MOLECULAR IDENTIFICATION OF *ECHINOCOCCUS* GENOTYPES FROM LIVESTOCK SLAUGHTERED IN SELECTED ABATTOIRS IN MERU AND ISIOLO COUNTIES IN KENYA

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MASTER OF SCIENCE

(Molecular Medicine)

A research thesis submitted in partial fulfillment for the award of Master of Science degree in Molecular Medicine in Jomo Kenyatta University of Agriculture and Technology

2015

DECLARATION

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

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                                                               Signature   Date

This thesis has been submitted for examination with our approval as university and research institutional supervisors.

PROF. JAPHET MAGAMBO   ………………………….   ………………………

MUST and JKUAT   Signature   Date

PROF. SAMMY NJENGA   ………………………….   ………………………

ESACIPAC, KEMRI.   Signature   Date

DEDICATION

I dedicate this work to my mother who has been my rock and has supported me both emotionally

and financially to realize my goals and my late father Robert Mbaya Murerwa who saw my

academic potential and nurtured and encouraged me since I was a young girl.

ACKNOWLEDGEMENT

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ABSTRACT

Cystic echinococcosis (CE) occurs in most regions of sub-Saharan Africa, but the frequency of this zoonosis differs considerably among and within countries in the continent. Research on cystic echinococcosis has a long history in Kenya, but has mainly concentrated on two discrete areas, Turkana and Maasailand, which are known to be foci of human CE in Africa.

In the present study genetic identification of *E. granulosus* isolates from selected abattoirs Eastern Kenya specifically, Meru and Isiolo counties were done. A total of 7831 livestock carcasses were surveyed. Hydatid cysts were collected from livestock (cattle, goats, sheep, and camels) from abattoirs and slaughter slabs. A DNA based molecular analysis was done to genotype cyst isolates using PCR-RFLP techniques targeting the mitochondrial gene nad-1.

Average CE prevalence was 1.92 % (number infected/number of animals sampled) in cattle, 6.94% (number infected/number of animals sampled) in camels, 0.37% (number infected/number of animals sampled) in goats and in sheep 4.62% (number infected/number of animals sampled).Majority of cysts occurred in the lungs in comparison to the liver for each of the hosts apart from goats. From a total of 284 recovered cysts, 258 could be identified as *E. granulosus* sensu stricto (n=160), *E. ortleppi* (n=51) and *E. canadensis* (n=47) by RFLP-PCR

The data indicate that the epidemiological situation in Eastern Kenya is clearly different from the well-studied pastoral regions of Turkana and Maasailand, and the apparently low number of human cases correlates with the infrequent occurrence of *E. granulosus* sensu stricto. With the finding of all the three genotypes prevailing in the small foci study areas further investigation needs to be done in other parts of the country to find out if the scenario is the same.

CHAPTER ONE

1.0 INTRODUCTION

Cystic echinococcosis (CE) is a zoonotic disease affecting livestock and humans. It is caused by the metacestodes of dog tapeworms of the *Echinococcus granulosus* complex. The metacestodes usually form fluid filled cysts 'hydatids' located in liver, lungs and other organs.

 Echinococcus granulosus belongs to the genera *Echinococcus* whose other members includes *E. multilocularis* which causes alveolar echinococcosis, *E. vogeli* which causes polycystic echinococcosis, *E. oligarthrus* which causes polycystic echinococcosis and recently described *E. shiquicus* that causes unicystic echinococcosis. Despite the frequency of CE, diversity among its etiological agents was ignored and they were collectively known as *E. granulosus*. Recent findings revealed that there are large morphological (adult worms), biological (host preference, developmental parameters) and genetic differences within the species (Romig *et al*., 2011).These findings on strain variation within the unilocular cystic hydatid disease organism *E. granulosus* have generated wide interest.

 Cystic echinococcosis is distributed worldwide, acquiring public health or economic significance in areas where extensive livestock production provides suitable conditions for the cyclic transmission between dogs and livestock animals. Cystic echinococcosis is considered an emerging disease in many parts of the world, in some regions re-emerging after initially successful control (Eckert *et al*., 2000; Jenkins *et al*., 2005). The global burden of CE in humans is estimated to be above 1, 000,000 DALYs (disability adjusted life years), which gives CE a greater impact than onchocercosis, Dengue fever and Chagas disease combined, and approaches

the burden caused together by human African Trypanosomiasis and Schistosomiasis (Budke *et al*., 2006).

Cystic echinococcosis has been reported from many of the countries in sub-Saharan Africa (Macpherson and Wachira, 1997). However, as it is typically a disease affecting pastoral communities who often live in remote areas and reliable data on prevalence of CE in humans or animals are only known from few regions. Previous studies done on CE in livestock showed that it is widespread and frequent especially in eastern and southern Africa. High-prevalence regions of human CE are focally distributed in Kenya, northern Tanzania and southern Sudan, where prevalence levels can reach 6% in nomadic populations (Macpherson *et al*., 1989).

Cystic echinococcosis (CE) in Kenya provides a valuable opportunity for studies on the molecular epidemiology of *E. granulosus* strains (Wachira *et al*, 1993). The disease is hyper endemic among two pastoral communities, the Turkana in the northwest and the Maasai in the southwest. These regions are geographically separated by a non-CE zone, the length of which varies between 250 and 800 km. The range of intermediate hosts for *E. granulosus* includes cattle, sheep, goats, pigs and humans in both regions and camels in Turkana, while domestic dogs are the main definitive hosts (Macpherson *et al*., 1985; Dinkel *et al*., 2004; Casulli *et al* 2010; Addy *et al* 2012).

In the current study three genotypes of *Echinococcus* (cattle (G5), camel (G6) and sheep (G1) strains have been identified as well as the prevalence of disease in each of the livestock species. This information could be useful for design of CE control programs in the areas.

1.1 Problem statement

Cystic echinococcosis (CE) is a neglected tropical disease with an impact on human health and the economy of endemic areas, which mostly consist of developing countries (Budke *et al*., 2006). Larval cysts may cause problems in host tissue because of the continual growth and expansion of the cyst (Jones and Pybus, 2001). Subsequent compression of tissues, such as the lung, may cause debilitation due to the animal's reduced ability to breathe if a sufficient number of cysts are involved. In humans, *E. granulosus* infection in the lungs may be associated with fever and difficulty breathing. *E. granulosus* cysts may also develop in other organs including the brain and cause severe problems because of the pressure on normal tissue.

Despite considerable economic losses, the political will to effectively control CE is often lacking, as CE does not seriously affect the health of the urban population or cause significant losses to intensely managed meat production schemes. It is a typical affliction of rural pastoralist societies with little access to health facilities, and whose economic losses are rarely considered and are difficult to quantify. Control programs against CE have been successfully implemented even in remote areas, but have often proven difficult to maintain because of financial and logistical reasons (Magambo *et al*., 2006).

Until recently, conventional means such as serological tests or microscopy were the only medium available for classifying the parasite which presents less accurate results. At present molecular techniques offer the viable option of effective characterization of the parasite *Echinococcus* in animals and humans. This study used molecular techniques to determine the genetic diversity of *Echinococcus* species in livestock in Meru and Isiolo areas, so as to enhance accurate diagnosis which leads to effective management of cystic echinococcosis.

Justification

Most of the studies done on *Echinococcus* in Kenya have been localized in Turkana in north-western Kenya and Maasailand in south-central Kenya and very few or none in other parts of the country. Meru and Isiolo counties are a new study site and no previous studies on the parasite have been done so far. They also provide an ideal platform for cystic echinococcosis studies in the North and North Eastern parts of the country because no mapping of the disease has been done in these areas. The G1 strain or sheep strain is the most common in other study sites in Kenya (Wachira *et al*., 1993, Dinkel *et al*., 2004).

By undertaking the project in the mentioned areas, we hope to identify the genotypes of *Echinococcus* present in livestock slaughtered in selected abattoirs. Identification of the locally prevailing *Echinococcus* taxa, their distribution, host preferences and pathogenicity is needed as it may help control and preventive efforts to those transmission cycles which are locally most important for human health and the production of vital animal resources.

1.2 OBJECTIVES

1.2.1 General Objective

To determine prevalence and identification of *Echinococcus granulosus* genotypes in livestock from selected abattoirs in Meru and Isiolo counties.

1.2.2 Specific objectives

i. To determine the prevalence and distribution of *Echinococcus* spp in livestock in Meru and Isiolo counties

ii. To determine the *Echinococcus* genotypes prevailing among livestock in Meru and Isiolo counties

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1.0 Life cycle

Refer to figures 1 and 2 for morphology of cyst and life cycle of the parasite respectively. *Echinococcus* causes disease in humans after accidental ingestion of cestode eggs from the environment. Taxa which infect livestock may cause considerable economic losses world-wide (Budke *et al*., 2006). Depending on the species, the life-cycles can be domestic, peridomestic (involving wild and domestic animals) or wildlife-based. Sylvatic (wildlife) cycles may be primary or secondary (caused by spill-over from domestic cycles) (Jenkins & Macpherson, 2003; Jenkins *et al*., 2005).

