia solium has a two-host life cycle which involves the pig as the normal intermediate host harboring the cysticerci and man as the definitive host harboring the adult form of the tapeworm (Nash and Neva, 1984) (Figure 2.1). In humans, harmful taeniosis may be acquired through ingestion of infected pork containing viable cysticerci. Consumption of contaminated food and water with taenia eggs can also lead to cysticercosis. The scolex of the metacestode then

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evaginates in the gut of man and attaches to the mucosa of the small intestines. The tapeworm matures over a period of 2-3 months to achieve a length of up to 10 metres. The adult worm begins to shed gravid segments which either degenerate in the rectum or are expelled with the feces (Brown, 1983). Self-contamination by man from an adult infection or internal autoinfection from reverse peristalsis has been reported, resulting in development of the larval stage of *T. solium* in humans (Beaver *et al.*, 1984; Pal *et al.*, 2000). Development of cysticerci in the brain or the spinal cord of humans leads to neurocysticercosis, which is a major cause of acquired epilepsy in the developing world (Mafojane *et al.*, 2003).

Pigs are infected when they ingest eggs shed in the feces by human tapeworm carriers, especially in environments characterized by the absence of latrines, and free roaming pigs. Once the eggs are ingested, oncospheres hatch in the intestines, invading the intestinal wall, and migrate via blood to the striated muscles as well as the brain, liver and other tissues, where they develop into cysticerci. The cysts form in the muscles within 3-6 months where they remain infective for one year (FAO, 2003).
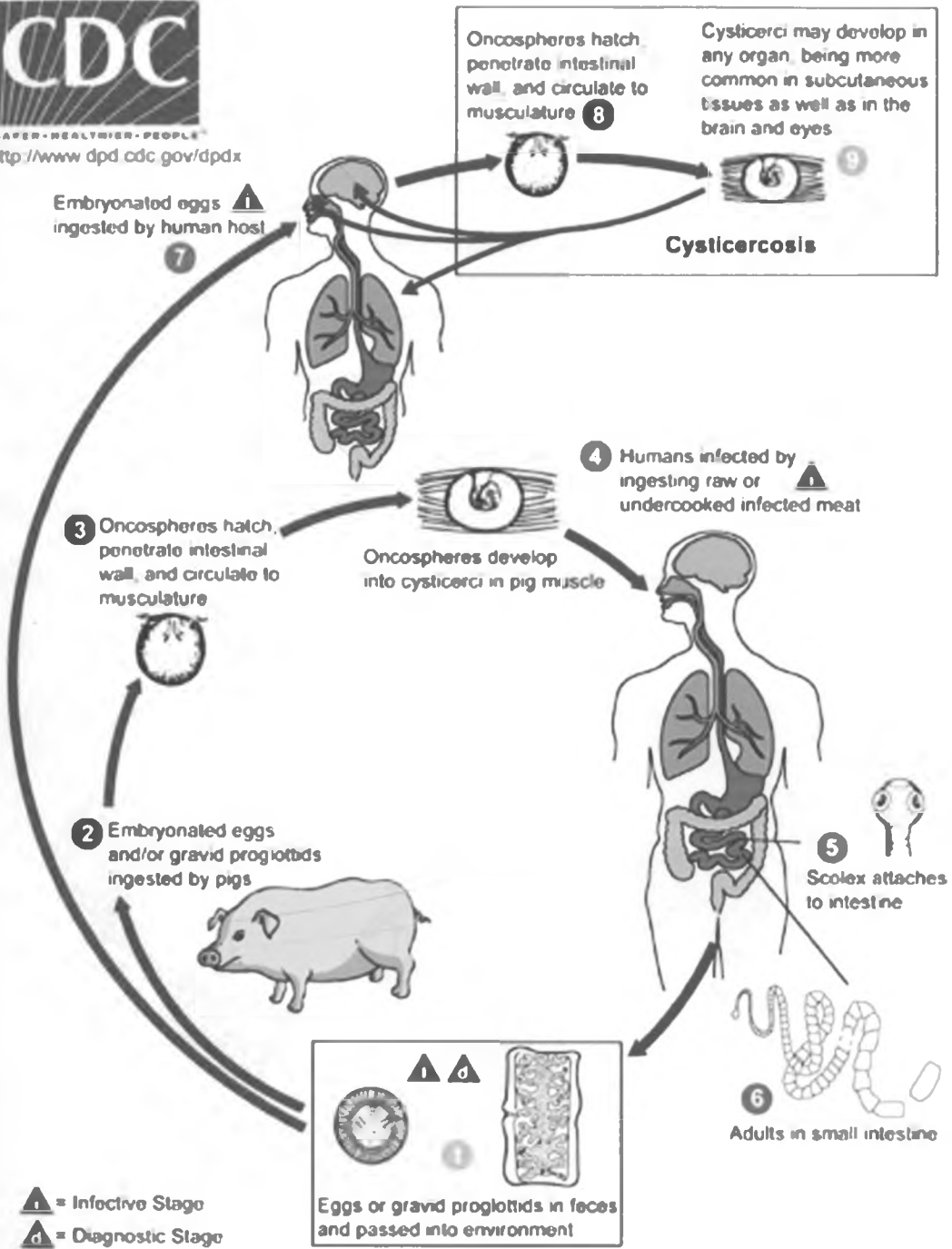
Figure 2.1: Life cycle of Taenia solium (CDC, 2011).

2.4   Occurrence of *T. solium* taeniosis/cysticercosis

Taenia solium is an emerging and expanding zoonosis in Africa (Zoli *et al.*, 2003). Infection with *T. solium* and its larvae is prevalent in human hosts in many developing countries of Latin America, Africa and Asia (Sarti *et al.,* 1992; Allan e*t al.,* 2003). Taenia eggs are very resistant and can live long in the environment (Schantz, 2002). Cases of human neurocysticercosis have been reported in non-endemic areas of Latin America indicating patterns of immigration from highly endemic countries (James, 2000). Neurocysticercosis is endemic mainly in China, Indonesia, India, Nepal and Korea, sub-Saharan Africa, Central America and parts of South America (Rajshekhar *et al.,* 2003). Figure 2.2 shows the worldwide distribution of cysticercosis.

■ Endemic (Full Lifecycle)
▨ Suspected Endemic
▢ Imported Cases (Human Cysticercosis Transmission)
▢ No information available/no evidence
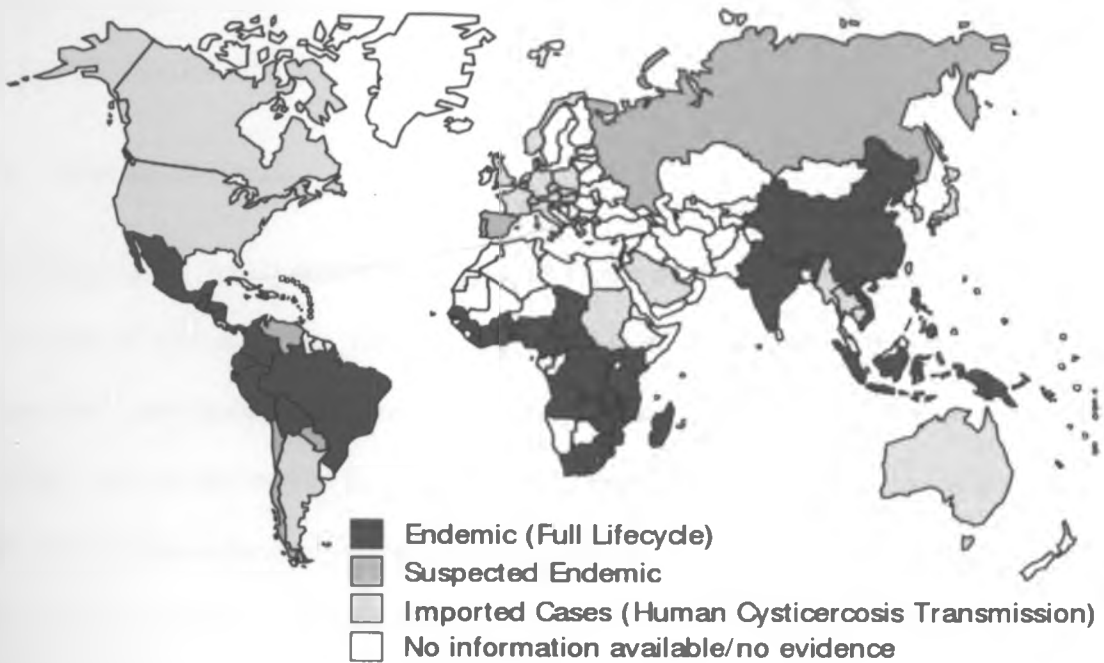
Figure 2.2: World map showing distribution of porcine cysticercosis (WHO/FAO, 2006).

Taenia solium cysticercosis in both humans and pigs is under-recognized in many developing countries of Africa especially those of central and western parts and very little epidemiological data are available (Tsang and Wilson, 1995). The infection reportedly occurs over most of the African continent with the exception of the strictly Muslim areas of the North and sub-Saharan areas. Both human and pig infections have been reported in South Africa, Zimbabwe, Gambia, Togo, Rwanda, Burundi, Malawi, Swaziland, Madagascar and Zaire (Zoli *et al.,* 2003). In Kenya Githigia *et al.,* (2006) reported 9%, 15% and 3% in Township, Funyula and Budalangi divisions respectively while Mutua et al., (2007) reported a prevalence of 6.5% in Teso District. In Tanzania, cases of porcine cysticercosis were first reported in Mbulu District (Boa *et al.,* 1995) where the prevalence was estimated at 17.4%. In Uganda, 9.4% of pigs surveyed were found positive by lingual palpation for cysticercosis with most cases coming from the rural areas (Kisakye and Masaba, 2002).

