PREVALENCE OF PORCINE CYSTICERCOSIS IN FREE RANGE PIGS AND ASSOCIATED RISK FACTORS 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

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LIST OF ABBREVIATIONS

$^0$C - 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

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

WHO - World Health Organization

Mg  - Micrograms

μl  - Microlitres

χ²  - Chi square
ABSTRACT

The present study was conducted in the year 2010 in Homa Bay District to investigate the status of 
*T. solium* cysticercosis, and its potential risk factors. Prevalence of infection was determined by 
ante-mortem lingual palpation of pigs and serological assay through antigen ELISA, while the risk 
factors for *T. solium* cysticercosis and taeniosis were determined by administration of a standard 
questionnaire at household level to respondents by face to face interview.

A total of two hundred and ninety nine households and three hundred and two pigs were sampled 
in this study. One member of the household familiar with the day to day management of the pigs 
was interviewed on the risk factors.

Porcine cysticercosis was found to be prevalent amongst the free range pigs in the district 5.6% 
(22/392). The household prevalence by lingual palpation was 7.36% and for pig prevalence while 
the household sero-prevalence by Ag-ELISA was 46.9% (84/179) and individual pig prevalence 
43.34% (101/233).

Farming was the predominant occupation (79.3%; 237/299) and a high proportion of farmers 
(88%; 263/299), kept pigs for sale. The pigs were mainly tethered all year round, (98%; 293/299) 
during the planting season, 98.3% (294/299) growing season and 98.3% (294/299) the harvesting 
season. Almost half of the respondents (46.9%; 140/299) stated that they fed their pigs on kitchen 
left over and pasture while 25.7% (77/299) respondents fed the pigs on kitchen left over, sweet 
potatoes and pasture. None of the farmers supplemented their pigs with commercial feeds.

Those households practicing home slaughter of pigs without official meat inspection were 13.98% 
(27 out of 193 households that consumed pork). The number of homes without latrines was one
hundred out of one hundred and ninety nine (51.8%; 155/299). Pig farming in Homa Bay District is mainly free-range, with only 1.5% (5/299) households housing the pigs and most farmers keeping between one and six pigs. More than half of the respondents (61.2%; 183/299) stated that they had at least one household member shedding tapeworm segments in stool. None of the respondents had knowledge on the transmission of *T. solium* and had seen and recognized *Cysticercus cellulosae* cysts in pork.

It was concluded that porcine cysticercosis is prevalent in free range pigs in Homa Bay District. No latrine use was the only risk factor found to be significantly associated with the presence of *T. solium* cysticercosis ($\chi^2 = 15.94, p = 0.00008$, Odds ratio (OR) = 3.56).

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.

Community education on the cause, mode of transmission, economic and social impact and methods of control of porcine cysticercosis infections is recommended in Homa Bay District.
CHAPTER ONE

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 of 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 (Renato and Ruberti, 2002).

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.

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 General objective

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

LITERATURE REVIEW

2.1 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, 1987). *Taenia solium* is a two-host zoonotic cestode. The adult stage usually up to 10 metres in length lives in the small intestine 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 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 cysticerci 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 (White, 1997; Del Brutto, 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).

2.2 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 evaginates in the gut of man and attaches to the intestinal mucosa. 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).

Figure 2.1: Life cycle of Taenia solium (CDC, 2011).
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).

### 2.3 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 *et 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 part of South America, (Rajshekhar *et al.*, 2003) Figure 2.2 shows the worldwide distribution of cysticercosis.
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 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).
2.4 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, e.g., the Ag-ELISA (Tsang and Wilson, 1995; Zoli et al., 2003).

2.5 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 et al., 2004). However, gastrointestinal discomfort including diarrhea, flatulence and abdominal pains are sometimes observed. Porcine cysticercosis produces generally 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 taking 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).

2.6 Diagnosis of *T. solium* taeniosis/cysticercosis

2.6.1 Lingual palpation and post mortem incisions in pigs

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 with Joshi et
al., 2006 reporting a sensitivity of 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, which are smaller.