Cystic echinococcosis (previously hydatidosis) is caused by adult or larval (metacestode) stages of cestodes belonging to the genus *Echinococcus* and the family *Taeniidae*. The adult *E. granulosus* (3 to 6 mm long) resides in the small bowel of the definitive hosts, dogs or other canids. Gravid proglottids release eggs that are passed in the feces. After ingestion by a suitable intermediate host (under natural conditions: sheep, goat, swine, cattle, horses, camel), the egg hatches in the small bowel and releases an oncosphere that penetrates the intestinal wall and migrates through the circulatory system into various organs, especially the liver and lungs. In these organs, the oncosphere develops into a cyst that enlarges gradually, producing daughter cysts that fill the cyst interior. The larval stage is a fluid-filled bladder or hydatid cyst that is unilocular, although communicating chambers also occur. Individual bladders may reach up to 30 cm in diameter and occur most frequently in liver and lungs, but may develop in other internal organs. The infection with this stage is referred to as cystic echinococcosis (Jones and Pybus, 2001).

The definitive host becomes infected by ingesting the cyst-containing organs of the infected intermediate host. After ingestion, the protoscolices evaginate, attach to the intestinal mucosa, and develop into adult stages in 32 to 80 days. There are rostellar hooks of various sizes on the protoscolex in two rows. The size of the hooks varies between 25 to 49 μm in the first row, and between 17 and 31 μm in the second row. The gravid uterus has well-developed sacculations.

The adult varies between 2 and 11 cm in length and usually possesses from two to seven segments, averaging from three to four segments. The penultimate segment is mature, and the genital pore normally opens posterior to the middle in both mature and gravid segments. The last (gravid) segment is usually more than half the length of the entire worm. The parasite is transmitted between the domestic dog and a number of domestic ungulate species. Sylvatic definitive and intermediate hosts also occur, e.g. wolf/cervid. Humans become infected by ingesting eggs, with resulting release of oncospheres in the intestine and the development of cysts in various organs (Elkin and Zamke, 2001).
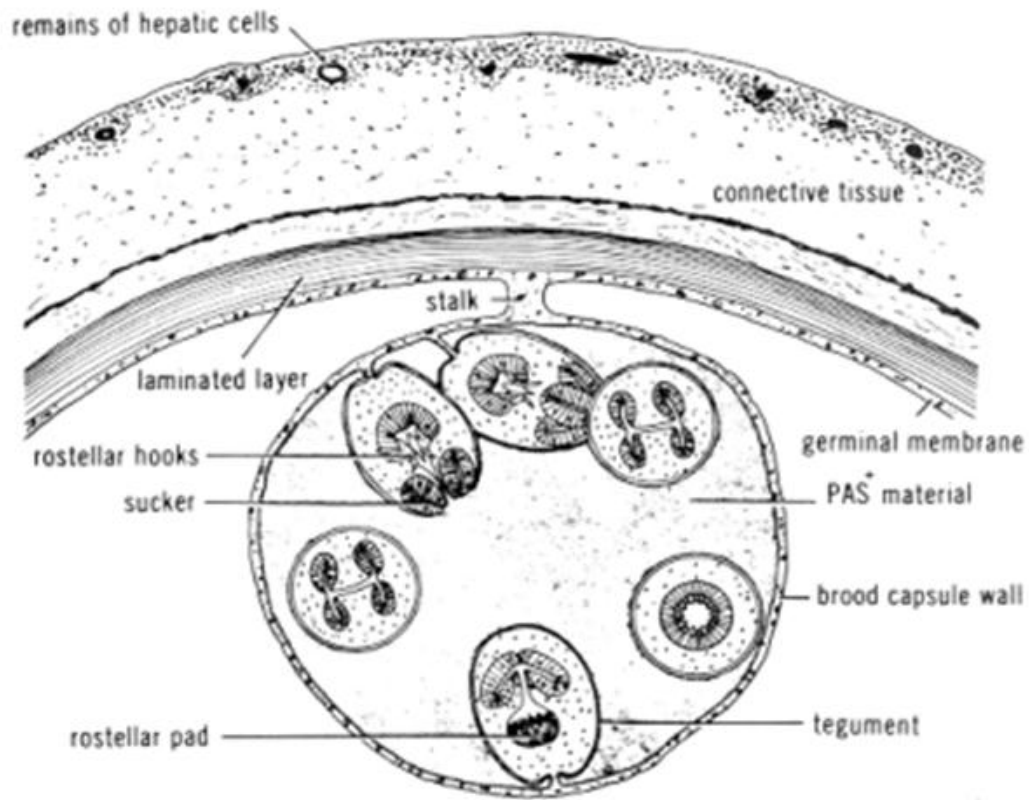
Figure 1: A diagrammatic representation of the transverse section of a hydatid cyst of *Echinococcus granulosus.* **Source:** www.dpd.cdc.gov/dpdx
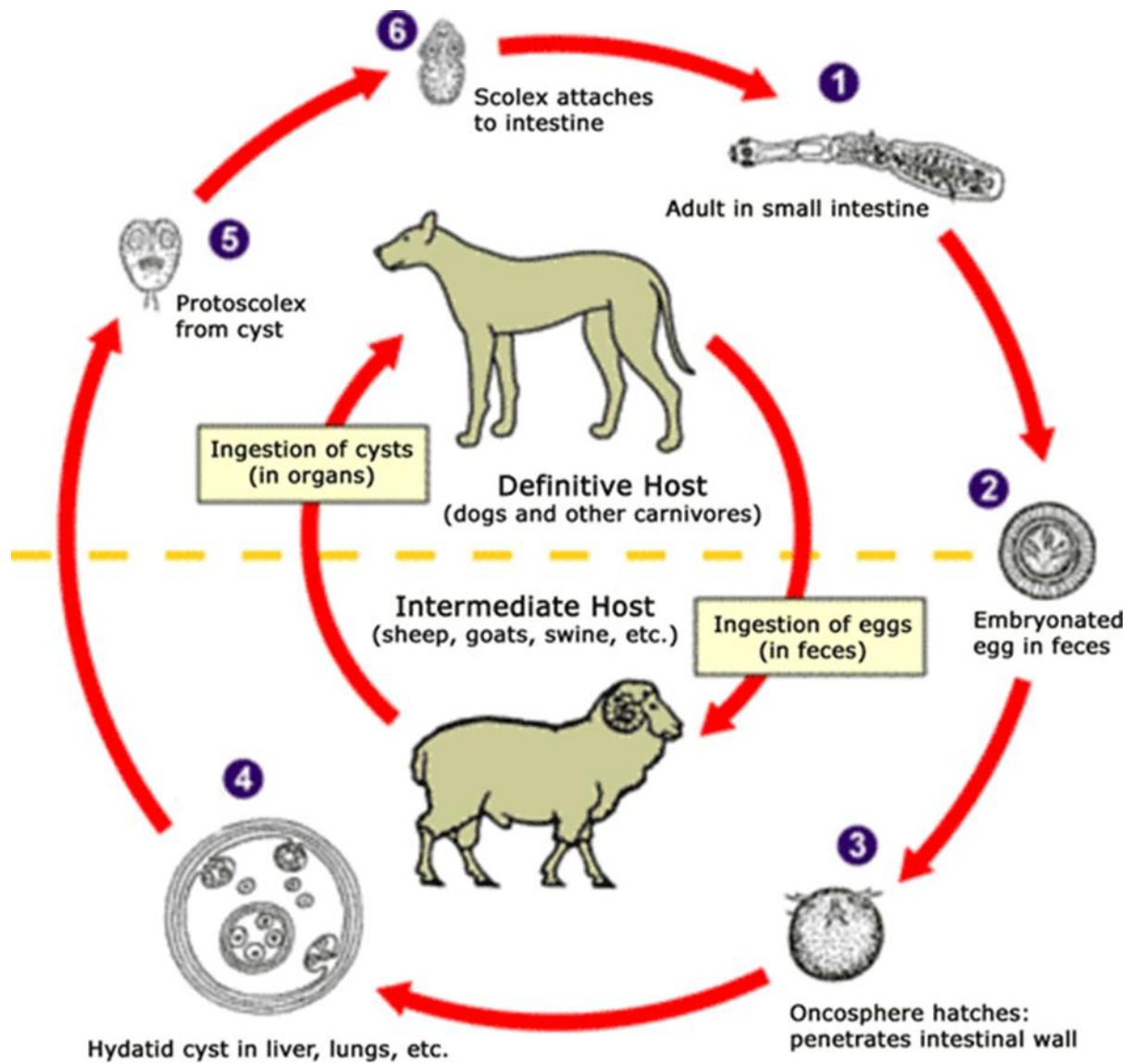
Figure 2: Life cycle of *Echinococcus granulosus.* **Source**: *www.dpd.cdc.gov/dpdx*

2.1.1 Epidemiology

Cystic echinococcosis has greater public health importance and economic impact in countries where livestock industry is an important segment of the agricultural sector and when livestock production is based on mainly extensive grazing system. Previous studies have reported that the disease is a problem in the areas where grazing, and in particular pastoralism, is widespread

(Garippa *et al*., 2004). The close relationship between dogs, livestock and man makes more likely that the life cycle of the parasite can be completed.

The disease is highly endemic in sub-Saharan Africa. It has been reported in West African as well as the East African countries. Due to lack of well-documented data from many countries in Africa, however, the sub-Saharan picture of the current disease situation is not complete (Magambo *et al*. 2006).

In Kenya, prevalence of CE in livestock varies between regions. Macpherson (1985) reported 8.9%, 8.1% and 7.1% prevalence levels in cattle, sheep and goats, respectively, in Maasailand. Njoroge *et al.* (2002) also reported the following prevalence levels: 19.4% in cattle, 3.6% in sheep and 4.5% in goat from Turkana.