2.5   Neurocysticercosis

Neurocysticercosis (NCC) occurs when cysts of *T. solium (Cysticercus cellulosae)* lodge in the central nervous system of man. This infection occurs more in areas where pigs are reared in poor sanitary and unhygienic conditions and recently in non-endemic areas due to migration of infected *T. solium* carriers into previously non endemic areas. It is recognized as a zoonosis of public health concern because it causes disability of the infected persons and could possibly be fatal if untreated (WHO, 1979). Signs and symptoms of this disease are dependent on the number and location of the cysts in the nervous system. Symptoms can occur months to years after infection, usually when the cysts are in the process of dying. When this happens, the brain can

swell. The pressure caused by swelling is what causes most of the symptoms of neurocysticercosis. Seizures and headaches are the most common symptoms. Other signs of *T. solium* cysticercosis in humans include nausea, vomiting, ataxia, and confusion. Focal neurological deficits are uncommon. Patients with cysts in the basal cisterns can present with meningeal signs, hydrocephalus, vasculitis, and stroke (Del Brutto, 1997). However, confusion, lack of attention to people and surroundings, difficulty with balance and swelling of the brain (called hydrocephalus) may also occur. Some 10%-20% of patients present with ventricular cysts, sometimes also with seizures or with meningeal inflammation. Rare neurological manifestations have also been reported including altered mental state; spinal cysticercosis with radicular pain or paraesthesiae, or progressive cord compression; ophthalmic cysticercosis; migraine headaches; and neurocognitive deficits (Del Brutto, 1997). Death can occur suddenly with heavy infections. It is the most common parasitic infection of the brain and a leading cause of epilepsy in the developing world, especially Latin America, India, Africa, and China (Carpio, 2002; Garcia *et al.*, 2003; Garcia *et al.*, 2004). Social consequences of NCC include stigmatization, incapacitation and decreased work productivity. Neurocysticercosis is of great economic relevance, resulting from the cost of medical treatment and lost working days (Roberts *et al.*, 1994). However, the true impact of the disease has been obscured by the unavailability of sensitive and specific diagnostic tools necessary for the collation of reliable epidemiological data in most parts of the world, for example, the Ag-ELISA (Tsang and Wilson, 1995; Zoli *et al.*, 2003).

2.6   Clinical signs of human taeniosis and porcine cysticercosis

Taenia solium causes two different diseases in man. When the adult cestode infects the human intestine, taeniosis develops. This is generally asymptomatic with the host becoming a continuous source of *Taenia* eggs expelled in feces each day (Brown, 1983; Julio, 2004). However, gastrointestinal discomfort including diarrhea, flatulence and abdominal pains are sometimes observed. Porcine cysticercosis generally produces no clinical signs (Gonzalez *et al.*, 2003). At the time of infection, pigs may have slight diarrhea due to the irritation of the intestinal mucosa by the migrating embryos. The establishment of the cysticerci may result in myositis, with locomotor disorders that may lead to progressive emaciation due to difficulty in ingesting and assimilating food. Encephalitic signs do occur if the cysticerci migrate to the brain. Abnormal skin sensitivity and myocardial failure may also result (Urquhart *et al.*, 1988).

2.7   Diagnosis of *T. solium* taeniosis/cysticercosis

2.7.1   Ante-mortem lingual palpation and post mortem incisions

Palpation of the tongue for *C. cellulosae* cysts in live pigs has been used (Phiri *et al.*, 2002; Githigia *et al.*, 2002; Ngowi *et al.*, 2004). However, this method is not very sensitive; Joshi *et al.* (2006) reported a sensitivity of only 50% in Chitwan and Kathmandu valley. Post-mortem meat inspection is more sensitive and specific and involves both palpation and incision of various parts of the carcass including the tongue for the presence of cysts. In light infections, both specificity and sensitivity of lingual palpation have been shown to be low (Sciutto *et al.*, 1998; Garcia *et al.*,

2003). The cysts seen during postmortem meat inspection need to be differentiated from those of sarcocystis (*Sarcocystis suihominis*), which are smaller.

2.7.2 Sero-diagnosis in pigs

Antibody detection methods including enzyme linked immunosorbent assay (Ab ELISA), Complement fixation test (CFT) and immunoblot have been used for diagnosis of porcine cysticercosis (Dorny *et al.*, 2003; Garcia *et al.*, 2003). Antigen-ELISA has been shown to be more specific and sensitive (Phiri *et al.*, 2002) but does not allow for the differentiation of metacestodes of *T. solium* and *T. hydatigena* (Dorny *et al.*, 2003). The test has the advantage over Ab-ELISA because antibodies are able to persist long after either body immune mechanisms or medical therapy has eliminated the active infection (Harrison *et al.*, 1989). In addition, the Ab ELISA could indicate exposure to infection and not necessarily the presence of an established viable infection resulting in transient antibodies and thus false positive results (Garcia *et al.*, 2001). The Ag-ELISA is reported to have a sensitivity of 85% (Garcia *et al.*, 2000) and a specificity of 94.7% in Zambia (Dorny *et al.*, 2004) and 84.1% in South Africa (Krecek *et al.*, 2008).

2.7.3 Diagnosis in humans

The direct recognition of proglottids in human feces is the best option for identification of *Taenia* infections but it may be difficult to differentiate eggs of *T. saginata* and *T. solium* which are similar morphologically (James, 1982). Obtaining gravid proglottids in saline for Indian ink injection or proglottids in formalin for sectioning and staining with Hematoxylin and Eosin has

been shown to be useful in differentiating the two *Taenia* species (Mayta *et al.*, 2000). Proglottids are usually identified as *T. solium* when 10 or fewer branches arose to each side from the central uterus and as *T. saginata* when there were 12 or more branches (Flisser, 1994). Recovery of tapeworm scolex lacking hooklets is usually indicative of *T. saginata* while armed scolex belongs to *T. solium* (Garcia *et al.*, 2003).

Antibody detection methods including complement fixation test, radioimmunoassay, ELISA, Latex agglutination and immunoblot techniques, have been used in diagnosis of human taeniosis (Tsang *et al.*, 1989; Chapman *et al.*, 1995; Garcia *et al.*, 2002). Methods used for the diagnosis of neurocysticercosis include Computerized Axial Tomography (CT scan) of the brain (Lotz *et al.*, 1988; Pal *et al.*, 2000) and Magnetic Resonance Imaging (MRI).

The CT scan and MRI provide objective evidence on the number and location of intracranial cysticerci, their viability, and the severity of the host inflammatory reaction against the parasites (Garcia and Del Brutto, 2003). However, the results may be nonspecific and the differential diagnosis with other infectious or neoplastic diseases of the central nervous system may be difficult. Neuroimaging findings in parenchymal NCC depend on the stage of development of the parasites (Garcia and Del Brutto, 2003). Vesicular (living) cysticerci appear as cystic lesions within the brain parenchyma. The cyst wall is thin and isodense with the surrounding tissues and is generally not visible on imaging studies. The cyst fluid is hypo-dense and is clearly demarcated. These cysts lack perilesional edema, do not enhance after contrast medium administration, and characteristically show a bright nodule (hole-with-dot imaging) in their interior that represents the scolex. When parasites begin to degenerate (colloidal cysts), their appearance in CT and MRI examinations changes to ill-defined ring-enhancing lesions

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surrounded by edema (acute encephalitic phase). Granular cysticerci are degenerated parasites seen as nodular hyperdense lesions surrounded by edema or a rim of gliosis after contrast medium administration, and calcified (dead) cysticerci appear on CT as small hyper-dense nodules without perilesional edema or abnormal enhancement after contrast administration. These lesions are usually not visualized by MRI. Conversely when calcified, they are associated with perilesional edema and contrast enhancement; these are better seen by MRI.

2.8 Treatment of *T. solium* taeniosis/cysticercosis

2.8.1 Treatment in man

Treatment of cysticercosis is tailored to the specific needs of the patient and may include medical therapy through anthelminthic drugs together with corticosteroids or surgery (White and Clinton, 2009).

Anthelmintics including praziquantel and albendazole have been used for the treatment of human taeniosis but can also act against the cystic larvae (Garcia *et al.*, 2002; Julio, 2004). Taeniosis is treated with praziquantel at 10 mg/kg bodyweight or niclosamide (Flisser *et al.*, 2003). Both albendazole and praziquantel have been shown to be effective for therapy of parenchymal brain cysticercosis, although albendazole is better and more effective in the penetration of the brain tissue (Sotello *et al.*, 1988; Nash, 2003; Julio, 2004). Between the second and fifth days of antiparasitic therapy, there is usually an exacerbation of neurological symptoms, attributed to local inflammation due to the death of the larvae. Thus, there is a need for combining the treatment with steroids to reduce the inflammation. For this reason, both albendazole and

praziquantel are generally given simultaneously with steroids in order to control the edema and intracranial hypertension that may occur as a result of therapy. Albendazole appears to be more effective and a safe drug for neurocysticercosis (Garcia *et al.*, 2004; Matthaiou *et al.*, 2008). Surgical intervention may at times be necessary to treat cysticercosis lesions.

2.8.2 Treatment of pigs

Anthelmintics such as dichlorvos, levamisole, mebendazole, oxfendazole and fenbendazole have all been demonstrated to be active against the major helminths in pigs (Brander *et al.*, 1991; Sarti *et al.*, 1997; Rajshekhar, 2003). As demonstrated by experiments in animals, praziquantel and albendazole are effective antiparasitic drugs against *T. solium* cysticerci. Initial studies with praziquantel noted that doses as low as 5 to 10 mg/kg/day had some effect against cysts, and doses as high as 50 to 75 mg/kg/day were well tolerated in infected pigs (Garcia *et al.*, 2002). Albendazole has been shown to lead to dissolution of cysts at a dosage rate of 15mg/kg/day for one week. Oxfendazole at 30mg/kg is effective against the cysts in pigs and is the drug of choice (Cederberg *et al.*, 2011)

2.9 Risk factors for infection in pigs and humans

The major risk factors for infection in pigs include conditions that allow pigs to access material contaminated with the faeces of a human with *T. solium* infestation. The conditions can be summarized as follows (Murrell, 2005; Pawlowski *et al.*, 2005; Kyvsgaard and Murrell, 2005):

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- Extensive/free range pig rearing and outdoor defecation by humans where pigs can have access to human faeces (lack of latrines or using latrines that allow pigs to access human stool);

- Use of pigs to scavenge/eat human feces (Use of pigs to cleanse neighborhoods by feeding on faeces);

- Connection of latrines to pig pens;

- Use of raw or improperly treated sewage effluent to irrigate vegetables and pastures where pigs feed; and

- Involvement of human *T. solium* carriers in pig care.

Infection in humans is through:

- Conditions that allow ingestion of *T. solium* eggs in contaminated food and water;

- Introduction of eggs from faeces into the mouth by contaminated hands (Oral route); and

- Eating pork with *T. solium* cysts, made easy through lack of pork meat inspection and eating improperly cooked pork with cysts.