2.6.2 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 owing to its draw back of antibodies being able to persist long after body immune mechanisms or medical therapy have eliminated the active infection (Harrison et al., 1989). The Ab ELISA could also 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).

2.6.3 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). 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 CAT 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 hypodense 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 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 perilesion edema and contrast enhancement; these are better seen by MRI.

2.7 Treatment of *T. solium* taeniosis/cysticercosis

2.7.1 Treatment of man

Treatment of cysticercosis is tailored to the specific needs of the patient and may include medical therapy through anthelminthic drugs and 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 10 mg/kg bodyweight praziquantel or using 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 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.

2.7.2 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.

2.8 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);

- Extensive/free range pig rearing and outdoor human defecation where pigs can have access to human faeces;

- 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.

### 2.9 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 slabs and slaughterhouses;
• 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 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 are being 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. This is because Homa Bay is known for free range pig husbandry 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 Km² and is made up of seven administrative divisions namely; Asego, Pala (Not shown in the map since the map had been drawn before sub-division of Riana to Riana and Pala Divisions), Riana, Rangwe, Nyarongi, Kobama and Ndhiwa (Figure 3.1). 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°C and 34.8°C. The total human population of Homa Bay District was estimated at 963,794 people (KNBS, 2009) with most of the residents being subsistence smallholder farmers (Government of Kenya, 2002).
Figure 3.1: Map of Kenya showing the location of Homa Bay District and its administrative divisions.
3.2 Sampling and sample size determination

The sample size of households and pigs that were to participate in the study was computed using the formula \( n = \frac{Z_a^2 pq}{L^2} \) (Martin et al., 1987). Where, \( n \) is the required sample size, \( Z_a=1.96 \) 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 one pig 40% of the pigs were randomly selected. To increase the geographic spread, all the 7 Divisions were included in the study. The number of households per division included in the survey was proportional to the total number of pig keeping households in the Division, so that Divisions with more pig keeping households had a higher number of households sampled. This was commonly a third of the total number of households. The sampling strategy was multistage whereby two locations per Division were randomly selected. Then a third of all the listed sub locations within the selected divisions were randomly selected at the second stage. A third of the villages within the selected sub locations were subsequently selected at the third stage. All pig-keeping households in the selected villages were then included in the study. The pig-keeping households were established with the help of the local extension officers and the local administration.
3.3 Data collection

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 examined for presence of *T. solium* cysticerci.

Plate 0.1: 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 0.2: Palpation of a tongue to check for *C. cellulosae* cysts

Blood for serology from the same pigs palpated for cysts was collected from the peri-orbital fossa using 18 gauge needles 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°C 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) and Fleury *et al.*, (2003). The reagents and buffers for the test are shown in Appendix 2. Prepare a 10μg/ml solution of McAb-HP10 in coating buffer and add 100μl to each of the wells of a flat bottomed Immunlon 1 ELISA plate. (20μl aliquot of a 5mg/ml solution to 10ml is enough for 1
Cover the plate with cling film or Saran wrap to prevent evaporation and incubate the plate overnight at 4°C. Wash out the wells of the plate twice with washing solution. Add 200μl of PBS/BSA/Tween to each of the wells to block any non reacted sites on the plate. Leave the plate for 1 hour at room temperature to block. Wash the plate 3 times with washing solution. Add the serum (or test samples) at 100μl/well, usually is used undiluted, but PBS/BSA/Tween can be used as a diluent for other samples if required. Cover the plate with cling film and incubate for 1 hour at 37°C. Empty the plate and wash the wells three times using washing solution. Add the biotinylated-McAb diluted 1:2,500 in PBS/BSA/Tween at 100μl/well. Cover the plate in cling film and incubate for 1 hour at 37°C. Wash the plate and add the Streptavidin Peroxiase conjugate diluted 1:10,000 (ie 0.1μg/ml) in PBS/BSA/Tween at 100μl per well. Add the Streptavidin Peroxiase conjugate diluted 1:10,000 (0.1μg/ml) in PBS/BSA/Tween at 100μl per well. Cover the plate in cling film and incubate for 1 hour at 37°C. Wash the plate and add the Streptavidin Peroxiase conjugate diluted 1:10,000 (0.1μg/ml) in PBS/BSA/Tween at 100μl per well. Cover the plate in cling film and incubate for 1 hour at 37°C then add the Streptavidin Peroxiase conjugate diluted 1:10,000 (ie 0.1μg/ml) in PBS/BSA/Tween at 100μl per well. Add 100μl TMB substrate and incubate the plate a room temperature for 15-30 minutes, checking that the background control wells remain negative. Stop the reaction with 100μl of 0.2M H₂SO₄ per well. Read the OD at 450nm on an ELISA plate reader. A sample was considered positive if the mean OD value was higher than the cut off value, which was calculated based on the mean of the OD plus 2 SD (0.25) samples from non-exposed controls.
3.3.3 Questionnaire survey