When Kenya is compared to other countries internationally such as southern Brazil, surveys of the previous five years resulted in *Echinococcus* prevalence estimates of 19% in cattle, 3% in sheep and 20% in rural dogs whereas prevalence of CE in domestic animals in Turkey ranges from 3.5% to 58.6%, varying widely among locations, with a higher overall prevalence in sheep compared to goats, cattle and water buffaloes (Umur 2003; Altintas 2008).Similarly, cystic echinococcosis is a major public health problem in Peru, with a prevalence of 6-9% in many areas of the country and numerous human cases reported every year (Moro *et al*., 1997, 2006). In China, *E. granulosus sensu stricto* and *E. multilocularis* are widespread in western, northern and central parts of the country (Wang *et al*., 2008), and hyperendemic foci exist within pastoral areas of the eastern Tibetan Plateau (Schantz *et al*., 2003; Li *et al*., 2008) and the Xinjiang Uyghur Autonomous Region (Wang *et al*., 2001).

2.1.2 *Echinococcus* taxa

WHO/OIE (2001) defined strain as group of individuals which differ statistically from groups of the same species in gene frequencies and in one or more characters of actual potential significance to epidemiology and control of hydatid disease. Molecular genetic studies in support of this designation have resulted in identification of 10 genotypes denoted as G1 to G10. This variability is reflected in characters which affect the life-cycle pattern, host specificity, development rate, pathogenicity, antigenicity and sensitivity to chemotherapeutic agents, transmission dynamics, epidemiology and control of echinococcosis (Thompson and McManus, 2001). Based on mitochondrial DNA analyses, the *E. granulosus* complex has been split into *E. granulosus* sensu stricto (G1 − G3), *E. equinus* (G4), *E. ortleppi* (G5) and *E. canadensis* (G6 − G10) (Thompson, 2008; Nakao *et al.,* 2007 and Scott *et al*., 1997).

The cystic morphology on metacestodes in CE may not be a sign of close relationship, but the result of adaptation to intermediate hosts and the mode of transmission: those taxa of *Echinococcus* which became adapted to large animals (e.g. sheep, horses, deer) developed fluid-filled cysts as metacestodes, those transmitted by small rodents (*E. multilocularis*) show a multivesicular growth pattern, and those using large rodents or leporids exhibit a morphology somewhere in-between (*E. vogeli, E. oligarthra, E. shiquicus*).

In sub-Saharan Africa, only CE is present. Affected livestock species are sheep, goats, cattle, camels, pigs, horses and donkeys. Cystic echinococcosis has additionally been recorded from a large number of wild herbivores, e.g. zebra, warthogs, buffalo and various species of antelopes. Wachira *et al* (1993) examined 208 larval isolates and 40 worm samples of *E. granulosus* from various hosts in Kenya using restriction fragment length polymorphism (RFLP) analysis of a segment of ribosomal DNA amplified by polymerase chain reaction (PCR). This was in an effort

to determine whether additional strains of *E. granulosus* occur in Kenya, to examine the level of genetic heterogeneity within the sheep/dog and camel/dog strains previously identified, and to map out their intermediate host range and geographic distribution in Kenya. They confirmed the existence of the two strains in Kenya and showed that the distribution of the camel strain appears restricted to the Turkana region, where camels are kept as livestock. The intermediate host range for both strains seems to be similar except that humans appear refractory to infection with the camel strain. They have also shown that although the life-cycle patterns of the two strains overlap both geographically and in intermediate and definitive hosts, the strains maintain their homogeneous genetic identity.

2.1.3 Diversity of *Echinococcus* in sub-Saharan Africa

From sub-Saharan Africa, more CE intermediate host species have been reported than from any other continent, owing to the large number of wild ungulate species (Macpherson & Wachira, 1997; Hüttner & Romig, 2009). Likewise, members of all genotypic clusters, or species, of *E. granulosus sensu lato* have been found, creating a very complex situation. In addition, there is no doubt that primary wildlife cycles exist at least in eastern and southern Africa, whose interaction with domestic lifecycles is unknown. Molecular screening tools to investigate this situation, however, have only recently become available so that data on the molecular epidemiology of CE are still limited to a few African countries south of the Sahara (Romig *et al*., 2011).

The species and strains of *Echinococcus* present in Africa are diverse and are evident in various studies that have been conducted within the continent. In Mauritania, all of 20 isolates from camels (17), cattle (1) and humans (2) were identified as *E. canadensis* G6 (Maillard *et al*., 2007; Maillard *et al*., 2009).In Sudan, 579 isolates analyzed revealed *E. canadensis* G6 (camel strain) to be the dominant taxon in camels, sheep, cattle and humans, whereas *E. ortleppi* occurs rarely

or locally in cattle (Dinkel *et al*., 2004; Omer *et al*., 2010). In the extreme southeast, near the border to Kenya, the presence of *E. granulosus sensu stricto* is highly probable, but published data are lacking. In central Ethiopia, only *E. granulosus* G1 has so far been found in 26 samples from cattle and sheep, whereas in northern Ethiopia (Makale) *E. granulosus* G1, *E. ortleppi* and *E. canadensis* G6/7 were found among 21 cysts from cattle (Maillard *et al*., 2007). In Somalia one isolate from a camel was identified as *E. canadensis* G6 (Bowles *et al*., 1992). In Kenya of 823 isolates, 623 belonged to *E. granulosus* G1 (sheep, goats, cattle, camels, pigs, humans and dogs), 199 to *E. canadensis* G6/7 (camels, cattle, goats, humans and dogs), and only one to *E. ortleppi* (pig). Most samples originated from the northwest of the country (Turkana area) (Wachira *et al*., 1993; Dinkel *et al*., 2004; Hüttner *et al*., 2009; Casulli *et al*., 2010). In Uganda, so far only isolates from wildlife in western Uganda have been examined *and E. felidis* was identified in 34 fecal samples of lions as definitive hosts, and from one cyst isolate in a warthog. An additional warthog cyst was genotyped as *E. granulosus* G1 (Hüttner *et al*., 2009).

In South Africa, one old isolate of *Echinococcus* worms from a lion preserved since the 1960s was identified as *E. felidis* (Hüttner *et al*., 2008). No isolates from livestock or humans have so far been examined whereas in Namibia there is one published record, *E. ortleppi* from a zebra (Obwaller *et al*., 2004).

2.1.4 Prevalence

Several studies have been done in Turkana, Kenya to establish the prevalence of CE in livestock. Njoroge *et al*. (2000) examined 1390 goats using ultrasonography and found 1.8% from Turkana, and 4.3% from Toposaland (Southern Sudan) infected with hydatid cysts. In another study, using slaughter data 19.4% of cattle, 3.6% of sheep, 4.5% of goats and 61.4% of camels were found infected with CE (Njoroge *et al*., 2002). In a recent survey from Maasailand, slaughter data

revealed a prevalence of 25.8% in cattle, 16.5% in sheep and 10.8% in goats (Addy *et al.*, 2012). These surveys show that CE is highly endemic and a major public health problem in eastern Africa.

When compared to other countries in Africa, varying prevalence of CE have been reported in cattle by several workers. In Sudan, CE has been reported to have a prevalence of 45% in camels, 3% in cattle and 7% in sheep (Elmahdi *et al.* 2004) while in Ngoro Ngoro district of Tanzania, a study showed that 48% of cattle, 34.7% of goat, 63.8% of sheep and 10% of dogs were infected with *E. granulosus* (Ernest *et al.*, 2004). In Egypt, over a five-year period CE prevalence was reported as 2.53%, 0.3% and 0.68% in camels, sheep and goats, and pigs, respectively (Haridy *et al.*, 2006). Other studies have also shown prevalences of 32% in the Niger Delta (Arene 1985), 5.6% in Libya (Mohammed 1985), 13.9% in Algeria (Bardonnet *et al.*, 2003), and 22.98% in Morocco (Azlaf and Dakkak 2006).

2.1.5 Diagnosis

2.1.5.1 Identification of the agent

In the intermediate host, diagnosis depends on the ultrasound detection of the larval cyst form, which can occur in almost any organ, but particularly in the liver and lungs. The diagnosis of CE in definitive hosts requires the demonstration of the adult cestodes of *Echinococcus spp.* in their faeces or the small intestine or the detection of specific coproantigens or coproDNA.

    a. **Detection of *Echinococcus* eggs in environmental samples**

This involves a concentration method in which a saturated solution is used to separate *Echinococcus* eggs from faeces. A faecal sample of 0.5–2 g is mixed with water or 0.3% Tween (20 in 1%) formalin in a 10–15-ml test tube and centrifuged (1000 g for 10 minutes) once or

twice until the supernatant is clear. Sediment is mixed with either zinc sulphate 33% (1.18 sp. Gr.) or sucrose solution (1.27 sp. Gr.) and centrifuged at 1000 g for 5–10 minutes. The test tube is filled to the top and a cover-glass is placed on the tube. The cover glass is examined microscopically 2–16 hours later (Ito 1980; Thienpont *et al*., 1979).

b. Diagnosis of larval echinococcosis

Whereas surveillance for *E. granulosus* in domestic animals may take place in licensed slaughter houses, that for *Echinococcus sp.* in wildlife must be done by field surveys. Specimens should be preserved by removal of tissue and fixation in 70% ethanol kept cool at +4°C and deep-frozen at –20°C for subsequent examination. Each specimen is preserved singly in a tube. When undertaking surveillance work for *E. granulosus* in intermediate hosts, it is vitally important that data are stratified and reported according to the age of animals slaughtered. Prevalence rates are strongly age dependent (Torgerson and Heath, 2003) and reports from abattoirs that may slaughter only young animals will substantially under-represent the true situation. This is because older animals may be heavily infected even when animals have very few larvae due to formation of multiple daughter cysts.