Reported prevalences of risk factors in Kenya, include:

In Funyula, Busia Githigia *et al.,* (2005) reported that 98% allow their pigs to roam freely while only 2% tethered their pigs. There was no pig housing (100%). Fried pork was preferred by 85% of these households and 33% were practicing home slaughtering of pigs with no official meat inspection. Pit latrines were present in 83.3% of these households, which were used by adults. Pit latrines were lacking in 15% of the households. Most households (89.6%) had previous tapeworm experiences. Those with active tapeworm infections composed 8.3% of the households. In Busia, Kenya Kagira *et al.,* (2010) reported that free-range pig keeping was practised by all of the

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households, history of human taeniosis infection in a family was reported by 51% of the households, slaughtering of pigs at home was carried out by (20%) of the households, lack of meat inspection was reported in (15%) of the households, and absence of latrines being reported in (15%) of the households, home slaughtering was carried out by (20%) of the households while Mutua *et al.*, (2011) reported that majority of farmers (73%) had no pig houses and pork was consumed by (31%) of the respondents in the villages of Busia and Kakamega district, Western Kenya.

2.10 Control and prevention of *T. solium* taeniosis/cysticercosis

The control and prevention of taeniosis/cysticercosis infections can be achieved through breaking the life cycle of the tapeworm. This can be achieved through a number of steps including:

- Mass treatment of humans and pigs in endemic areas;
- Enforcement of proper meat inspection of pig carcasses in slaughter points;
- Proper treatment and disposal of human waste including use of latrines and toilets to avoid pigs ingesting *T. solium* eggs;
- Total confinement of pigs;
- Adopting proper hygienic standards such as washing hands before handling food;
- Proper processing and disposal of infected pork; and
- Vaccination of pigs against porcine cysticercosis.

2.10.1 Vaccine against porcine cysticercosis

Considering that pigs are the intermediate hosts in the life cycle of *T. solium*, vaccination of pigs is another viable point of intervention to eliminate cysticercosis. This is supported by the fact that many immune cell types are found to be capable of destroying cysticercus (Scuitto *et al.*, 2008). A number of approaches have been used by different groups towards the development of a vaccine against *T. solium* infection (Cai *et al.*, 2001). The approach that has been most successful in development of vaccines against other taeniid cestode parasites has been the use of recombinant oncosphere antigens (Lightowlers and Gauci, 2001). The test vaccines are extracted from antigens of different cestodes such as *T. solium*, *T. crassiceps*, *T. saginata*, *T. ovis* and target oncospheres and/or cysticerci. Vaccines extracted from genetically engineered 45W-4B antigens have been successfully tested in pigs under experimental conditions and have been shown to protect against cysticercosis in both Chinese and Mexican type of *T. solium* (Luo *et al.*, 2009). However, its effectiveness in endemic field conditions is not known (Scuitto *et al.*, 2008).

The S3PVAC Vaccine, a tri-peptide which is synthetically produced, has proven its efficacy in natural conditions of transmission (Huerta *et al.*, 2001). The vaccine so far, can be considered as the best vaccine candidate to be used in endemic areas such as Mexico (Gilman *et al.*, 1999). The vaccine consists of three protective peptides, KETc12, KETc1 and GK1, whose sequences belong to native antigens that are present in the different developmental stages of *T. solium* and other cestode parasites (Scuitto *et al.*, 2008).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

The study was conducted in Homa Bay District in Nyanza Province of Kenya. The district was chosen because it is known to have pigs kept under free range husbandry system and no previous studies on porcine cysticercosis had been conducted in the district. The district is located approximately 500km from Nairobi and lies 0°31'S 34°27'E . The district covers an area of 1160 Km^2 and is made up of seven administrative divisions, namely, Asego, Pala (Not shown in Figure 3.1 since the map had been drawn before sub-division of Riana to Riana and Pala Divisions), Riana, Rangwe, Nyarongi, Kobama and Ndhiwa. The District lies within the Lake Victoria Basin and the altitude ranges between 1163m and 1219m above sea level. Most parts of Homa Bay district receive 500–1000mm mean annual rainfall, which is bimodal occurring in April to May and November to December. The temperatures in the district range between 17.1^0 C and 34.8^0 C. The total human population of Homa Bay District was estimated at 963,794 people (KNBS, 2009a). The total population of cattle, sheep and goats is estimated at 589,400 animals while that of pigs is estimated at 20,800 (MOLD, 2007). However this is an underestimation considering a census carried out estimated the pig population at 28,400 in Riana, Pala and Ndhiwa divisions only (DVO personal communication 2010).
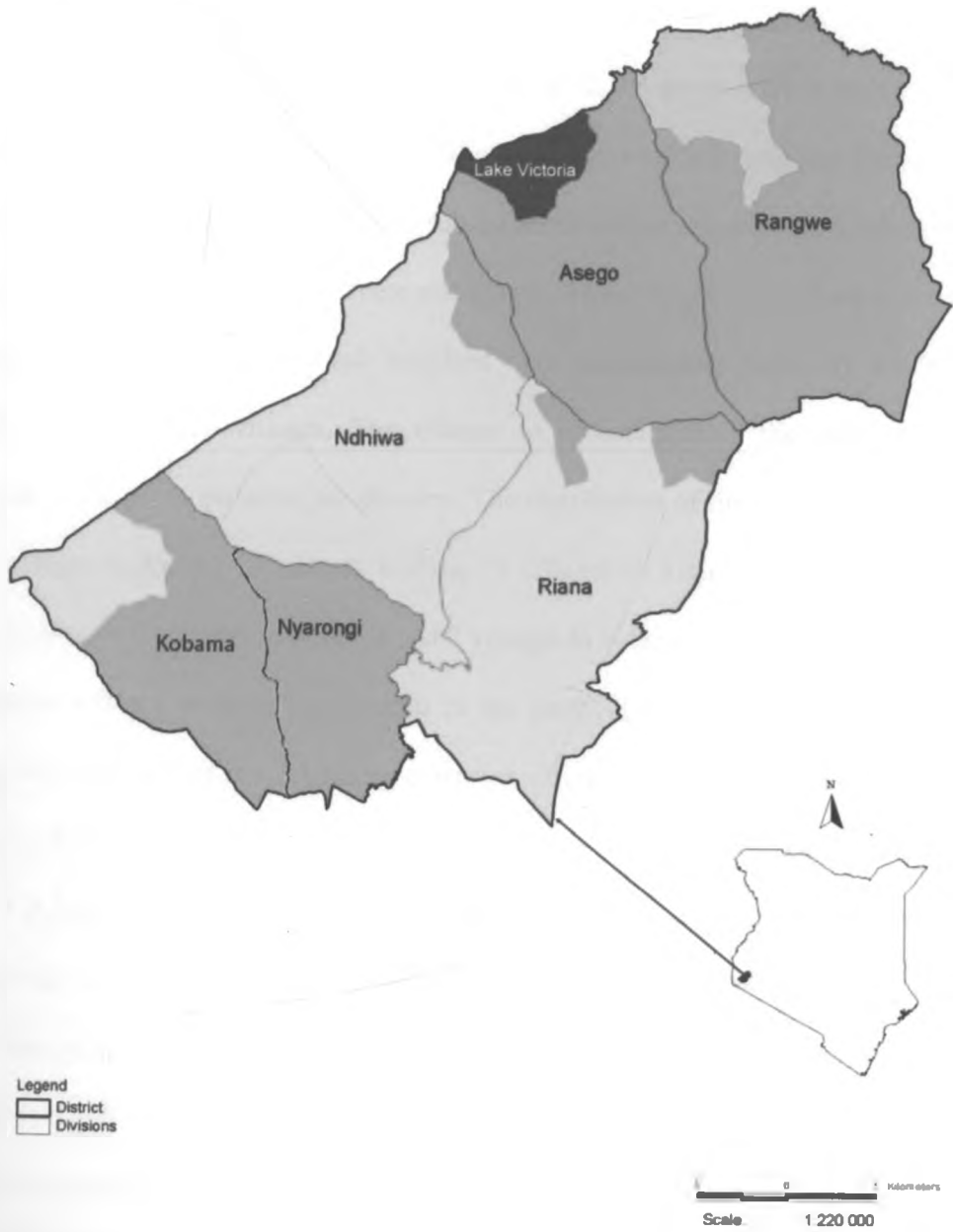
Figure 3.1: Map of Kenya showing the location of Homa Bay District and its administrative divisions.

3.2   Sample size determination

The sampling strategy was random multistage whereby all the seven were selected to increase the geographic spread. Then two locations per division were randomly selected thus resulting in fourteen locations. One third of the sixty six sub-locations within the selected fourteen locations were randomly selected at the second stage resulting in 22 sub-locations. A further one third of the 126 villages within the selected sub locations were subsequently randomly selected at the third stage resulting in 42 villages. The villages to be included in the study per division proportionate to the pig population per division. The distribution of the villages selected was as follows; 1 village in Asego, 12 villages in Pala, 15 villages in Riana, 2 villages in Rangwe, 3 villages in Nyarongi, 2 villages in Kobama and 7 villages in Ndhiwa. All pig-keeping households in the selected villages were to be included in the study. The pig-keeping households were established with the help of the veterinary extension officers and the local administration. The sample size of households and pigs that were to participate in the study was computed using the formula $n = Z_\alpha^2 pq/L^2$ (Martin et *al.*, 1987). Where, n is the required sample size, $Z_\alpha=1.96$ is the standard normal deviate at 5% level of significance, p is the estimated prevalence, q=1-p, and L is the precision of the estimate. Setting p = 0.1 (Githigia et *al.*, 2002) and L at 5%, the required sample size was 138 households. To control for clustering within households, more than double the calculated sample size was included and thus 299 households were selected (Martin et *al.*, 1987). In households with more than two pigs, a proportion of the pigs were randomly selected for sampling. For instance where there were three pigs two were sampled and where there were five, three were sampled.

3.3 Sample collection

The following procedure of collecting samples was carried out upon acquisition of consent from the pig owner.

3.3.1 Lingual examination and blood collection from pigs

Each pig was held using a hog restrainer and maintained in standing position during handling. A strong stick was used to keep its mouth open, by passing it across the mouth as shown in Plate 3.1. The tongues of the pigs were then palpated for presence of *T. solium* cysticerci.

Plate 3.1: Gagging of a pig before lingual palpation for *C. cellulosae* cysts.

The method used in the study was as follows: the tongue was grasped gently but firmly with a gauze swab and extended from the mouth cavity using one hand and the other used to palpate for the cysts as shown in Plate 3.2.

Plate 3.2: Palpation of a tongue to check for *C. cellulosae* cysts

Blood for serology from the same pigs palpated for cysts was then collected from the anterior venacava and drawn into vacutainer tubes with clot activator. The blood samples were labeled and left to stand at room temperature and the serum separated and stored in labeled cryogenic vials at -20^0C until use. Sex and age of pig, blood collection and lingual palpation data were recorded.