A pretested structured questionnaire (Appendix 1) 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 written in English and administered by the investigator, 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; housing of pigs, no latrine use, history of shedding tapeworm segments in stool by a household member, history of epilepsy in a household member, pork inspection, were similar to those reported by Murrell, 2005; Pawlowski *et al.*, 2005; Kyvsgaard and Murrell, 2005 and those reported by Kagira *et al.* (2010). 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 questionnaire data were combined with pig serology data in a MS Excel (Microsoft Corporation) spreadsheet and then exported to SPSS (PASW Statistics 18) software for analysis. Summary statistics were generated using the same software. For the purpose of modeling these
data, explanatory variables were first explored for any associations with the serology result using \( \chi^2 \) test. A liberal \( p \)-value (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 (\( >0.5 \)) 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 are strongly significant. The likelihood ratio test statistic (G2) was used to test the goodness of fit of the final model; confounding interactions were assessed following the procedures outlined in Dohoo et al. (2003) in the final model. The strength of the associations was determined using the odds ratio (OR). The agreement between individual pigs’ lingual palpation and Ag ELISA results was done using the Kappa statistic as explained by Viera and Garrett (2005). Risk factors found to be significant were had their population attributable fraction calculated. The population-attributable fraction was calculated according to the formula \( \left( \frac{(RR - 1)}{RR} \right) \times \) the proportion of cases in the exposed population, where RR was the risk in the exposed population (Miettinen, 1974).
CHAPTER FOUR

4. RESULTS
4.1 Characteristics of households.

The number of household sampled per Division was proportionate to the pig population in the Division, as guided by pig populations recorded by the District Veterinary Office in Homa Bay District and information from the local administration. 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.
The mean age of the respondents was 40.74 years (range 12 to 88 years). 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. The farmers reared only few pigs ranging from one to five pigs per homestead. The mode was two pigs per homestead. Majority (79.3%; 237/299) of the interviewed respondents depended entirely on farming for their livelihood while a few (3%; 9/299) engaged in other businesses and 7% (21/299) were in formal employment. Fourteen percent (42/299) were unemployed.

4.2 Reasons for keeping pigs and duration

Eighty one percent (242/299) of the respondents kept pigs for sale to raise money for other expenses, 1.3% (4/299) respondents 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 mature pigs for slaughter or piglets. In addition to pigs, cattle, poultry, sheep, goats and donkeys were also reared by the farmers.
Figure 4.2: Duration of pig rearing by 299 residents of Homa Bay District, 2010.

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)

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 hybrid pigs. These were predominantly females (59%; 178/302) and about 75% (227/302) were less than 12 months old.
4.3.2 Pig housing

The pigs in the district were mainly not housed. A good case is depicted in Plate 4.2 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 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 on the grass in 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 pigs were
tethered to small shrubs or pegs sunk into the ground. Not all the pigs were tethered, with piglets commonly being let free. A good case is in Plates 4.2 and 4.3 in which a pig pen constructed using affordable locally available materials was not in use and the pigs were tethered outside the pen.