Larvae can be observed in many organs, but in large animals, such as sheep and cattle, palpation or incision should be done. Pigs, cattle, sheep and goats may be infected with larval *Taenia hydatigena*, and it is sometimes difficult to differentiate between these two parasites when they occur in the liver. In wild animals, such as ruminants and rodents, several other larval cestodes should be considered for differential diagnosis (Varcasia *et al*., 2007).

Formalin-fixed material can be stained by conventional histological techniques. The presence of a periodic acid-Schiff (PAS) positive acellular laminated layer, underlying a connective tissue

layer, and with or without an internal cellular, nucleated germinal membrane can be regarded as a specific characteristic of the metacestodes of *Echinococcus* spp. The presence of protoscoleces within brood-capsules or in hydatid sand is also diagnostic for the genus (Lightowlers *et al.,* 1995).

c. **Serological tests**

For intermediate hosts serological tests include Enzyme linked immunosorbent assay (ELISA), an indirect haemagglutination test, a complement fixation test and a Western Blot system. Immunological tests, useful in humans, are less sensitive and specific in livestock and at present cannot replace necropsy (Murray *et al*., 1990).

 In definitive hosts following ingestion of a cyst, dogs will be exposed at the intestinal level to various antigens during the establishment of the parasite and its development and oogenesis. Specific antibodies against oncosphere and protoscolex antigens can be readily detected in the serum of infected dogs. This methodology has not reached a practical stage as it does not differentiate between current and previous infections and false positives may occur with infections of *Taenia* species (Schantz *et al*., 1990).

i.   **Enzyme Linked Immunosorbent Assay (ELISA)**

Diagnosis of *E. granulosus* using ELISA involves use of a 96 wells plate standard kit which primarily uses IgG antibodies using standard methodologies. Wells coated with antigens from *E. granulosus* hydatid fluid are used (Gottstein *et al*., 1993).

ii.   Polymerase Chain Reaction (PCR)

Genotyping of *E. granulosus* is usually done on DNA derived from protoscolices or larval tissue material that is frozen, refrigerated or preserved in 70% ethanol.

One format of PCR is specific for cestode DNA. This is carried out to demonstrate the presence of cestode DNA in samples, which amplifies the target sequence from all cestode species and genotypes which are tested (Dinkel *et al*., 2004).

Another format of PCR is specific for various genotypes for example the g1 PCR selectively amplify the G1 genotype of *E. granulosus* with a specific band of 254 base pairs (bp). To prove specificity of the g1 PCR, the amplified 254 bp fragments of the G1 reference isolates are sequenced and compared with the G1 sequences deposited in GenBank (accession number AY462129) using the National Center for Biotechnology Information BLAST programs and databases (Dinkel *et al*., 2004).

The g5/6/7 PCR is found to amplify *E.ortleppi* and the G6 and G7 genotypes of *E. granulosus* with a characteristic band of 254 bp. Specificity of the 254 bp fragment is also confirmed by sequencing of the g5/6/7 PCR products obtained from all G5, G6 and G7 reference isolates. The obtained sequences are similarly compared with G5, G6 and G7 sequences deposited in GenBank (accession numbers AY462126–AY462128) using the National Center for Biotechnology Information BLAST programs and databases (Dinkel *et al*., 2004).

2.1.6 Treatment for Echinococcosis

Treatment for human CE is difficult because most cysts or cystic lesions develop in the liver, lungs, or other organs (McManus *et al*., 2003:- Ci-Peng *et al*., 2005). Surgery remains the main

treatment, but medico-surgical approaches are becoming more widespread, along with percutaneous drainage for hepatic cystic echinococcosis.

i) Chemotherapy

Two benzimidazolic drugs, mebendazole and albendazole, are the only anthelminthics effective against cystic echinococcosis. Albendazole and mebendazole are well tolerated but show different efficacy. Albendazole is significantly more effective than mebendazole in the treatment of liver cysts. Benzimidazole treatment alone requires prolonged administration over many weeks, with an unpredictable outcome in terms of response rates in individuals (Stojkovic M *et al*., 2009).

Treatment with albendazole in *E. granulosus* infection can result in an apparent cure in as many as 30% of patients, with a further 40-50% of patients showing objective evidence of response when observed short term. Patients who do not show obvious initial evidence of response may be found to be cured when observed over several years.

Duration of therapy and doses are also important. Albendazole efficacy increases with courses of up to 3 months in the more common cyst sites. Patients once received these drugs in cycles of 4 weeks separated by 1-2 weeks without drugs. This regimen is no longer advocated given the parasitostatic activity of benzimidazoles and their safety as shown by cumulative data from several retrospective studies. Continuous treatment is preferred and has been administered for periods of up to 2 years without significant side effects (Liu *et al*., 2000, Brunetti *et al*., 2010). The safety profile shows that liver function abnormalities are common, although they rarely limit treatment, while occasional hematologic changes affecting white cells may be more serious. The safety data supply the rationale for monitoring patients during treatment.

Overall, albendazole has been demonstrated to be a useful advance in the management of cystic echinococcosis when used as sole treatment or as an adjunct to surgery or other treatments.

Praziquantel has recently been suggested, administered additionally once per week in a dose of 40 mg/kg during treatment with albendazole. However, available data are limited (Bygott JM., *et al* 2009).

ii)   Surgery

Surgery was the only treatment available before the introduction of anthelmintic drugs. It is considered the first choice of treatment for CE but is associated with considerable mortality (up to 2% in some series, increasing with second and further operations), morbidity, (Elmalki *et al*., 2008) and recurrence rates (2-25%). Given the more frequent detection of early and asymptomatic *E. granulosus* liver lesions, a widened indication for chemotherapy exists.

Several procedures have been described for the treatment of hepatic echinococcal cysts, ranging from simple puncture to liver resection and transplantation, although the most commonly used technique is total or partial cystopericystectomy.

Usually, radical surgery (total pericystectomy or partial hepatectomy) is indicated for liver cysts. Conservative surgery (open endocystectomy with or without omentoplasty) or palliative surgery (simple tube drainage of infected cysts or communicating cysts) is also an option. More radical interventions have higher intraoperative risks but less numerous relapses. With the inclusion of chemotherapy prior to or after surgery, less-aggressive surgery may be possible (Bagheri *et al*., 2011)

Surgery for pulmonary cysts includes extrusion of cysts using Barrett technique (intact endocystectomy without preliminary aspiration), pericystectomy, and lobectomy (Bagheri *et al*., 2011). Peripheral and unilobar echinococcal cysts, regardless of how complicated they are, can also be treated with laparoscopic surgery using partial cystopericystectomy and drainage. When surgery cannot be avoided, presurgical use of albendazole reduces risk of recurrence and facilitates surgery by reducing intracystic pressure.

iii)   Percutaneous treatment

The puncture of echinococcal cysts has long been discouraged because of risks of anaphylactic shock and spillage of the fluid; however, as experience with ultrasonography-guided interventional techniques has increased since the early 1980s, an increasing number of articles have reported its effectiveness and safety in treating abdominal, especially liver, echinococcal cysts. A recent systematic review of the literature found that the overall fatality rate due to lethal anaphylaxis from puncture of echinococcal cysts is 0.03% (2 in 5943 procedures) for procedures and 0.04% (2 in 5517 cysts) for cysts respectively (Neumayr *et al*., 2011).

Under albendazole coverage, cysts are punctured under ultrasonographic or CT guidance either with a needle or with a catheter according to their size. Usually, a small quantity of fluid is first aspirated and examined by light microscope to observe for the presence of viable protoscolices. If they are present, the cyst is aspirated completely. As happens with drug therapy, positive responses include both a decrease in cyst size and a progressive change in echo pattern (generally solidification) (Filice *et al*., 1998, Paksoy *et al*., 2005, 2003).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1.1 Study design

This was a cross-sectional study involving livestock from selected abattoirs and slaughter slabs in Meru and Isiolo. A sample size was calculated for both Meru and Isiolo areas and thereafter each slaughterhouse allocated a sample size that would contribute to attainment of target sample. The study was nested within an ongoing study SSC number 1684 but additional approval was given by KEMRI SSC and ERC and JKUAT Board of Post Graduate studies.