3.3.2 The antigen-ELISA test

This procedure was carried out at the International Livestock Research Institute (ILRI), Nairobi. The HP10 antigen was detected by Ag-ELISA as described previously by Harrison *et al.* (1989). The reagents and buffers for the test are shown in Appendix 1. The procedure was as follows: A 10µg/ml solution of McAb-HP10 in coating buffer was prepared fresh each day and 100µl added to each of the wells of a flat bottomed Immunlon 1 ELISA plate and incubated the plate overnight at 4°C. The wells were washed out twice with washing solution. Two hundred micro

liters of PBS/BSA/Tween was added to each of the wells and incubated at room temperature for 1 hour to block any non reacted sites on the plate. The plates were then washed three times with washing solution. 100μl of undiluted sera was then added to each well, with each sample running in duplicate. The plate was incubated for 1 hour at 37°C, followed by emptying and washing three times. Biotinylated-McAb diluted 1:2,500 in PBS/BSA/Tween was added at 100μl/well, covered and incubated for 1 hour at 37°C followed again by washing three times. Streptavidin Peroxidase conjugate diluted 1:10,000 (i.e. 0.1μg/ml) in PBS/BSA/Tween added at 100μl per well, covered and incubated for 1 hour at 37°C. After a further three washes 100μl TMB substrate was then added to each well and incubated at room temperature for 15-30 minutes. The reaction was then stopped with 100μl of 0.2M H_2SO_4 per well and read at 450nm on an ELISA plate reader. A sample was considered positive if the mean OD value of the duplicates was higher than the cut-off value, which was calculated based on the mean of the OD plus 3 Standard Deviations of the 5 samples from non-exposed controls. In this case, controls were piglets from a clean, indoor pig unit in Kitengela in Kajiado District, Kenya.

3.3.3   Questionnaire survey

The questionnaire was pretested on twelve pig farmers of Homa Bay who were then to be excluded from the actual study. The structured questionnaire (Appendix 2) with both closed and open-ended questions was administered to a member of the selected household who was familiar with the day to day raising of the pigs owned by the household. The questionnaire survey obtained data on pig production and husbandry and risk factors for occurrence of porcine cysticercosis and *T. solium* cysticercosis and taeniosis. The questionnaire was administered

through personal interviews with the respondents. Where the person being interviewed could not understand English or Swahili, the local *dholuo* language was used with the assistance of an interpreter. Risk factors considered in this study included, housing of pigs, no latrine use, history of shedding tapeworm segments in stool by a household member, history of epilepsy in a household member and pork inspection. These were similar to those reported by Murrell (2005), Pawlowski *et al.* (2005), Kyvsgaard and Murrell. (2005), and Kagira *et al.* (2010). Additional information collected included: the person's identity, age, education status, farming activities, division and village, source of drinking water, reason for keeping pigs, pig management practices, presence or absence of latrines, consumption of pork, home slaughter of pigs, preferred method of preparing pork, length of time the household had kept pigs, history of cysticercosis and taeniosis, knowledge of *T. solium* transmission and the history and presence of epilepsy in the household and neighborhood.

3.4 Data handling and analysis

The unit of statistical analysis was the individual pig. The questionnaire data were merged with the serology data in a MS Excel® (Microsoft Corporation) spreadsheet and exported to SPSS (PASW Statistics 18®) software for statistical analysis. The overall and household seroprevalence were computed as the number of households with at least one pig testing positive for cysticercosis in the Ag-ELISA divided by the total number of households sampled. The level of agreement between the lingual palpation test and the Ag-ELISA test for the detection of *C. celulosae* was assessed using the Kappa statistic (Byrt *et al.*, 1993). The bivariate analysis where each factor was assessed for association with a positive Ag-ELISA result using χ^2 (5% level of significance) and the strength of association using the Relative Risk. The responses to the

questionnaire were modeled in a logistic regression model using the serology test results of the pigs as the outcome. For the purpose of modeling these data, explanatory variables (lack of pork inspection, lack of evidence of latrine use, history of tapeworm segments shed by a household member and history of a household member with epilepsy and lack of pig housing) were first explored for any associations with the serology results using χ^2 test and a liberal p-value of 0.15 was used to determine significance (Dohoo et $al.$, 2003). The strength of the associations was determined using the odds ratio (OR). Correlations between the explanatory variables were assessed to identify highly correlated variables, if highly correlated, then one variable was dropped ($p>0.5$). This was then followed by a backwards elimination logistic regression proceeding from the variables with the highest p-values to arrive at the most parsimonious model. A threshold p-value of 0.1 was used in order to include only those variables that were strongly significant. The likelihood ratio test statistic was used to test the goodness of fit of the final model. Confounding and interactions were assessed following the procedures outlined in Dohoo et $al.$ (2003) in the final model. The population attributable fraction (reduction in population cysticercosis that would occur if exposure to a risk factor were reduced) for those factors found to be significantly associated with a positive Ag-ELISA result was computed according to Miettinen. (1974) as: $((RR-1)/RR)$ × the proportion of cases in the exposed population; RR was the risk in the exposed population.

4. RESULTS

4.1 Characteristics of households

A total of 299 respondents from 299 households were interviewed of which 35.4% (106/299) were male and 64.6% (193/299) female (Figure 4.1).
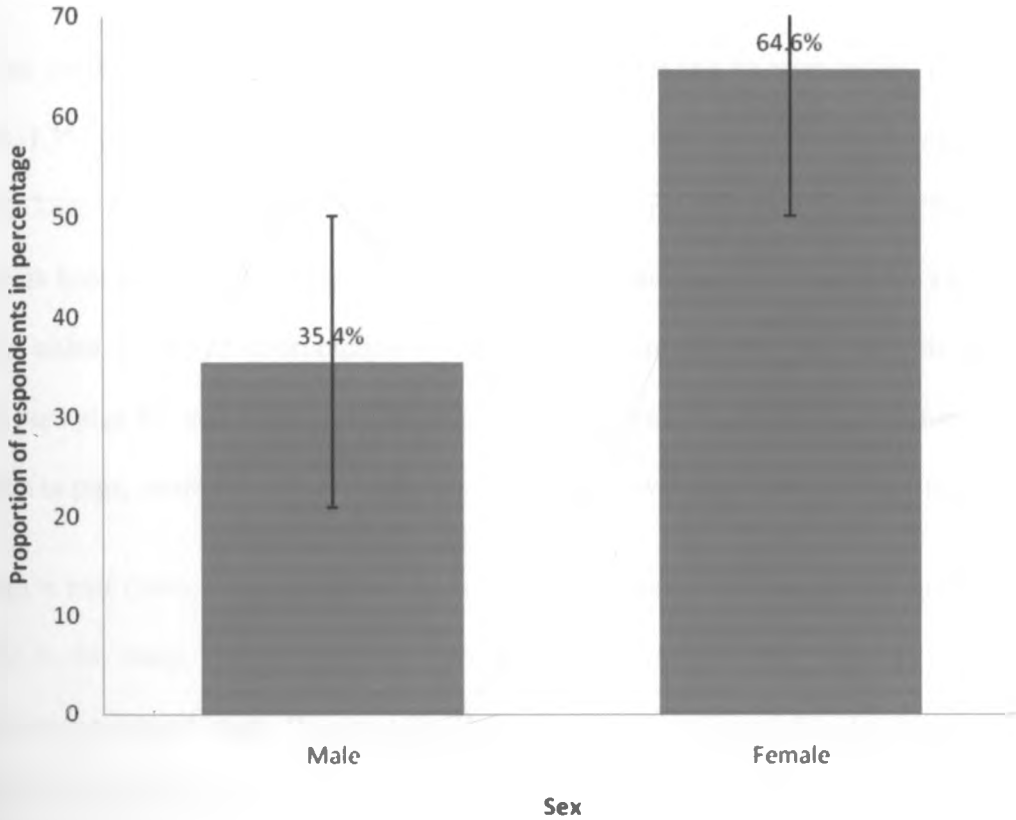
Figure 4.1: Distribution of respondents to the questionnaire survey by gender in Homa Bay District, 2010.

The mean age of the respondents was 40.7 years (range 17.5 to 88 years with only one aged 12). Twenty four percent of the respondents had no formal education, 65% had attained education to primary level and 11% had received at least high school level education. Each farmer reared only few pigs ranging from one to five in number per household. The mode was two pigs per

homestead. Majority (93.3%; 279/299) of the respondents depended entirely on farming for their livelihood while a few (3%; 9/299) engaged in businesses and 7% (21/299) were in formal employment.

4.2 Reasons for keeping pigs and duration

Eighty one percent (242/299) of the respondents kept pigs for sale to raise money for domestic expenses, 1.3% (4/299) kept them in order to produce piglets for sale and a very small proportion (0.006%) kept pigs for home slaughter and consumption. Six percent (18/299) of the respondents kept pigs both for home consumption and at times for sale while 1.3% (4/299) kept their pigs either for home consumption or for sale of mature pigs or their piglets while 8% (24/299) kept pigs for sale of either the piglets to other pig keepers or mature pigs for slaughter. In addition to pigs, cattle, poultry, sheep, goats and donkeys were also reared by the farmers.