Plate 4.2: A model pig house constructed by a researcher in Homa Bay District.
4.3.3 Pig feeding

Most of the respondents (40.4%; 121/299) fed their pigs on natural pasture together with kitchen left over. None of the respondents fed their pigs on commercial feeds. Other feed types included guavas, brewers waste, paw paw, sweet potato vines and tubers, cassava, and corn flour (Table 4.1). Plate 4.4 shows a pig feeding on *ugali* (cooked corn flour meal) and waste water. The waste water had been used for washing cooking and eating utensils.
Table 4.1: Types of pig feeds in Homa Bay District, 2010.

<table>
<thead>
<tr>
<th>Feed type</th>
<th>Number of households</th>
<th>Proportion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kitchen leftovers</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Kitchen leftovers and pasture</td>
<td>121</td>
<td>40.4</td>
</tr>
<tr>
<td>Kitchen leftovers, pasture and sweet potato tubers</td>
<td>98</td>
<td>33</td>
</tr>
<tr>
<td>Kitchen leftovers, pasture and sweet potato vines</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Kitchen leftovers, pasture and guavas</td>
<td>20</td>
<td>7</td>
</tr>
<tr>
<td>Kitchen leftovers, pasture, sweet potato tubers and vines</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>Kitchen leftovers, pasture and brewers mash</td>
<td>4</td>
<td>1.3</td>
</tr>
<tr>
<td>Kitchen leftovers, pasture and paw paw</td>
<td>2</td>
<td>0.7</td>
</tr>
<tr>
<td>Kitchen leftovers, pasture and corn flour</td>
<td>2</td>
<td>0.7</td>
</tr>
<tr>
<td>Kitchen leftovers, sweet potato tubers and flour</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Pasture, sweet potato tubers, guavas, and cassava</td>
<td>4</td>
<td>1.3</td>
</tr>
<tr>
<td>Pasture and flour</td>
<td>2</td>
<td>0.7</td>
</tr>
<tr>
<td>Kitchen leftovers, pasture, sweet potato vines and guavas</td>
<td>19</td>
<td>6.4</td>
</tr>
<tr>
<td>Commercial feeds</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>299</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>
Plate 4.4: Pig feeding on ‘ugali’ (cooked corn flour) in used 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 severe mange due to *Sarcoptes scabei* and plate 4.5(B) shows a piglet with pediculosis due to *Hematopinus suis*. 
4.4 Water sources.

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, 41% (123/299) boiled the water and a further 10% (30/299) used the water without any treatment.
4.5 Toilet facilities

Of the 299 households surveyed, only 48% (144/299) reportedly had latrines. However by direct observation by the survey team, only 77% (111/144) of the households that reported ownership of the latrines had evidence of being used. Thus, use of latrines for defaecation was not a common practice in Homa Bay District at the time of 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. 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. Note the skinning (usually fed to dogs) of the carcass and the proximity of the slaughter point to a bush. There is chicken feeding on the blood.

Plate 4.6: Slaughter point in the bush in Riana Division of Homa Bay District, 2010.
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 450Km away.

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 is market for the Homa Bay pigs and has 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.
4.9 Prevalence of cysticercosis

4.9.1 Lingual palpation prevalence

A total of 392 pigs from two hundred and ninety nine households were examined for lingual cysts and the households were distributed across the seven divisions as shown in Table 4.2. The number of households sampled was proportionate to the pig population in the Division. Of the pigs examined, 22 had palpable cysts converting to a prevalence of 5.6% (3.3%, 7.9%; 95% C.I.). Plate 4.8 shows the tongue of a pig with a visible and palpable cyst. A further single 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 7.36% (4.4%, 10.32%; 95% C.I.). The household prevalence was not significantly different from the individual pig prevalence by lingual palpation.
Table 4.2: Distribution of households with pigs with palpable lingual Cysticercus cellulosae cysts by divisions in Homa Bay District, 2010.