3.1.2 Study area

The study was conducted in abattoirs and slaughter slabs in and around Meru and Isiolo. A total of twelve abattoirs were sampled from both areas. These included Thimangiri, Gikumene, Katheri, Nkubu, Ruiri, Isiolo, Kianjai, Timau, Ntirimiti, Ntugi, Kiirua, Mudajune and Maua. Thimangiri, Gikumene, Katheri, Kiirua, Ntugi, Ntirimiti, Maua and Nkubu abattoirs are found in the wet areas of Meru with cooler temperatures. Ruiri, Isiolo, Kianjai and Mudajune abattoirs are located within the drier areas of Isiolo County. These were chosen so as to investigate variation of cystic hydatid disease with regard to geographical distribution. Four abattoirs were chosen in the drier areas particularly Isiolo as compared to eight from Meru county due to insecurity that prevented sampling from the interior parts of the county.
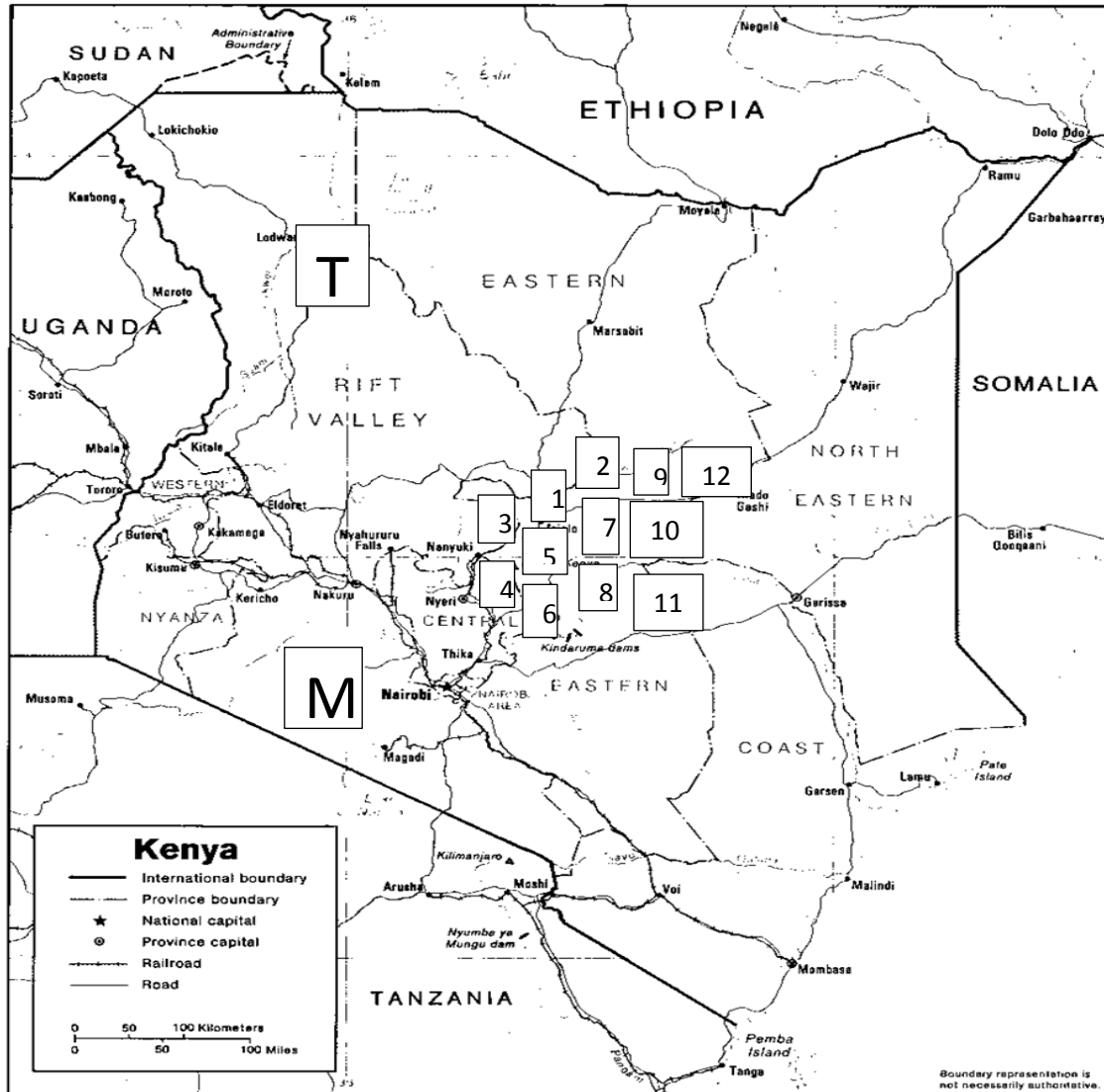
Figure 3: Map of Kenya, showing the position of the study sites (1-12) in relation to the Turkana region (T) and Maasailand (M). **Source of background map**: www.worldofmaps.net

List of abattoirs: 1-Isiolo, **2**-Ruiri, **3**-Timau, **4**-Kibirichia, **5**-Kiirua, **6**-Ntugi, **7**-Mudajune, **8**-Kianjai, **9**-Thimangiri, **10**-Gikumene, **11**-Nkubu, **12**-Maua

The study area is shown in Figure 4. Meru town is located at 0.047035 degrees north and 37.649803 degrees east, on the northeast slopes of Mount Kenya and is situated about five miles north of the equator, at an altitude of approximately 5,000 feet, in an area of mixed forest and clearings, small towns, villages and rural farms. The area receives rainfall of about 1,366mm per

annum. Majority of the animals slaughtered in abattoirs in Meru are not of local origin. This is because majority of livestock kept by the people are mainly for dairy purposes such as production of milk rather than meat. Livestock such as cattle and goats are bought in livestock markets in Isiolo or Kianjai and then brought by traders for slaughter in local abattoirs and the meat sold to butcheries.

Some of the origins of livestock slaughtered in abattoirs in Meru included Isiolo, Tharaka, Kianjai, Katheri, Kibirichia, Gikumene, Gatunga, Ntumburi, Samburu and a few from the locality of the abattoir. Isiolo is the gateway to the northern corridor of Kenya and has a livestock market where communities from the area that practice pastoralism come to sell.

In Isiolo the local topography is arid to semi-arid low plains and Ewaso Nyiro River flows through the county and partly bounds it. The main Livestock markets in the area are in Isiolo town and Kangeta market in Igembe District (Nyambene County).The origin of livestock slaughtered in abattoirs in Isiolo include the locality, Samburu, Moyale, Laisamis and as far away as Garissa.

3.1.3 Study population

The study population included cattle, camels, goats and sheep brought to the abattoirs for slaughter. The animal organs of interest for this study were liver and lungs from infected animals.

3.1.4 Specimen collection

Sampling was done during the months of March to June 2013 after approval from the KEMRI-ERC was given in February 2013. The director of veterinary services and district veterinary

officers of respective areas were also approached by the investigator and the overall objective of the study explained with a purpose of obtaining permission to conduct the study in the respective abattoirs.

Animals brought for slaughter were monitored from the point of reception until slaughter. Details such as species, age, sex and origin of the animals will be obtained from the abattoir records Animals were identified on the basis of enumerated marks on their body surface using ink and this marking was transferred to all visceral organs after slaughter. During inspection the liver, lungs, heart, spleen and kidneys were examined by observation, palpation and incision for the presence of hydatid cyst. Cysts obtained from diseased organs were preserved in 70% ethanol for subsequent laboratory analyses.

3.1.5 Sample size

Sample size was computed using Cochran's Formula (1963, 1975).

Statistical formula

$$n_0 = \frac{Z^2 pq}{e^2}$$

Where n=required sample size

  Z=1.96(confidence level at 95%)

  p=prevalence rate of *Echinococcus* (50% assumed because prevalence is unknown in study area)

  q=1-p

  e=level of precision at 5% (standard value of 0.05)

  i. $n = \frac{(1.96)^2 \, 0.5(1-0.5)}{0.05^2}$

ii. n= $\dfrac{(1.96)^2 0.5(0.5)}{0.05^2}$

iii. n= $\dfrac{3.814 \times 0.25 = 0.9604}{0.0025}$

iv. n= 384.16 approximately 385

Therefore, 385 of cattle, goat, sheep and camel, respectively were to be sampled from each of the two areas.

3.1.6 Statistical Analysis

Statistical analysis was carried out using Epi Info 3.4.3. Data was first entered in Microsoft Office Excel spread sheet (2010) and then converted to Epi Info compatible mode in Microsoft Office Access. The Microsoft Office Access datasheet was imported into Epi Info for analysis. Descriptive statistics was first carried out to summarise the data using mean, mode, median and standard deviation. Prevalence of CE, abundance and infection intensity of *Echinococcus* cysts were then determined.

3.2 LABORATORY METHODS

Lab work for this project was done at the Centre for Microbiology Research (CMR) parasitology laboratory at the Kenya Medical Research Institute (KEMRI), Nairobi. Cysts were processed by cutting open using a sterile scalpel-blade and the contents transferred into a sterile test tube. The volume of the cyst fluid was measured and thereafter a small amount transferred to a microscope slide. The fluid in microscope slide was observed to check on fertility of the cyst. Presence of brood capsules containing protoscolices implied that the cyst was fertile while absence of protoscolices or brood capsule implied that cyst was infertile. The infertile cysts were further

classified as sterile (fluid filled cyst without any protoscoleces) or calcified (cyst already calcified) as previously described by (Macpherson 1985).

3.2.1 DNA extraction from protoscolices

Single protoscolices were transferred using a pipette to tubes containing 10µl of 0.02N NaOH. The tubes were incubated at 95°C for 10 min in an Applied Biosystems Gene Amp PCR System 9700 Thermocycler. Lysed protoscolices were used directly as template for polymerase chain reaction assay (Nakao *et al*., 2003).

3.2.2 DNA extraction from tissue

DNA from cyst wall tissue material and cell debris in sterile cysts was picked from petri-dishes containing cyst fluid and transferred with a pipette to tubes containing 10µl of 0.02N NaOH. The tubes were subjected to temperatures of 95°C for 10 minutes in an Applied Biosystems Gene Amp PCR System 9700 Thermocycler. Lysed material was used directly as a template for polymerase chain reaction assay (Nakao *et al,* 2003).