More than a half (56%; 167/299) of the respondents reportedly begun rearing pigs less than a year prior to the study. Only 20% (60/299) of the respondents had kept pigs for more than 5 years before the current study (Figure 4.2). The respondents attributed this development to the increase in demand for pigs from markets such a Ndumbuini and Kisumu.
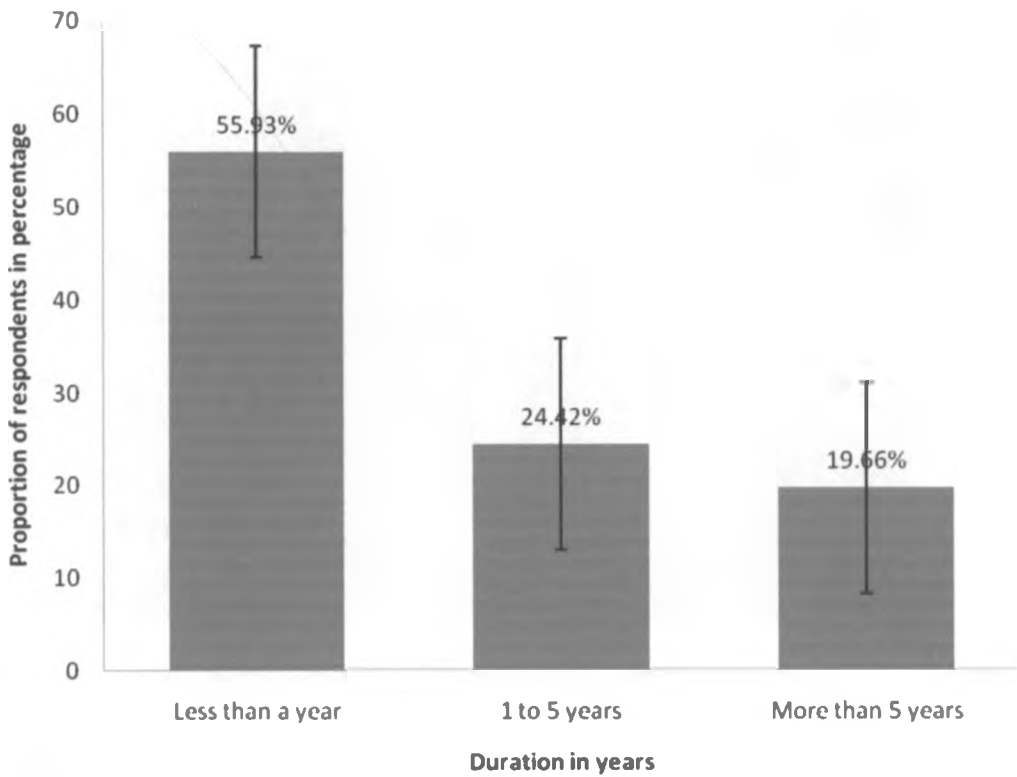
Figure 4.2: Duration of pig rearing by 299 residents of Homa Bay District, 2010.

4.3 Pig husbandry practices

4.3.1 Pig breeds

Most (98.31%; 294/299) of the farmers kept non-descript pigs (Plate 4.1), while 1.67% (5/299) kept exotic breed of pigs. These were predominantly females (59%; 231/392) and about 75% (294/392) were less than 12 months old.

Plate 4.1: Free ranging piglets in Homa Bay District, 2010.

4.3.2   Pig housing

The pigs in the district were mainly not housed. A good case is depicted in Plate 4.2 in which a

pig pen constructed using affordable locally available materials was not in use and the pigs were

tethered outside the pen. The preferred method of confining pigs was by tethering, practiced by

98% (293/299) of the farmers during the crop planting season, 98.3% (294/299) during the

growing season and 98.3% (294/299) during harvesting and 86.5% (259/299) in the fallow

season. The pigs were let to graze around the homesteads. Tethering was intended to stop crop

destruction that could lead to neighborhood conflicts. Few pig farmers (1.6%; 5/299) practiced

total confinement of their pigs. The pigs were left to scavenge after harvesting of the crops. The

tether ropes were observed to be very weak and would easily be broken and allow the pigs to

roam. The ropes also inflicted wounds that were often septic on the pigs' legs and necks. The

adult pigs were tethered to small shrubs or pegs sunk into the ground but the piglets were commonly let free (Plate 4.3).

Plate 4.2: A model pig house constructed by a researcher in Homa Bay District 2010. Notably the pigs in the homestead are tethered outside this pen.

Plate 4.3: A tethered sow and her piglets grazing.

4.3.3 Pig feeding

Most of the respondents (40.4%; 121/299) fed their pigs on natural pasture together with kitchen left-overs. None of the respondents used commercial feeds. Other feed types included guavas, brewers mash, paw paw, sweet potato vines and tubers, cassava roots, and corn flour (Table 4.1). Plate 4.4 shows a pig feeding on *ugali* (cooked maize flour meal) and waste water. The waste water had been used for washing cooking and eating utensils.

Table 4.1: Types of pig feeds used in Homa Bay District, 2010.

Feed type	Number of households	Proportion (%)
Kitchen leftovers	6	2
Kitchen leftovers and pasture	121	40.4
Kitchen leftovers, pasture and sweet potato tubers	98	33
Kitchen leftovers, pasture and sweet potato vines	8	2.1
Kitchen leftovers, pasture and guavas	20	7
Kitchen leftovers, pasture, sweet potato tubers and vines	10	3.1
Kitchen leftovers, pasture and brewers mash	4	1.4
Kitchen leftovers, pasture and paw paw	2	0.7
Kitchen leftovers, pasture and corn flour	2	0.8
Kitchen leftovers, sweet potato tubers and flour	3	1
Pasture, sweet potato tubers, guavas, and cassava	4	1.4
Pasture and flour	2	0.7
Kitchen leftovers, pasture, sweet potato vines and guavas	19	6.4
Commercial feeds	0	0
Total	**299**	**100**

Plate 4.4: Pig feeding on 'ugali' (cooked corn flour) in waste water in Homa Bay District.

4.3.4 Disease control

Only a few (2.3%; 7/299) of the surveyed pig farmers sought advice from veterinarians for disease problems affecting pigs. The vast majority (81.8%; 245/299) did not seek veterinary advice while 16% (47/299) consulted extension personnel. Plate 4.5 (A) shows a pig with severe mange due to *Sarcoptes scabei* and plate 4.5(B) shows a piglet with pediculosis due to *Hematopinus suis*.

A) Mange due to *Sarcoptes scabei*
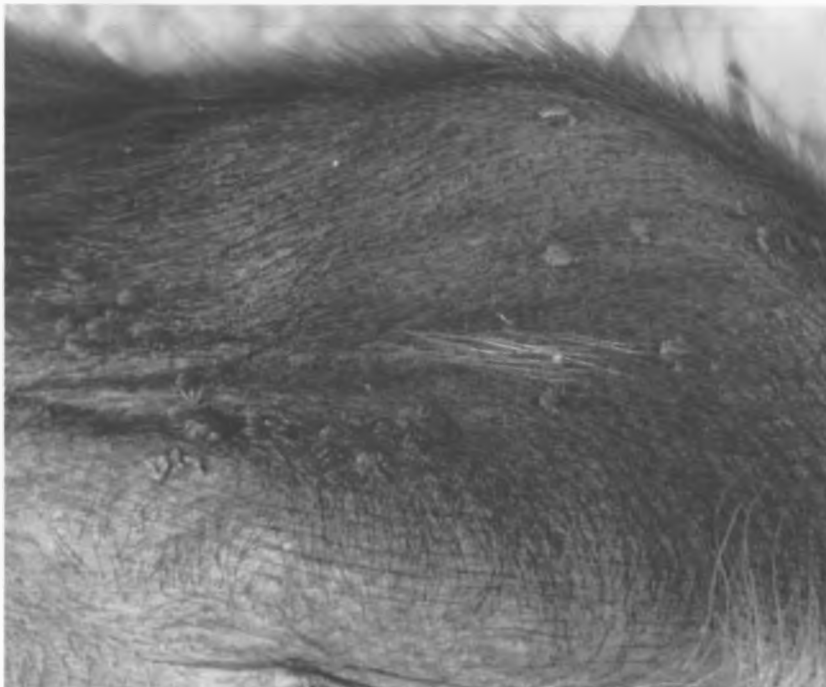
B) Pediculosis due to *Hematopinus suis*

Plate 4.5: Some forms of external parasitism diagnosed in surveyed pigs in Homa Bay District, 2010.

4.4   Water sources and treatment

Water for domestic use was from various sources including ground wells, rivers, bore-holes and rain water (Fig 4.3). About a half (49%; 147/299) of the households treated their water with chemicals (Chlorine), 41% (123/299) boiled the water and a further 10% (30/299) used the water without any treatment.
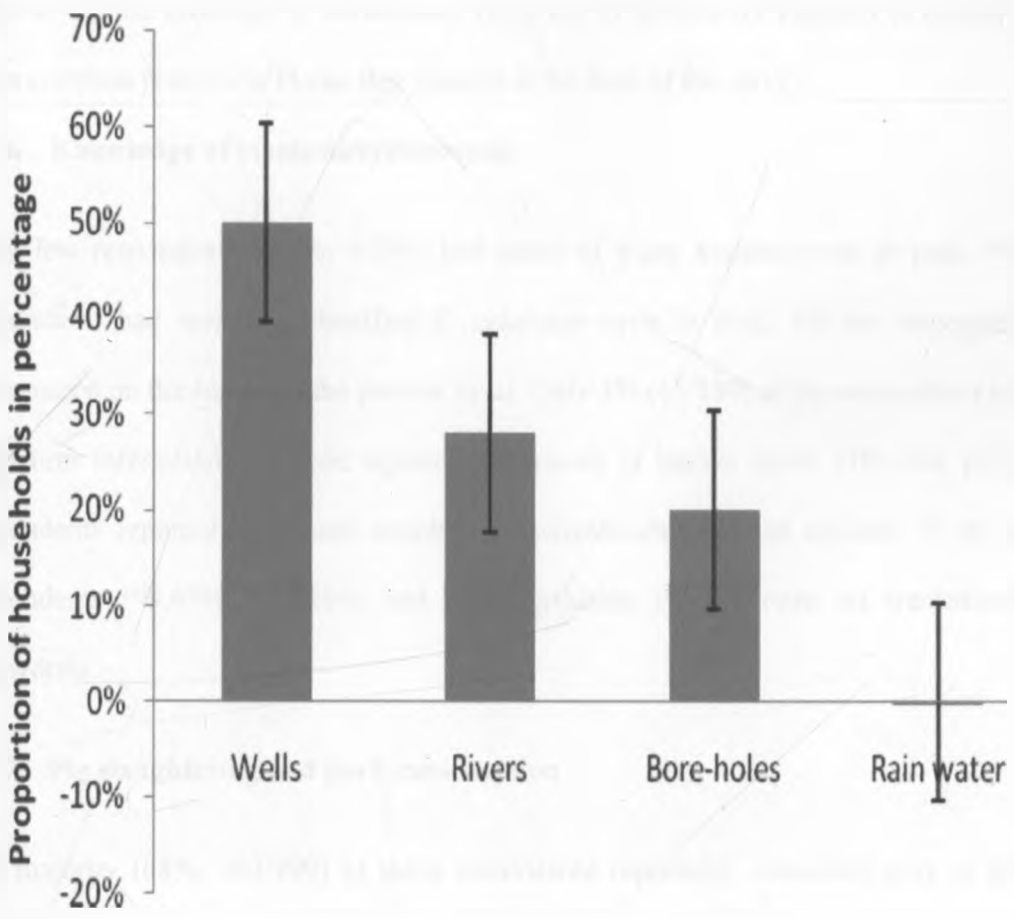
Figure 4.3: Water sources for 299 households in Homa Bay District, 2010.