<table>
<thead>
<tr>
<th>Division</th>
<th>Number of households sampled</th>
<th>Number of households with pigs with palpable lingual cysts</th>
<th>Proportion of households with a pig with palpable lingual cysts (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kobama</td>
<td>3</td>
<td>0</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Rangwe</td>
<td>5</td>
<td>0</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Asego</td>
<td>4</td>
<td>0</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Nyarongi</td>
<td>10</td>
<td>0</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Ndhiwa</td>
<td>37</td>
<td>6</td>
<td>16.2 (4.3, 28.1)</td>
</tr>
<tr>
<td>Pala</td>
<td>100</td>
<td>8</td>
<td>8 (2.7, 13.3)</td>
</tr>
<tr>
<td>Riana</td>
<td>140</td>
<td>8</td>
<td>5.7 (1.9, 9.6)</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>299</strong></td>
<td><strong>22</strong></td>
<td><strong>7.36 (4.4, 10.32)</strong></td>
</tr>
</tbody>
</table>

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

Serum samples that were adequate for antigen detection were only 233 from 179 households. Serum samples from 120 households were lost in the laboratory due to handling errors. Of the 179 households sampled, 84 had a pig which tested positive for *T. solium* antigens converting to a household prevalence of 46.9% (39.6%, 54.2%; 95% C.I.) (Table 4.3), while 101 pigs out of 233 tested positive for circulating *T. solium* antigens converting to an overall pig prevalence of 43.34% (36.98%, 49.7%; 95% C.I.). The household prevalence was not significantly different from the individual pig prevalence for circulating *T. solium* antigens. The household prevalence of *T. solium* antigens was not significantly different in Kobama, Nyarongi, Ndhiwa and Pala Divisions.

Table 4.3: Distribution of households with pigs testing positive for Cysticercus cellulosae on Ag-ELISA test by division in Homa Bay District, 2010.

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

4.10 Comparison for agreement between lingual palpation and Ag-ELISA prevalence in Homa Bay District.

A kappa value of 0.02 ($p = 0.333$) was obtained using the kappa inter-rater agreement for two unique raters procedure (Appendix 3), indicating poor agreement between serological detection of circulating T. solium antigens and the lingual palpation method.

4.11 Risk factors of taeniosis/cysticercosis

4.11.1 Bivariate analysis

Of the 4 risk factors considered the only one that was significantly ($p < 0.05$) associated with circulating antigens of *T. solium* was lack of latrine use ($\chi^2 = 15.94, p = 0.00008$). The detection of circulating antigens of *T. solium* was more than twice in households where there was lack evidence of latrine use relative to households where there was evidence of latrine use (Risk ratio = 2.05), (Table 4.4).
Table 4.4: Factors associated with a positive Ag-ELISA test result in Homa Bay District, 2010.

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Level</th>
<th>Ag-ELISA +</th>
<th>Prevalence (%)</th>
<th>Relative risk</th>
<th>$\chi^2$</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No latrine use</td>
<td>Yes</td>
<td>64</td>
<td>58.7</td>
<td>2.05</td>
<td>15.94</td>
<td>0.00008</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>20</td>
<td>28.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pork inspection</td>
<td>Yes</td>
<td>21</td>
<td>35</td>
<td>0.74</td>
<td>0.34</td>
<td>0.5583</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>63</td>
<td>47.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>History of tapeworm shedding</td>
<td>Yes</td>
<td>80</td>
<td>49.1</td>
<td>1.96</td>
<td>3.37</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>4</td>
<td>25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>History of epilepsy</td>
<td>Yes</td>
<td>18</td>
<td>43.9</td>
<td>0.92</td>
<td>0.194</td>
<td>0.66</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>66</td>
<td>47.8</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4.11.1 Risk estimates.