3.2.3 Polymerase Chain Reaction Assay of *nad*-1

Nested polymerase chain reaction assay was performed to essentially amplify the *nad*-1 (1073 – 1078 bp) using the primer pairs previously used by Hüttner *et al*. (2009).Briefly, the 25µl reaction mixture consisted of 10X PCR buffer, 2mM MgCl2, 100µM of each dNTPs, 6.25pmol of each primer and 1.25 units of Taq polymerase and 1µl of DNA template. The primary PCR included 2 µl (100-300 µg/ml) of the lysate while the secondary PCR used 1 µl of the primary PCR product. In both PCR assays, amplification reactions were performed in the Applied Biosystems Gene Amp PCR System 9700 Thermal Cycler. Reactions were run for 35 cycles

with cycling conditions as follows: denaturation (94°C for 30 s), annealing (55°C for 30 s), elongation (72°C for 1 min) and then post cycling final elongation (72°C for 5 min).

For the primary PCR Forward primer was nad A (TGT TTT TGA GAT CAG TTC GGT GTG) and reverse primer was nad C (CAT AAT CAA ACG GAG TAC GAT TAG). For the secondary PCR forward primer was nad B (CAG TTC GGT GTG CTT TTG GGT CTG) and reverse primer was nad D (GAG TAC GAT TAG TCT CAC ACA GCA).

All PCR reactions included negative control (no DNA to control contaminations) as well as positive control samples of *E. granulosus* G1, *E. ortleppi* and *E. canadensis* G6. After amplification, PCR products were visualised on 1.5% (w/v) agarose gel with the aid of Gel Red® (Biotium, Inc.) dye in a Bio-Rad Power Pac 300 gel electrophoresis machine and subsequently photographed using transilluminator.

3.2.4 Restriction Fragment Length Polymorphism (RFLP) of *nad*-1

The *nad*-1 PCR amplicons were digested with the 5 bp Hph I endonuclease (Fermentas, Germany) according to the method used by Hüttner *et al*. (2009). In summary, a total reaction mixture 30.5 μl was constituted which contained 10 μl *nad*-1 PCR amplicons , 18 μl nuclease free water, 2 μl digestion buffer (supplied with enzyme) and 0.5 μl of the Hph I endonuclease. Reaction mixture was incubated at 37°C for 3 h in the Applied Biosystems GeneAmp PCR System 9700 Thermal Cycler and enzyme inactivated for 20 min at 65°C. The resultant restricted fragments were separated in 3% agarose gel stained with Gel Red® (Biotium, Inc.) dye. Genotyping of samples were done by comparing their banding patterns with the defined patterns of *E. granulosus* G1, *E. ortleppi* and *E. canadensis* G6 used as reference. Reference banding patterns were based on Hüttner *et al*. (2009) as follows: *E. granulosus* G1, (485, 320, 204, 64

base pairs) *E. ortleppi* and (867, 107, 102 base pairs) and *E. canadensis* G6 (442, 425, 107, 102 base pairs).

CHAPTER FOUR

4.0 RESULTS

A total of 7,831 carcasses of cattle (4595), camels (216), sheep (65) and goats (2955) were inspected for cysts in all organs of the pleural and abdominal cavities in abattoirs in Meru and Isiolo areas and out of these 117 animals were positive for CE. In Meru region a total of 73 cattle, 8 goats and three sheep were positive for the disease while in Isiolo 14 cattle, 15 camels and 3 goats were positive.

4.1.0 Prevalence and distribution

Cysts of *Echinococcus* spp. were found in 117 animals. This corresponds to prevalences of 1.92% in cattle (88 of 4,595), 6.94 % in camels (15 of 216), 0.37 % in goats (11 of 2,955) and 4.62 % in sheep (3 of 65) (Table 1). The number of cysts per infected animal was low—only two cattle and two camels contained more than five cysts (Table 2). The majority of cysts occurred in the lungs, particularly in cattle and camels (86 and 87 % of infected animals) (Table 3).

Of the 284 cysts collected, 258 could be characterized molecularly. Across all livestock species, 160 cysts belonged to *E. granulosus,* 51 to *E. ortleppi* and 47 to *E. canadensis*. When looking at fertile cysts only, the picture is different: There, *E. canadensis* and *E. ortleppi* (40 and 35 cysts) predominate over *E. granulosus* (20 cysts) (Table 3).

Table 1 below shows a summary of prevalence for various species of livestock from abattoirs in Meru and Isiolo.

Table 1: Prevalence of three *Echinococcus* spp. and total CE in livestock of Eastern Kenya

Livestock species	*Echinococcus* species	n infected /n examined	Prevalence (%)
Cattle	*E. granulosus* s.s.	62 / 4595	1.35
	E. ortleppi	23 / 4595	0.50
	E. canadensis	5 / 4595	0.11
	Unidentified	3 / 4595	-
	Total CE (*E.* spp.)	88 / 4595	1.92
Camel	*E. granulosus* s.s.	3 / 216	1.39
	E. ortleppi	0 / 216	0.00
	E. canadensis	11/ 216	5.09
	Unidentified	1 / 216	-
	Total CE (*E.* spp.)	15 / 216	6.94
Goat	*E. granulosus* s.s.	4 / 2955	0.14
	E. ortleppi	3 / 2955	0.10
	E. canadensis	1 / 2955	0.03
	Unidentified	3 / 2955	-
	Total CE (*E.* spp.)	11 / 2955	0.37
Sheep	*E. granulosus* s.s.	1 / 65	1.54
	E. ortleppi	2 / 65	3.08
	E. canadensis	0 / 65	0.00
	Unidentified	0 / 65	-
	Total CE (*E.* spp.)	3 / 65	4.62

The figures in the table show that prevalence of the *Echinococcus* species varied across the species of livestock from the abattoirs in both counties. *Echinococcus granulosus* sensu stricto (G1) appears more prevalent in sheep (1.54%) as compared to cattle, (1.35%), camels (1.39) and goats (0.14). This can be attributed to the low number of sheep that were sampled (65) as opposed to those of cattle (4595), camels (216) and goats (2955).In reality, *E. granulosus* sensu stricto was identified in 62 cattle, 3 camels, 4 goats and I sheep. *E. canadensis* was most prevalent in camels (5.09%) as compared to cattle (0.11%) and goats (0.03%). This genotype was not identified in sheep. The genotype *E.ortleppi* also appears most prevalent in sheep (3.08%) compared to cattle (0.5%) and goats (0.1%) due to the low number of sheep sampled. The genotype was positively identified in 23 cattle, 3 goats and 2 sheep was not identified in camels. The low number of sheep sampled is due to the fact that majority are slaughtered at home and not abattoirs.

Table 2: Distribution of *Echinococcus* spp. cysts in infected animals

EG = *E. granulosus*; EO: *E. ortleppi*; EC: *E. canadensis*; (?): Species undetermined

Infected livestock animals (n)	Number of cysts per animal and *Echinococcus* species		
	1 cyst(n animals)	**2-5 cysts(n animals)**	**6-10 cysts(n animals)**
Cattle(88)	28 (16 EG, 9 EO, 2 EC, 1?)	58 (40 EG only, 10 EO only, 1 EC only, 3 EG/EO, 1 EG/EC, 1 EO/EC, 2?)	2(2 EG only)
Camels(15)	6 (2 EG, 3 EC, 1?)	7(1 EG only, 6 EC only)	2(2 EC only)
Goats(11)	6 (1 EG, 1 EO, 1 EC, 3?)	5 (3 EG only, 2 EO only)	0

| Sheep(3) | 2 (2 EO) | 1(1 EG only) | 0 |

The various *Echinococcus* species were highly aggregated in individual host animals (Table 2): out of 75 animals with multiple cysts, 68 contained only cysts of a single species (47 *E. granulosus*, 12 *E. ortleppi*, 9 *E. canadensis*), only five animals (all cattle) had mixed infections (three *E. granulosus/E. ortleppi*, one *E. granulosus*/*E. canadensis*, and one *E. ortleppi/E. canadensis*). Five out of seven *E. granulosus* cysts found in all camels came from a single individual.

4.2 Genetic Characterisation

Two of the three species of *Echinococcus* were unequally distributed within the study area. All 17 animals infected with *E. canadensis* (camels, cattle and goats) originated from the northern part (Isiolo area); whereas, *E. ortleppi* was found in a far larger proportion of infected livestock in the southern part (Meru area) compared to the North (45 vs. 13 % of infected cattle, goats and sheep). The distribution of *E. granulosus* did not differ significantly (55 vs. 62 % of infected cattle, camels, goats and sheep).

In cattle, most cysts were sterile or calcified *E. granulosus* cysts (126 of 194), mainly in the lungs. Out of 54 fertile cattle cysts, 32 belonged to *E. ortleppi* (all except one from the lungs), only 16 to *E. granulosus* (lungs) and six to *E. canadensis* (lungs). In camels, most cysts belonged to *E. canadensis* (39 of 46), 31 of them were fertile lung cysts; *E. granulosus* was less frequent in camels (7 of 46), *E. ortleppi* did not occur in this sample. In sheep, only non-fertile cysts were found (2 of *E. granulosus*, 2 of *E. ortleppi*). In goats, the four fertile cysts (from the lungs)

belonged to *E. ortleppi* (3) and *E. canadensis* (1), while all *E. granulosus* cysts (9) were non-fertile (Table 3).