4.5   Toilet facilities

The pit latrines ranged between 20 to 25 feet deep. Of the 299 households surveyed, only 48% (144/299) reported that they had latrines. However, direct observation during the survey revealed that only 77% (111/144) of the households that reported ownership of the latrines had evidence of being used while 62.8% (188/299) lacked evidence (beaten path, presence of a lockable door, signs of regular cleaning) of latrine use. Thus, use of latrines for disposal of human waste was not a common practice in Homa Bay District at the time of the survey.

4.6   Knowledge of taeniosis/cysticercosis

Very few respondents (1.4%; 4/299) had heard of white nodules/cysts in pork. None of the respondents had seen and identified *C. celulosae* cysts in pork. All the respondents had no information on the source of the porcine cysts. Only 5% (15/299) of the respondents had heard of tapeworm infestation and seen tapeworm segments in human stool. Fifty-five percent of the respondents reported household members or neighbours who had epilepsy in the past. Most respondents (99.67%; 298/299) had no information on the route of transmission of the tapeworms.

4.7   Pig slaughtering and pork consumption

The majority (68%; 203/299) of those interviewed reportedly consumed pork at least once a year. The preferred method of preparing pork was by frying (83%; 248/299), boiling (14%; 42/299) and roasting (3%; 9/299). Only a small proportion (4%; 12/299) said they slaughtered pigs at home. Of those that slaughtered pigs 67% (8/12) reportedly sought meat inspectors whenever they planned to slaughter pigs at home. Plate 4.6 shows an outdoor slaughter point at

which an average of two pigs was slaughtered every week for sale to the residents of Ombo and neighbouring villages. The levels of hygiene were noted to be very poor as shown by the blood on the ground. Untreated water for cleaning the carcass was sought from a nearby stream. Pork inspection was never carried out.

Plate 4.6: Slaughter point for pigs in the bush in Riana Division of Homa Bay District, 2010.

4.8 Transport of pigs to destinations outside Homa Bay District.

Plate 4.7 shows pigs being loaded onto a truck in preparation for transportation to a slaughterhouse in Kiambu District on the outskirts of Nairobi. This shows that there was market for the Homa Bay pigs with the implication of spread of *T. solium* cysticercosis to other parts of the country. The common complaint by the respondents was the low prices that the buyers pay for the finished pigs.

Plate 4.7: Pigs being loaded onto a truck in Homa Bay District for transport to a slaughterhouse in Ndumbuini, in the outskirts of Nairobi, approximately 450 Km away.

4.9.1   Prevalence by lingual palpation

A total of 392 pigs from 299 households were examined for lingual cysts and their distribution across the seven divisions is as shown in Table 4.2. Of the pigs examined, 22 had palpable cysts converting to a prevalence of 5.6% (95% CI. 3.3%, 7.9%). Plate 4.8 shows the tongue of a pig with a visible and palpable cyst. A further pig (0.003%) had a scar on its tongue presumably from a healed cyst while 0.008% (3/392) pigs had calcified cyst-like tissue and were considered suspect.

Pigs with palpable lingual cysts were only detected in households in three divisions, namely, Riana (5.7%; 8/140), Pala (8%; 8/100) and Ndhiwa (16.2%; 6/37) (Table 4.2). Household prevalence by lingual palpation was 7.4% (95% CI. 4.4%, 10.3%). The household prevalence was not significantly different from the individual pig prevalence by lingual palpation.

Table 4.2: Distribution of households with pigs having palpable lingual *Cysticercus cellulosae* cysts by divisions in Homa Bay District, 2010.

Division	Number of households sampled (Estimated pig keeping households)	Number of households with pigs with palpable lingual cysts	Proportion of households with a pig with palpable lingual cysts (95% CI)
Kobama	3 (10)	0	0 (0)
Rangwe	5 (20)	0	0 (0)
Asego	4(10)	0	0 (0)
Nyarongi	10 (30)	0	0 (0)
Ndhiwa	37 (150)	6	16.2 (4.3,28.1)
Pala	100 (400)	8	8 (2.7,13.3)
Riana	140 (550)	8	5.7 (1.9,9.6)
TOTAL	**299**	**22**	**7.4 (4.4,10.32)**
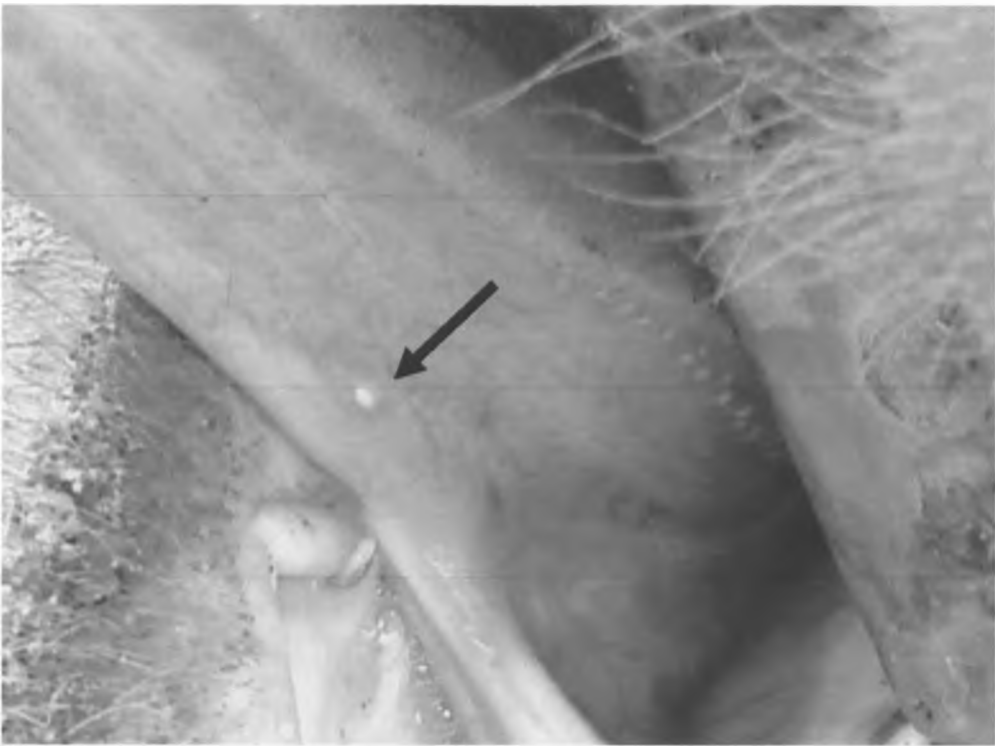
Plate 4.8: A palpable *Cysticercus cellulosae* lingual cyst (arrow) under the tongue of a free range pig in Homa Bay District.

4.9.2   Antigen ELISA prevalence

Only 233 from 179 households were tested and analysed according the procedure 3.3.2. Out of the 233 pigs tested, 101 had circulating *T. solium* antigens thus converting to an overall pig prevalence of 43.3% (95% CI. 37%, 49.7%). While the 84 households out of the 179 had at least one pig that tested positive for circulating *T. solium* antigens converting to a household prevalence of 46.9% (95% CI. 39.6%, 54.2%) (Table 4.3). The household prevalence was not significantly different from the individual pig prevalence for circulating *T. solium* antigens. The household prevalence proportions of *T. solium* antigens were not significantly different across the divisions (Table 4.3).

Ag-ELISA test by division in Homa Bay District, 2010.

Division	Number of households sampled	Number of households with pigs testing positive for *C. cellulosae* cysts on Ag-ELISA	Proportion (%) positive for *C. cellulosae* (95% CI)
Kobama	5	3	60 (17.1,1.029)
Rangwe	4	2	50.0 (1.0,99.0)
Asego	6	0	0 (0)
Nyarongi	10	6	60 (29.6,90.4)
Ndhiwa	19	13	68.4 (47.5,89.3)
Pala	52	34	57.7 (44.3,71.1)
Riana	83	26	31.3 (21.3,41.3)
TOTAL	**179**	**84**	**46.9 (39.6,54.2)**

.10   Agreement between lingual palpation and Ag-ELISA tests.

kappa statistic value of 0.02   was obtained using the kappa inter-rater agreement for two

ique raters procedure (Appendix 3), indicating poor agreement between serological detection

circulating T. *solium* antigens and the lingual palpation method.

4.11 Risk factor analysis for the association with cysticercosis

4.11.1 Bivariate analysis

Of the risk factors considered, the only one that was significantly ($p < 0.05$) associated with circulating antigens of *T. solium* was lack of latrine use ($p = 0.032$) (Table 4.4). The prevalence of circulating antigens in pigs from households with no evidence of latrine use (36.7%), was significantly (p<0.05) more than the prevalence in households with evidence of latrine use (23.4%). Pigs from households without evidence of latrine use were 1.6 times more likely to test positive for circulating *C. celulosae* antigens relative to pigs from households with evidence of latrine use (Table 4.4)

Table 4.4: Bivariate analysis of factors associated with a positive Ag-ELISA test result in Homa Bay District, Kenya, 2010.

Risk factor	Level	Ag-ELISA +	Ag-ELISA -	Prevalence (%)	Risk ratio (RR)	*p* - value
Absence of evidence of latrine use	Yes	29	50	36.7	1.6	0.032
	No	36	118	23.4		
Pork inspection	Yes	21	40	34.4	1.3	0.19
	No	44	128	25.6		
History of tapeworm shedding	Yes	61	148	29.2	1.8	0.2
	No	4	20	16.7		
History of epilepsy	Yes	18	42	30	0.9	0.98
	No	50	116	30.1		

4.11.1 Risk estimates.

The population attributable fraction (PAF) for absence of evidence of latrine use was 54.5% indicating that 54.5% of the Ag-ELISA positive results in the pig population of Homa Bay District was due to lack of latrine use.

4.12   Multivariate analysis

The full model was:

$Y_i = \beta_0 + \beta_1$ (lack of pig housing) $+ \beta_2$ (pork inspection) $+ \beta_3$ (lack of evidence of latrine use) $+ \beta_4$ (shedding tapeworm segments) $+ \beta_5$ (epilepsy in household) $+ \varepsilon_i$

Of the variables introduced in the logistic regression model, lack of evidence of latrine use remained the only significant variable associated with seropositivity for porcine cysticercosis in the study (Table 4.5). The final model was:

$Y_i = \beta_0 + \beta_3$ (lack of latrine use) with an odds ratio (OR) of 1.9 (95% CI; 1.05,3.43) indicating that pigs from households with no evidence of latrine use were almost twice as likely to give a positive Ag-ELISA test results relative to pigs from households with evidence of latrine use.

Table 4.5: Description and contingency test results for explanatory variables used in logistic regression analysis.