Results showed that porcine cysticercosis prevalence in Homa-Bay is contributed to by 39.2%, by lack of latrine use (Table 4.5).
Table 4.5: Risk-Based Estimates and 95% Confidence Intervals

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Population attributable fraction and 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>No latrine use</td>
<td>39.2% (20.24, 57.99)</td>
</tr>
</tbody>
</table>

4.12 Multivariate analysis

Logistic regression analysis was conducted to predict the risk factors contributing to the occurrence of *T. solium* circulating antigens in pigs. Five predictors were entered stepwise by forward method into the analysis: presence or lack of pig housing, lack of pork inspection, no latrine use, history of tapeworm segments shed by a household member and history of a household member with epilepsy.

Table 4.6: Description and contingency test results for explanatory variables used in logistic regression analysis.

<table>
<thead>
<tr>
<th>Category</th>
<th>Variable</th>
<th>Level</th>
<th>ELISA</th>
<th>Chi-square</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Household characteristics</td>
<td>No latrine use</td>
<td>Yes</td>
<td>64</td>
<td>45</td>
<td>15.94</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>20</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Pork inspection</td>
<td>Yes</td>
<td>21</td>
<td>39</td>
<td>0.34</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>63</td>
<td>56</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tapeworm shedding</td>
<td>Yes</td>
<td>80</td>
<td>83</td>
<td>3.37</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>4</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>History of epilepsy</td>
<td>Yes</td>
<td>18</td>
<td>23</td>
<td>0.35</td>
<td>0.66</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>66</td>
<td>72</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preparation of pork</td>
<td>Frying</td>
<td>Yes</td>
<td>48</td>
<td>50</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>36</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Roasting</td>
<td>Yes</td>
<td>1</td>
<td>4</td>
<td>1.49</td>
</tr>
</tbody>
</table>
The initial full model was:

\[ Y_i = \beta_0 + \beta_1 (\text{lack of pig housing}) + \beta_2 (\text{pork inspection}) + \beta_3 (\text{lack of latrine use}) + \beta_4 (\text{shedding tapeworm segments}) + \beta_5 (\text{epilepsy in household}) + \epsilon_i \]

Of the variables introduced in the logistic regression model lack of latrine use remained in the model after backward selection as a significant risk factor for pigs seropositive for porcine cysticercosis (Table 4.7). The final model was:

\[ Y_i = \beta_0 + \beta_3 (\text{lack of latrine use}) \]

\[ \log (p/1-p) = -1.187 + 0.642* \text{latrine use} \]

Table 4.7: The variable remaining in the final model with odds ratios, confidence interval of odds and the \( p \)-value for the term.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Level</th>
<th>Odds ratio (OR)</th>
<th>95% C.I.</th>
<th>( p )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knowledge of cysticercosis</td>
<td>No</td>
<td>83</td>
<td>91</td>
<td>0.008</td>
</tr>
<tr>
<td>Boiling</td>
<td>Yes 18</td>
<td>9</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Free range</td>
<td>75</td>
<td>86</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pig management practices</td>
<td>Coralled</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Housing</td>
<td>Free range</td>
<td>65</td>
<td>168</td>
<td></td>
</tr>
<tr>
<td>Feeding</td>
<td>Supplemented</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Not supplemented</td>
<td>65</td>
<td>168</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Source of infection to humans</td>
<td>Undercooked</td>
<td>9</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Location on the carcass</td>
<td>Subcutaneous</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Risk of people getting infected with these</td>
<td>Yes, no, not sure.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Knowledge of existence of larval stages of worms in pork/viscera: Yes, no, not sure.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The table shows the variable remaining in the final model with odds ratios, confidence interval of odds and the \( p \)-value for the term.
### Table

<table>
<thead>
<tr>
<th>No latrine use</th>
<th>Yes</th>
<th>3.56</th>
<th>1.88-6.74</th>
<th>0.00003</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Objective:* Estimation of the seroprevalence of porcine cysticercosis and the determination of the risk factors in households in Homa Bay Kenya (2010). Sample size: Households. N = 179, Likelihood = 58.41, d.f = 1, $P = 0.0003$. 