Table 3: Fertility status and organ location of *Echinococcus* spp. cysts in different livestock species

EG: E. granulosus; EO: E. ortleppi; EC: E. canadensis

Livestock species	Cyst fertility	Liver		Lungs	
		n(cysts)	*Echinococcus spp*	n(cysts)	*Echinococcus spp*
Cattle	Fertile (54)	1	1 EO	53	16 EG; 31 EO; 6 EC
	Non-fertile (140)	21	19 EG; 2 EO	119	107 EG; 11 EO; 1 EC
Camel	Fertile (37)	2	2 EC	35	4 EG; 31 EC
	Non-fertile (9)	0	-	9	3 EG; 6 EC
Sheep	Fertile (0)	0	0	0	0
	Non-fertile (4)	0	0	4	2 EG; 2 EO
Goats	Fertile (4)	0	0	4	3 EO; 1 EC
	Non-fertile (10)	7	6 EG; 1 EO	3	3 EG

From the above results *E. granulosus* sensu stricto (G1) was isolated from cattle, camel, sheep and goats, *E. ortleppi* (G5) from cattle sheep and goats, while *E. canadensis* (G6) was identified in camels, goats and cattle.
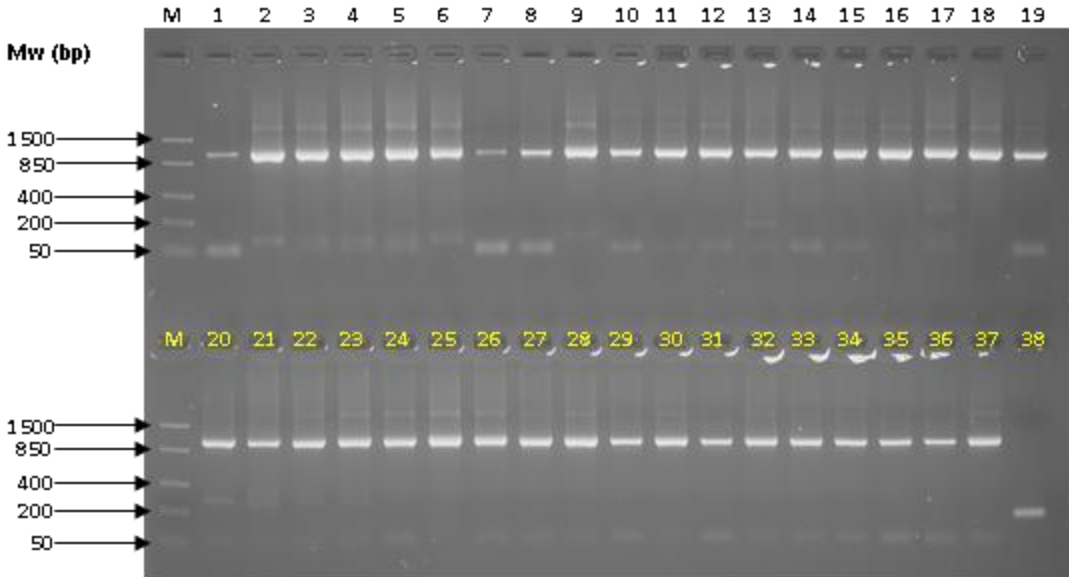
Figure 5: Agarose gel photo of nad-1 amplicons (~1073 – 1078 bp). Lane **M:** Molecular ladder (FastRuler middle range), Lane **1 – 14:** Cattle samples; **15** - **22:** Goat samples; **23-30:** Camel samples; **31-34:** Sheep samples **35, 36, 37**: Positive samples of *Echinococcus granulosus* G1, *Echinococcus ortleppi* G5 and *Echinococcus canadensis* G6, respectively; **38:** Negative control (no DNA).
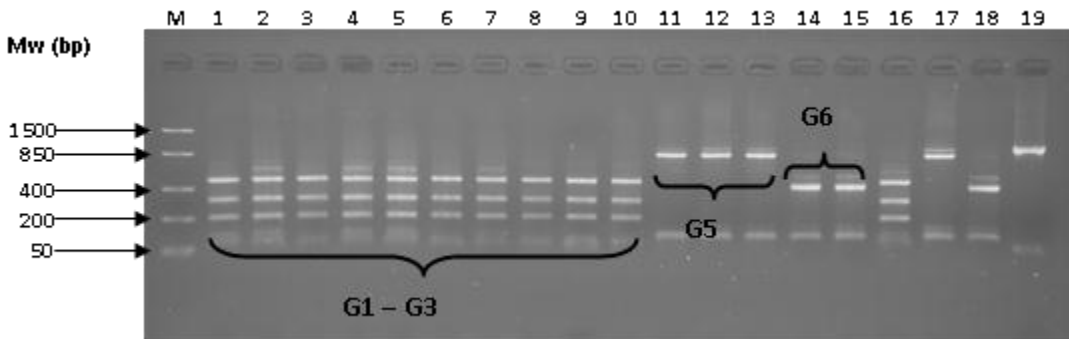
Figure 6: Agarose gel photo of PCR – RFLP of nad-1 using Hph I. Lane **M:** Molecular ladder (FastRuler, low range); **1-10:** *E. granulosus* G1; **11,12 and 13:** *E. ortleppi*; **14 and 15:** *E. canadensis* G6;**16,17 and 18:** References of *E. granulosus* G1 (485bp-320bp-204bp-64bp), *E. ortleppi* (867bp-107bp-102bp) and *E. canadensis* G6 (442bp-425bp-107bp-102bp)-positive controls; **19:** undigested *nad*-1 amplicons(negative control)

Table 4: Comparative CE prevalence levels of livestock in Kenya: Eastern Kenya, Turkana and Maasailand

Livestock species	Eastern Kenya (this study) (% prevalence)	Turkana (Njoroge *et al*., 2002) (% prevalence)	Maasailand (Addy *et al*., 2012) (% prevalence)
Cattle	1.9 n = 4595	19.4 n = 381	25.8 n = 587
Camel	6.9 n = 216	61.4 n = 70	- -
Sheep	4.6 n = 65	3.6 n = 588	16.5 n = 430
Goats	0.4 n = 2955	4.5 n = 5752	10.8 n = 194

The table above shows comparison of prevalences of CE in livestock from this study, one from Turkana and another in Maasailand. The eastern Kenya study had a CE prevalence of 19% in cattle as compared to 19.4% in Turkana and 25.8% in Maasailand. Prevalence of CE in camels was 6.9% in this study as compared to 61.4% in Turkana, while in sheep it was 4.6 % for this study as compared to 3.6% in Turkana and 16.5% in Maasailand. For goats CE prevalence was 0.4% for this study as compared to 4.5% in Turkana and 10.8% in Maasailand.

<center>**CHAPTER FIVE**</center>

5.0 DISCUSSION

5.1 Prevalence of Cystic Echinococcosis in Livestock from Meru and Isiolo

The results demonstrate the presence of cystic echinococcosis in Meru and Isiolo, Eastern Kenya. The CE prevalences in livestock of my study area are far lower than those reported from other parts of Kenya (Table 4). In the country's hot spot for human CE, northern Turkana, prevalences in cattle, camels and goats were higher by approximately the factor 10. Only the prevalence level in sheep was comparable with the present study, which may be an artifact due to the low number of sampled sheep (Njoroge *et al*. 2002). The difference is even more pronounced when comparing our data to the most recent survey in the Maasailand of southern Kenya, where 25.8% of cattle, 16.5 % of sheep and 10.8 % of goats were infected; camels are not kept there in significant numbers (Addy *et al*. 2012). The low prevalence levels obtained from Meru and Isiolo can be attributed to the fact that communities in both areas practice primarily agriculture as opposed to nomadic pastoralism. This means that some livestock are housed in animal sheds where feed is provided as opposed to allowing them to graze freely. This reduces the likelihood of them ingesting the parasite eggs from the grass. The number of animals slaughtered also depends on the demand for beef products. It is difficult to conclude whether data obtained reflects true prevalence as a number of factors might have influenced the outcome of surveys including pre-selection of livestock to be slaughtered. This means cattle are clearly overrepresented in the study as they are sold to abattoirs more often than small stock which is usually slaughtered at home.

The taxonomy used for the various members of the *E. granulosus* (sensu lato) complex is done in accordance with the most recent treatment of the group (Nakao *et al.* 2013a, b). Due to various inconsistencies, the genotype-based nomenclature which has been used previously for the agents of CE (G1-10) was replaced by grouping closely related genotypes (G1-3 and G6-10) as species. This distinction is supported by genetic data as well as morphological criteria of the adult worm (Eckert *et al.* 1989, 1993). The metacestode (cyst) stage, however, cannot be allocated reliably to any one species, as morphological criteria of the larval hooks overlap, and no distinctive structures exist in non-fertile cysts. The PCR-RFLP used in this study had been evaluated for each of the *Echinococcus* spp., which cause CE (*E. granulosus* sensu stricto, *E. felidis*, *E. equinus*, *E. ortleppi*, *E. canadensis*), but does not distinguish between intraspecific variants and genotypes (Hüttner *et al.* 2008, 2009).

The low prevalence of CE may also be a result of dilution effect by animals from non-pastoralist areas in Meru. Farmers in Meru primarily keep livestock in zero-grazing units which reduces the opportunities for infection through free-grazing. In contrast, farmers in Isiolo practice nomadic pastoralism which increases the opportunities for infection through ingestion of *E. granulosus* eggs deposited in vegetation by definitive hosts. The dog-keeping habits also differ in both of these areas. In Isiolo, dogs are used to guard livestock due to their large numbers during grazing and live in close proximity to the people. The interaction of dogs and livestock during grazing ensures the domestic life cycle of *E. granulosus* parasite is maintained. In Meru, dogs are mostly used to guard homesteads and are housed in kennels and rarely let out until dark. This means that they have little interaction with the livestock kept and people in the homesteads which reduces the chances of infection.