Category	Variable	β coefficients	Chi-square	p-value
Household characteristics	Absence of evidence of latrine use	0.626	4.59	<0.05
	Pork inspection	0.419	1.74	>0.05
	Tapeworm shedding	0.479	1.67	>0.05
	History of epilepsy	- 0.684	0.89	>0.05

5. DISCUSSION

This study determined the prevalence of porcine cysticercosis in pigs in Homa Bay District using both lingual palpation and Ag-ELISA tests. Porcine cysticercosis was prevalent in the pig population at 5.6% using the lingual palpation method. These results are in agreement with results reported for endemic areas worldwide of between 5% and 30% (Craig *et al.*, 1996). Prevalence proportions ranging from 4% to 12.9% were reported by Nsadha *et al.* (2010) in several regions of Lake Kyoga basin of Uganda. The prevalence was however lower than that found in previous studies in Western Province and parts of South Nyanza in Kenya that ranged between 10% and 14% (Githigia *et al.*, 2002).

The overall prevalence of 43.34% based on detection of circulating antigens using the Ag-ELISA indicated that porcine cysticercosis is highly prevalent in the pigs in Homa Bay District. Similar results were reported in Angonia District in Mozambique by Pondja *et al.* (2010) where prevalence of 12.7% by lingual palpation and 34.9% by Ag-ELISA were estimated. Elsewhere, reported Ag-ELISA prevalence proportions include, 40.6% in Eastern Cape Province of South Africa (Krecek *et al.,* 2008), 23.3% in the Eastern, Southern and Western provinces of Zambia (Sikasunge *et al.,* 2008), 29% in Yucatan Peninsula, Mexico (Widdowson *et al.* 2000) and 38.4% in Congo (Praet *et al.,* 2010).

The low lingual palpation prevalence compared to Ag-ELISA was most likely due to the lower sensitivity of the lingual palpation method. Lingual palpation does not pick infested pigs at the onset of the infection unlike Ag-ELISA that detects antigens even before cyst formation. The

Ag-ELISA has been reported to have a sensitivity ranging between 76.3% and 86.7% and a specificity ranging between 84.1% and 98.9% in pigs in South Africa, Zambia and West Cameroon (Krecek *et al.,* 2008; Dorny *et al.,* 2004; Poudet *et al.,* 2002). The prevalence values obtained in this study indicate that pigs in Homa Bay District are exposed to *T. solium* eggs. The prevalence could possibly be even higher since the HP10 Ag-ELISA test used can detect heavily infected pigs 29 days post infection while lightly infected ones can be detected from day sixty one to ninety seven post infection (Scuitto *et al.,* 1998). There is a high probability therefore that in this study, lightly infected pigs were not detected if they were sampled within two months post infection.

The majority of the farmers interviewed in this study were females. This was similar to findings in northern Tanzania whereby 61% of respondents were women (Ngowi *et al.,* 2009). This possibly indicates that either it is women who are directly responsible for pig rearing or they are commonly left in the homesteads to tend to household chores and farming. This has an implication on the target group for training on cysticercosis control methods in a bid to control infections.

Socio-economic status of the residents of Homa-Bay District was generally characterized by: unemployment or employment with very low wages, limited access to safe and clean water, toilet facilities and proper refuse disposal and free-range pig farming. The pigs were mainly left loose to scavenge and were tethered when the farms had been cultivated and crops grown. Most of the farmers were small scale with majority owning two pigs per homestead which is similar to western Kenya (Githigia *et al.*, 2005 and Mutua *et al.,* 2007). Majority of the farmers' reason for keeping pigs was for selling to supplement income for the households.

Most of the respondents had received some formal education. This has the implication on the ease of educating the farmers on *T. solium* control strategies. Majority of the farmers kept small bodied, cross bred pigs with multicoloured coats while few farmers kept exotic breeds. Institutions that kept pigs in the study area on the contrary realised fast maturity for the hybrid pigs but complained about market access. Despite the challenges faced by the pig farmers, more and more are slowly embracing pig farming in the district. This could be due to the increasing demand for pork in the country. The increased pig farming in Homa Bay appears to be determined by the demand for pigs by Ndumbuini slaughter house which is located in Kiambu District on the outskirts of Nairobi. The demand for pigs in Ndumbuini was high and the farms close to the slaughter house can not meet the demand and pigs are therefore sourced from far away places such as Eldoret, Kakamega, Nakuru and Homa Bay.

Cysticercosis was detected by Ag-ELISA in all the divisions of Homa Bay District except Asego. This is possibly due to the fact that Asego is an urban division and has toilet facilities and potable water supply. However, this is discounted by observation during data collection that human stool was accessible to pigs. The likely reason for this low prevalence is that the human beings do not harbour the tapeworm possibly because they easily access medical care and potable water.

From direct observation, about three quarters of the households had no latrines. Previous studies in Tanzania have reported an association between not having a farm latrine and occurrence of porcine cysticercosis (Ngowi *et al.*, 2004). In this study, absence of a toilet or latrine supported by lack of evidence of use in homesteads with latrines was the only significant risk factor. Surveys conducted in Tanzania and Zambia showed that the prevalence of porcine cysticercosis

was considerably higher in pigs reared in households lacking latrines than in those reared in households that had latrines in use. Kagira *et al.* (2010) also found that lack of latrines at household level was the only significant risk factor associated with the occurrence of cysticercosis in Busia District of Kenya. This study suggests that either the households visited were not using the latrines, or pigs had access to the latrines since most of them were open and easily accessible to roaming pigs to eat human faeces with tapeworm eggs. Based on the population attributable fraction, the prevalence of cysticercosis amongst the pigs can be reduced by more than half through proper use of latrines. This finding is similar to that reported in the Gambia and Senegal by Sercka *et al.* (2010) who reported that porcine cysticercosis incidence might be suppressed by the generalised use of toilets and latrines.

Free-range husbandry system was not a significant risk factor for porcine cysticercosis in this study. These results showed that pigs were almost entirely kept on free-range and semi-intensive husbandry systems which permitted them to have access to human faeces with tapeworm eggs. Similar findings have been reported in Zambia (Sikasunge *et al.* 2007) and Mbulu District, Tanzania (Ngowi *et al.,* 2004). Even though practiced, free range system is however, against the Kenyan laws (GOK, 1972).

History of an individual in the household shedding tape worm segments in the stool was not a significant risk factor for porcine cysticercosis in this study. The segments shed in stool should however be distinguished from those of *T. saginata.* Egg morphology (shape, maximal diameter) provide an appropriate differentiation between *T. solium* and *T. saginata* eggs compared to using Ziel Nielsen staining (Jimenez *et al.,* 2010). Shedding tape worm segments coupled with failure

to use latrines for defecation and failure to house the pigs facilitated the completion of the life cycle of *T. solium* in this study area.

A very high proportion of the farmers were not housing their pigs; they were either tethered or left to roam about exposing them to possible ingestion of human faeces with tapeworm eggs. Most respondents fed their pigs on pasture and kitchen left-overs. None of the respondents supplemented their pigs with or solely fed their pigs on commercial feeds. Considering pigs are affected by levels of energy intake and adverse climatic factors such as hyperthermia, hypothermia and wind (Shrestha *et al.*, 2002), lack of housing for the pigs is presumably one of the factors reducing the profitability of pig ventures in the district. The low levels of nutrition provided by the poor quality feeds partly explains the slow growth rate and low mature weight also reported by Mutua *et al.*, (2010). Most farmers in Homa Bay believed that pigs can feed on anything, including waste water.

Potable water was not accessible to majority of the respondents. None of the respondents had piped, treated water. A significant proportion of the respondents sourced drinking water from rivers or uncovered shallow wells with a possibility of contamination with human stool with *T. solium* eggs. Boiling of drinking water which fully destroys *T. solium* eggs was carried out by less than half of the respondents interviewed. This implies that more than half of the households were at risk of drinking water with *T. solium* eggs and subsequently, this may lead to neurocysticercosis manifested by epilepsy.

Almost three quarters of the households reportedly consumed pork at least once a year in their households. This indicates availability of ready local market for the pigs reared in the District. Pork is reported to be the most popular meat (FAO, 2001) with pork and pork products

accounting for 44% of meat consumed worldwide. This, however, has a setback locally, where respondents did not eat pork citing religious reasons. Most respondents who consumed pork preferred frying it to other cooking methods. This was attributed to the taste of fried pork that is not produced by boiling or barbequing. Frying however, does not guarantee the destruction of the *C. celulosae* cysts in the pork.

A few respondents carried out home or local slaughter of pigs. More than half of the respondents that carried out home slaughter had no official meat inspection. This implied that more than half of the households in Homa Bay are at risk of acquiring taeniosis by eating uninspected pork with *Cysticercus celulosae*.

Pig diseases such as colibacilosis, pneumonia, wounds, fractures, agalactia, as well as infestations including mange, helminthosis and pediculosis (DVO, personal communication, 2010) coupled with poor accessibility to veterinary services in the District was a finding similar to that reported by Mutua *et al.* (2010) in Kakamega District. Kagira *et al.* (2010) reported that diseases are a major constraint to free range pig production in western, Kenya. The entire Homa Bay District had two qualified veterinarians in Government service and one in private practice. The divisions were manned by either holders of diploma or certificate in animal health (DVO, personal communication, 2010). The common problem faced by these staff was low knowledge of pig husbandry and management techniques and lack of means of transport to get to the farms. This was attributed to the low levels of funding to the Ministry of Livestock Development as reported by Oruko *et al.* (2003).

Risk factors for *T. solium* cysticercosis/taeniosis such as absence of latrines/failure to use latrines in some households, presence of free roaming pigs, frying of pork and home slaughter with no

official inspection were found to be most prevalent in Riana Division and this may in part explain the high prevalence of porcine cysticercosis in the division compared to the other six divisions. Unlike in this study, Secka *et al.* (2010) found no association between absence of latrines/failure to use latrines and the prevalence of porcine cysticercosis in pigs in the Gambia and Senegal. This was due to the fact that almost all the households had either a latrine or a toilet that was regularly used.

Access to markets was not readily available to the pig farmers in Homa Bay. A huge proportion of the respondents kept their pigs for sale at maturity so as to supplement family income. The most ready market was the Ndumbuini slaughterhouse which is more than 450 kilometres away from the farms in Homa Bay.

The transportation to and slaughter of pigs in Ndumbuini which is located in Kiambu District on the outskirts of Nairobi, posses the potential of spread of *T. solium* cysticercosis to the human population in the outskirts of Nairobi, who consume the pork from Homa Bay. Most of these consumers prefer fried or barbecued pork especially at refreshment points where pork was consumed in large quantities.