**CHAPTER FIVE**
DISCUSSION

This study determined the prevalence of porcine cysticercosis in pigs in Homa Bay District using both lingual palpation and Ag-ELISA. Porcine cysticercosis was prevalent in the pig population at 5.6% using the lingual palpation method which is in agreement with results reported for endemic areas worldwide of between 5% and 30% (Craig et al., 1996), for instance, prevalence ranging from 4% to 12.9% was reported by Nsadha et al. (2010) in several regions of Lake Kyoga basin of Uganda. The prevalence was 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 indicates 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 prevalences 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 due to the lower sensitivity of the lingual palpation method. Lingual palpation does not diagnose 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 and 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 that in this study, lightly infected pigs were not detected if they were sampled within two months post infection. In this study 37.3% of the households visited had at least one pig with circulating antigens for *T. solium*.

The majority of the farmers interviewed in this study were females. This is 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, meaning therefore, that in this study, those responsible for the day to day maintenance of the pigs were mostly women and 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 locals of Homa-Bay was generally characterized by: unemployment or employment with very low wages; limited access to health care; 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 hybrid pigs. 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 farmers were slowly embracing pig farming in the District. This could be due to the increasing demand for pork in the country. The demand for pigs in Homa Bay is commonly determined by the demand from 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 could not meet the demand. The pigs were thus being sourced mainly from 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 treated potable water.

From direct observation almost three quarters of the households had no latrines. Previous studies have reported an association between not having a farm latrine and occurrence of porcine cysticercosis (Ngowi *et al.*, 2004). In this study, absence of toilet or latrine supported by evidence of no use in homesteads with latrines, was similarly 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 to be 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. Based on the population attributable fraction, the prevalence of cysticercosis amongst the pigs can be reduced by 39.2% through proper use of latrines. This finding is similar to that reported in Gambia and Senegal by Sercka et al. (2010) who reported that porcine cysticercosis incidence might be suppressed by the use of toilets and latrines.

Free-range husbandry system was not a significant risk factor for porcine cysticercosis in this study. These results show that pigs were mostly kept on free-range and semi-intensive husbandry systems may have permitted them to have access to eating human faeces with tapeworm eggs. Similar findings have been reported by Sikasunge et al. (2007) and Ngowi et al. (2004). Free range system is however, against the Kenyan laws (G.O.K., 1972).

History of an individual in the household with epilepsy was found to be insignificant in this study. This is possibly supported by the fact that just a few respondents confirmed to keep their pigs for home consumption. This therefore indicates that epileptic cases are not necessarily directly from the pigs to the household members but resulting from other etiological agents.

History of an individual in the household shedding tape worm segments in the stool was found to be insignificant 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 complete 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 infection with tapeworm eggs. Most respondents fed their pigs on pasture and kitchen left over. 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 as reported by Mutua, (2010). Most farmers in Homa Bay believe 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 40.5% of the respondents interviewed. This implies that more than half of the households are at risk of being infected with *T. solium* by drinking water with *T. solium* eggs. This may lead to neurocysticercosis manifested by epilepsy.

Almost three quarters of the households consume pork at least once a year in their households. This indicates availability of ready local market for the pigs reared in the District. The local pork
consumption level is low unlike in western Kenya where Mutua et al., (2010) found that most pork is consumed in the villages of western Kakamega. 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 sizzling taste of fried pork that is not produced by boiling or barbequing. Frying however, does not guarantee the destruction of the \textit{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 implies that very few households in Homa Bay are at risk of being infected with \textit{T. solium} by eating uninspected pork with \textit{Cysticerus celulosae} cysts.

Disease prevalence 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). 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 explains 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 Gambia and Senegal.