From table one (1) above, the prevalence of CE appears greater in sheep than in cattle. This may be due to the low number of sheep that were sampled in comparison to cattle. Beef is the preferred animal protein of choice for most people hence the high numbers of cattle that are slaughtered. Mutton on the other hand is not consumed often and when people do, home slaughter of sheep is commonly done resulting to low numbers of sheep at the abattoirs.

5.2 Genetic Characterisation

Cattle may have a higher prevalence compared to the rest of the livestock but the low levels of cyst fertility means that they do not play a major role in disease transmission as compared to the smaller livestock. The exception is *E. ortleppi* that exhibits high cyst fertility in cattle but the isolation of the same in goats will mean that they too can play a role in transmission. High cyst fertility means that once a cyst is ingested by definitive host there are higher chances for establishment of infection. This may be through brood capsules and daughter cysts that are released into the gut of definitive host once the cyst ruptures and consequently grow into adult worms. The dogs shed eggs from adult *E. granulosus* worms in their faeces which are ingested by intermediate hosts while feeding on vegetation and this ensures continuation of the cycle of transmission. However, if the cyst ingested is sterile the cycle of transmission is stopped because there are no daughter cysts to develop in the gut of the definitive host .Sheep and goats slaughtered at home without inspection can easily pass on the parasites to dogs and consequently perpetuate the disease in the population (Macpherson 1985).

Echinococcus species infects a wide range of organs (Abdul *et al*., 2010; Omer *et al*., 2010 and Varcasia *et al*., 2007) but in our case it was mostly localized to the liver and the lungs of infected livestock. This translates into greater economic loss due to condemnation of affected organs as

well as reduction in market value of entire carcasses which explains reluctance by meat inspectors to discard entire organ but instead cut away the infected area.

The relative frequency of *E. ortleppi* was conspicuous. Thirty-two of 54 fertile cysts from cattle belonged to this cattle-adapted species. Despite this, the absolute prevalence of *E. ortleppi* was low in cattle (0.50 %), goats (0.10 %) and sheep (3.08 %). To my knowledge, the latter two small stock species have not been recorded before as hosts for *E. ortleppi*; notably, three of four cysts in goats were fertile, so goats seem to be suitable hosts. No *E. ortleppi* was found in camels; there is only one previous record of that species in camels, from central Sudan (Ahmed *et al.* 2013). The only other record of *E. ortleppi* from cattle in Kenya comes from Maasailand (Addy *et al.* 2012), where only one of 201 cattle cysts belonged to that species. Despite the much higher CE prevalence in Maasai cattle, the calculated *E. ortleppi* prevalence is probably lower than in our study area (some 0.1 vs. 0.5 %). The sporadic occurrence of the cattle-adapted *E. ortleppi* in most parts of the world has been discussed before (Addy *et al.* 2012). Even in remote places, cattle are usually sold alive and transported to distant abattoirs for slaughter, which means that the local dog population has no access to offal and re-infection of livestock is inhibited. Prior to the identification of *E. ortleppi* in Kenyan cattle, this taxon had only been reported from pigs in the West of the country (Dinkel *et al.* 2004); it is unknown, however, if pigs play any epidemiologically important role to maintain this parasite. In camels, the proportion between *E. canadensis* and *E. granulosus* was approximately 5:1 in our sample, which corresponds well with data from the Turkana region of Kenya (no significant numbers of camels are kept in Maasailand) (Hüttner *et al.* 2009). However, the prevalence of both species was far lower in our study region. In Turkana, camels are the most frequently infected livestock species (61.4 % CE prevalence— Njoroge *et al.* 2002), which is at a similar level as in neighbouring Sudan (44.6–

59.8 %), where camels were found exclusively infected with *E. canadensis* in two large surveys (Elmahdi *et al*. 2004, Omer *et al*. 2010). In our area, the *E. canadensis* prevalence in camels was only 5.1 %. To all appearances, the environmental conditions and type of camel husbandry are similar to the high prevalence regions, so the reason for this drastic difference remains to be explored. Interestingly, *E. canadensis* (in camels, goats and cattle) was restricted to the northern part of our study area, where semiarid conditions prevail and camels are the most important species of livestock. This may suggest that the presence of camels is important for the persistence of this parasite, but data from Maasailand indicate that it may also be transmitted in a dog-goat lifecycle (Addy *et al*. 2012). The suitability of goats to maintain *E. canadensis* lifecycles is also known from southern Europe (Varcasia *et al*. 2007). The most significant difference between our study area and the high prevalence regions is the rarity of *E. granulosus* sensu stricto, whose prevalence in cattle is decreased by a factor of approximately 20 compared to both Turkana and Maasailand (Addy *et al*. 2012, Hüttner *et al*. 2009). Although cattle are not good hosts for *E. granulosus* sensu stricto (most cysts remain sterile or degenerate into calcified lesions), they can serve as indicators for the infection pressure from the environment, which seems to be low in our study area. Even sheep, which are considered to be particularly well adapted as hosts of this parasite, seem to be rarely infected (one of 65 sheep), although the low number of sampled animals does not allow further conclusions on this. If we disregard non-fertile cysts (which have no consequence for transmission of the parasite), *E. granulosus* sensu stricto was the least frequent of the three *Echinococcus* spp. in the area (only 20 fertile cysts in >7,000 examined animals). The reason for this remains speculative. I could not obtain reliable data on the frequency of sheep (relative to goats) kept in the area, but anecdotal observations indicate that they are much rarer. If this, or some other factor, is responsible for the low number

of livestock infected with *E. granulosus* sensu stricto, needs further study. *E. granulosus* sensu stricto is considered to be the principal *Echinococcus* species causing human CE (Alvarez Rojas *et al*. 2014), which coincides with the apparent rarity of human CE in eastern Kenya (Zeyhle, personal communication). This places our study area in an excellent position to investigate the factors that keep the transmission of this species at a low level. When animals with multiple cysts were considered, *Echinococcus* species did not occur in individual host animals in proportion to their frequency in the area. Instead, individuals were mostly infected with several (up to 10) cysts of the same species, while mixed infections were rare. This is suggestive of a single infection event as the usual source of disease rather than the accumulation of cysts over a prolonged period—at least under conditions of low endemicity, as in this study area.

E. granulosus sensu stricto was isolated from cattle, camel, sheep and goats, *E. ortleppi* from cattle sheep and goats, while *E. canadensis* was identified in camels, goats and cattle. The identification of these strains is in support of earlier reports of these species/genotypes isolated from African livestock (Maillard *et al*. 2009; Dinkel *et al*. 2004; Wachira *et al*. 1993; Omer *et al*. 2010).

The isolation of *E. ortleppi* (G5) from the goat and high fertility rate of the cysts (4 out of 5) shows that the parasite is well adapted to the new hosts which are able to maintain the lifecycle in places where cattle are absent. The identification of mixed infections is also a first of its kind in Kenya. They can occur when an animal ingests two eggs shed by adult *Echinococcus* worms belonging to different genotypes during grazing. An observation that was made in regards to the mixed infections was that one of the cysts was fertile and the other was sterile. In the case of the cattle with G1/G5 infection the G5 cyst was fertile and for the G1/G6 mixed infection only the cyst with G6 was fertile.

Twenty-three cyst samples from CE positive livestock did not amplify after Polymerase Chain Reaction assay. This could be due to the fact that some cysts contained calcified material in place of cyst fluid and hence it was difficult to retrieve the germinal layer that is required for DNA extraction.

CHAPTER SIX

6.0 CONCLUSION

i. The present survey provides baseline data for the infection of livestock with *Echinococcus* species in Eastern Kenya. This is the first large-scale molecular survey outside the high endemicity regions of Turkana and Maasailand.

ii. The results obtained—low prevalence levels, in particular of *E. granulosus* sensu stricto—will help to evaluate the epidemiological situation of CE in Kenya from a different perspective. Rather than being the rule, the situation in the high endemicity regions of the northwest and south of Kenya may be exceptional, when considering the country as a whole. This survey cannot provide the reasons for this disparity.

iii. There are no obvious differences in climatic conditions and the general lifestyle of the population; in all three regions, pastoralism is the major occupation, and people have similar lifestyles (including frequent home slaughter). A smaller number of sheep kept may be a factor for the less frequent occurrence of *E. granulosus* sensu stricto, but the trend towards low prevalence is similar for all *Echinococcus* species. and livestock species. The number of dogs kept and their access to offal may be a key element, which is in need of closer study.

iv. The data shows presence of three distinct *Echinococcus* species with *E. ortleppi* as a frequent strain in these areas in addition to *E. granulosus* sensu stricto and *E. canadensis*, which are also common in other parts of Kenya.

v. The data also shows the first report of *E. ortleppi* in goats (3) in the world and reports for the first time mixed infections of *Echinococcus* species in livestock (cattle) in Kenya.

6.1 RECOMMENDATION

i. Although low levels of prevalence in livestock were obtained in both Meru and Isiolo, it is important to conduct surveys in other parts of the country to identify the genotypes that are present. This will help in the design of control programmes for the disease in both livestock and humans.

ii. Surveys should be conducted in the abattoirs located near game reserves and national parks to investigate the possibility of transmission of the parasite from the wild definitive hosts to the domestic livestock.

CHAPTER SEVEN

7.0 REFERENCES

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