CHAPTER SIX

6. CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

Conclusions drawn from the findings are as follows:

a) Porcine cysticercosis is prevalent among the free range pigs of Homa Bay District with lingual palpation prevalence of 5.6% and an Ag-ELISA seroprevalence of 43.3.

b) The following risk factors were found to occur in Homa-Bay District in 2010:

1. Free range system of rearing pigs which exposes them to human faeces and *T. solium* eggs.

2. Very low usage of latrines and lack of other effective methods of disposal of human faecal matter.

3. Home slaughter and lack of pork inspection at most slaughter places that expose the humans to ingesting *Cysticercus cellulosae*.

Very low usage of latrines the only risk factor significantly associated with the presence of circulating antigens of *T. solium* in the pigs' sera.

6.2   RECOMMENDATIONS

a) Pig keepers should be educated on the importance of housing their pigs by construction of pig pens using locally available and affordable materials.

b) Improvement of sanitation through construction and strict use of pit latrines alongside provision of piped potable water to the households.

c) More slaughter slabs to be established and licensed to curb clandestine slaughtering of pigs.

d) The government should employ more veterinary staff and enhance communication to boost capacity for extension and meat inspection.

e) Education of farmers to create awareness of porcine cysticercosis to aid in lowering its prevalence.

f) Vaccination of the pigs against cysticercosis to be employed in the district as one of the control methods.

g) A study be conducted to determine the prevalence of taeniosis as well as an investigation on the etiology of the epilepsy reported amongst the residents of Homa Bay.

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8. APPENDICES

Appendix 1: Materials used for HP10 Ag-ELISA

HP10 Ag-ELISA materials

1. 2x0.5ml McAbHP10 5mg/ml
2. 2x250µl McAb HP10-Biotin conjugate at 2.5mg/ml
3. 30 ml Positive Control
4. 30 ml Negative Control
5. 500 µg Streptavidin Peroxidase conjugate Sigma S-5512

Other Sigma reagents employed:
Bovine serum albumin (A-4503)
Phosphate buffered saline tablets ph7.3
TME liquid substrate (T-8665)
Carbonate Buffer tablets (C3041)

Plastics
Immulon 1 flat bottomed 96 well ELISA plates Thermo Life Sciences Ltd Cat No 3355

Plastic vials for storage 1.5ml and 0.5 ml or smaller for the 20µl, plastic pipette tips, storage boxes, multichannel and single channel pipettes.

McAb-HP10 Ag-ELISA Reconstitution of reagents.

1. McAb-HP10 (5mg/ml) 0.5 ml lyophilized

Add 0.5ml pure distilled water to the vial and dissolve the lyophilized McAb. Prepare 20µl

aliquots and store frozen at -20°C. Use at 10µg/ml dissolve in coating buffer, 100µl per well on

Immunlon 1 flat bottomed plates. (i.e. one 20µl aliquot per 10ml of coating buffer).

2. McAb HP10 Biotin (2.5mg/ml) 250µl lyophilized

Add 250µl pure distilled water to the vial and dissolve the lyophilized McAb Biotin conjugate.

Prepare 20µl aliquots and store frozen at -20°C. Use at a dilution of 1:2,500 in PBS/BSA/Tween

ie 1µg/ml. Once defrosted store the aliquot at 4°C until it is finished. Avoid freezing and thawing the conjugate.

3. Control Serum (1 ml aliquots)

1ml aliquots are store frozen at -20°C until use. Use undiluted at 100µl per well according to the plan provided.

4. Streptavidin Peroxidase conjugate Sigma lyophilised 500µg supplied

Add 500µl pure distilled water and dissolve the lyophilised conjugate. Prepare 20µl aliquots and store at -20°C until use. Use at 1:10,000 ie 0.1µg/ml in PBS/BSA/Tween. Do not freeze thaw repeatedly. Once an aliquot is defrosted, store it at 4°C until it is finished.

Other Reagents for the Ag-ELISA

1. Washing Solution - 0.9% (w/v) NaCl-0.05%(w/v) Tween 20

NaCl   9.0g
Tween (20 Sigma)   0.5g
Make up to 1 litre with distilled water

Usually a x5 concentrated stock solution is prepared which can be diluted to the required volume as desired.

2. PBS/BSA/Tween (Blocking solution, diluent and for background estimateion wells)

Prepare Phosphate Buffered Saline pH7.3 (PBS) solution using the Sigma Tablets (1 Tablet to 100ml pure water)

To approximately 900ml of PBS add:

0.5g Tween 20 Sigma then place

10.0g Bovine Serum Albumin (Sigma A-4503)

on top of the solution. Leave the BSA to dissolve slowly into the PBS/Tween.

Then make the solution up to 1 litre with PBS.

Prepare 25ml aliquots in plastic universal bottles and store at -20°C

3. Coating Buffer

Carbonate/Bicarbonate Buffer capsules (Sigma C-3041)

Dissolve the contents of 1 capsule in 100ml of pure distilled water to give a 0.05M Carbonate/Bicarbonate Buffer pH 9.6. Use only freshly prepared buffer each day.

4. Tetramethylbenzidine Substrate Sigma T8665

Liquid substrate supplied by Sigma Ltd.

5. 0.2M H_2SO_4 Stop Solution

2ml concentrated H_2SO_4 (Aristar 11M to 100ml pure water).

Appendix 2: Cysticercosis questionnaire

CYSTICERCOSIS QUESTIONNAIRE

Last name: _____ First name: _____

Questionnaire number: _____

District: _____ Division_____

Location: _____ Sub location_____

Village _____ Hut (House) number: _____

How long have you lived in this village? _____ Yrs

1. How old are you? _____ years

   1.1. Sex   Male   ☐   Female   ☐

   1.2 What is the highest schooling grade you have completed?

       ☐   None   ☐   Primary School

       ☐   High School

   1.3 What is your occupation?

       ☐   Farmer   ☐   Employed   ☐   Unemployed   ☐   Other(Specify)_____

2. Have you ever owned pigs? (If they answer "yes", ask when they owned the pigs)

     ☐   Yes, in the past year   ☐   Yes 1 to 5 years ago

     ☐   Yes more than 5 years ago

   2.1. What kind of pigs were they?

     ☐   Foreign   ☐   Native

     ☐   Both foreign and Native   ☐   I don't know

   2.2. Of the pigs that you have, how many are for? (*Read each choice and record the number*)

     Home consumption_____   Trading_____
     Reproduction_____   Others (specify) _____

   2.3. Other animals owned by the household

     Cattle: ____ Poultry (specify):_____ Sheep: _____
     Goats: _____ Donkeys: _____ Others (specify):_____

3. Where do you get your drinking water?

    ☐ River   ☐ Bore-hole

    ☐ Well   ☐ Other (please specify) _____

    3.1. Do you boil your drinking water?

        ☐ Always   ☐ Almost always

        ☐ Sometimes   ☐ Never

4. How often do you eat pork?

    ☐ At least once a month   ☐ Less than once a month but at least once a year

    ☐ Less than once a year   ☐ Never

    4.1 How is the pork that you eat prepared? (*Check all that apply*)

    ☐ Boiling   ☐ Barbeque

    ☐ Fried   ☐ Others (specify) _____

5. Do you have a latrine at home?

    ☐ Yes   ☐ No

    5.1. How often do you use a latrine when you have to defecate?

        ☐ Always   ☐ Sometimes

        ☐ Never

6. How do you keep your pigs

Season	In a pen	Free ranged	Tethering	Other (Specify)
Planting season				
Growing season				
Harvesting season				
Fallowing season				

    6.1 If the pigs are housed, how many pigs share the same pen? _____

7. What do your pigs eat?

    ☐ Pasture   ☐ Kitchen left overs

    ☐ Commercial feeds   ☐ Other (specify) _____

8. Do you slaughter pigs *at home*? If yes how often?

    ☐ At least once a month   ☐ Less than once a month but at least

    ☐ Less than once a year   ☐ Never

    8.1. If ever, how often was the meat inspected by a meat inspector?

        ☐ Always   ☐ Almost always

        ☐ Sometimes   ☐ Never

9. Have you ever seen or heard of white nodules (rice) in pig carcasses?

☐ Yes　　　　　　　　　　☐ No

9.1. Where can you find nodules on a live pig?

☐ It's not possible to find them on a live pig　　☐ Under the tongue
☐ Under the skin　　　　　　☐ Somewhere else (Specify) _____

9.2. How do pigs get these nodules?

☐ By eating human faeces　　☐ By eating pig faeces
☐ From an infected pig　　　☐ Other (Specify) _____

10. Have you ever heard of tapeworm infection in humans?

☐ Yes　　　　　　　　　　☐ No

10.1 Have you ever seen tapeworm segments in the stools of the household member?
☐ Yes　　　　　　　　　　☐ No

10.2 How does a person get tapeworm infection?
☐ They don't wash their hands　　☐ They eat undercooked pig meat
☐ They are in contact with an infected person　☐ Other (Specify)_____
☐ I don't know

11. Do you know of any person in the neighborhood who has had epilepsy?
☐ Yes　　　　　　　　　　☐ No

12. If yes, when_____ (Year)

The following two items be completed for ALL respondents after direct observation of latrine.
20. Presence and type of latrine **(to be asserted by direct observation)**

☐ Absent　　　　　　　　☐ Present and completely closed
☐ Present and partially closed　　☐ Present and open (easily accessible to roaming pigs)

21. Is there evidence of recent use of the latrine (by anyone) **(to be asserted by direct observation)**
☐ Yes　　　　　　　　　　☐ No

THIS IS THE END OF THE INTERVIEW. THANK YOU VERY MUCH FOR YOUR CO-OPERATION!

INTERVIEWER: _____ DATE OF INTERVIEW: _____

Appendix 3: Kappa statistic computation

	Lingual Palpation		
Ag-ELISA	+	-	**Total**
+	7	58	65
-	15	153	168
Total	22	211	233

Apparent prevalence = (65/233) = 0.28 = 28%

Apparent prevalence = (22/233) = 0.09 = 9%

Observed proportion agreement	160/233 = 0.687
Chance proportion agreement (Both positive)	(22/233) x (65/233)= 0.026
Chance proportion agreement (Both negative)	(168/233) x (211/233) = 0.653
Chance proportion agreement	0.026 + 0.653 = 0.6799
Observed minus chance agreement	0.687 – 0.6799 = 0.0071
Maximum possible agreement beyond chance level	1 – 0.6548 = 0.3452
Kappa	0.0071/0.3452 = 0.02

The Kappa statistic = 0.02