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 is the Ndumbuini slaughterhouse which is more than four hundred and fifty kilometres away from the farms in Homa Bay. The respondents complained about the meagre returns from their pigs. A mature pig of approximately 36 months of age and weighing approximately 70 kg live weight fetched the farmers around Kshs. 3000 (USD 37.5) in Homa Bay. The pig traders then transported the pigs to Ndumbuini slaughterhouse, where such a pig would be sold for Kshs. 9000 (USD 112.5). This implied that the pig traders benefited most. 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 preferred fried or barbecued pork especially at refreshment points where pork is consumed in large quantities.
6. CONCLUSIONS

This study aimed at estimating the prevalence of porcine cysticercosis by lingual palpation and antigen detection through Ag-ELISA and also to determine the risk factors for its prevalence. 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% (3.3%, 7.9%; 95% C.I.) while Ag-ELISA prevalence of 43.34% (37%, 49.7%; 95% C.I.).

b) Home slaughter and lack of pork inspection, no latrine use and history of a member of the household with active infection and shedding tapeworm segments were found to occur in Homa Bay. Lack of latrine use was the only risk factor significantly associated to circulating antigens of *T. solium* in the pigs.
7. RECOMMENDATIONS

a) Education of farmers to create awareness of porcine cysticercosis to lower its prevalence.

b) Farmers to be encouraged to house their pigs by construction pig pens using locally available and affordable materials.

c) Improvement of sanitation through construction and use of latrines.

d) The farmers to be assisted in creating proper marketing channels for their pigs so as to increase their income margins.

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

f) A longitudinal study to be carried out to establish the true prevalence.

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

h) More slaughter slabs to be established and licensed to curb clandestine slaughtering of pigs.
8. REFERENCES


CDC (2011) Life cycle of *T. solium*. 

www.dpd.cdc.gov/dpdx/HTML/ImageLibrary/Cysticercosis_il.htm


Current Consensus Guidelines for Treatment of Neurocysticercosis


KNBS (2009) www.knbs.or.ke/Census%20Results/KNBS%20Brochure.pdf


9. APPENDICES

Appendix 1: 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
☐ Well
☐ Bore-hole
☐ Other (please specify) _______________

3.1. Do you boil your drinking water?

☐ Always
☐ Sometimes
☐ Almost always
☐ 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

<table>
<thead>
<tr>
<th>Season</th>
<th>In a pen</th>
<th>Free ranged</th>
<th>Tethering</th>
<th>Other (Specify)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Planting season</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Growing season</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Harvesting season</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fallowing season</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

7. What do your pigs eat?

☐ Pasture
☐ Commercial feeds
☐ Kitchen left overs
☐ 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 once a year
☐ 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 2: Kappa statistic computation

Lingual Palpation

<table>
<thead>
<tr>
<th>Ag-ELISA</th>
<th>+</th>
<th>-</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>7</td>
<td>58</td>
<td>65</td>
</tr>
<tr>
<td>-</td>
<td>15</td>
<td>153</td>
<td>168</td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
<td>211</td>
<td>233</td>
</tr>
</tbody>
</table>

Apparent prevalence = 0.094

Observed proportion agreement \[ \frac{160}{233} = 0.687 \]

Chance proportion agreement (Both positive) \[ 0.094 \times 0.28 = 0.026 \]

Chance proportion agreement (Both negative) \[ 0.906 \times 0.687 = 0.622 \]

Chance proportion agreement \[ 0.026 + 0.622 = 0.648 \]

Observed minus chance agreement \[ 0.687 - 0.648 = 0.039 \]

Maximum possible agreement beyond chance level \[ 1 - 0.648 = 0.352 \]

Kappa \[ \frac{0.007}{0.326} = 0.02 \]

The Kappa statistic = 0.02 (p = 0.333)
Appendix 3: Materials used for the 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 lyophilised

Add 0.5ml pure distilled water to the vial and dissolve the lyophilised 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. (ie one 20μl aliquot per 10ml of coating buffer).

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

Add 250μl pure distilled water to the vial and dissolve the lyophilised 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 estimation 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.

Please note that the coating information in the application information is NOT to be followed for this Ag-assay

4. Tetramethylbenzidine Substrate Sigma T8665

Liquid substrate supplied by Sigma Ltd.
6. **0.2M H₂SO₄ Stop Solution**

2ml concentrated H₂SO₄ (Aristar 11M to 100ml pure water).