GENETIC DIVERSITY AND PREVALENCE OF *ECHINOCOCCUS* SPECIES IN LIVESTOCK IN MAASAILAND AND TURKANA, KENYA

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Genetic Diversity and Prevalence of *Echinococcus* Species in Livestock in Maasailand and Turkana, Kenya

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DECLARATION

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

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DEDICATION

To the memory of my late Grandmother, Christiana Maku and Aunt, Eva Akumaki.

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LIST OF ABBREVIATIONS AND ACRONYMS

***act* II**	Actin II gene
AMREF	African Medical Research Foundation
BLAST	Basic Local Alignment Search Tool
CE	Cystic echinococcosis
CESSARi	Cystic Echinococcosis in sub-Saharan Africa Research initiative
CO 1 or *cox* – 1	Cytochrome c Oxydase Subunit 1 gene
DALYs	Disability Adjusted Life Years
dNTP	Deoxynucleotide triphosphate
DTT	Dithiothreitol
EDTA	Ethylene-Diamine-Tetra-Acetic acid
G	Genotype
***hbx* 2**	Homeobox gene
HCl	Hydrochloric acid
Hph I	*Haemophilus parahaemolyticus* endonuclease I
ITS	Internal Transcribed Spacer
KEMRI	Kenya Medical Research Institute
mtDNA	Mitochondrial DNA
NaCl	Sodium Chloride
ND 1 or *nad* – 1	Nicotinamide Adenine Dinucleotide Dehydrogenase 1
OIE	Office International des Epizooties

OR	Odds Ratio
PCR	Polymerase Chain Reaction
RAPD	Random Amplified Polymorphic DNA
rDNA	Ribosomal DNA
RFLP	Restriction Fragment Length Polymorphism
rRNA	Ribosomal RNA
SDS	Sodium dodecyl sulphate
SSCP	Single-Strand Conformation Polymorphism
Taq	*Thermus aquaticus*
WHO	World Health Organisation

ABSTRACT

Cystic echinococcosis (CE) is a cosmopolitan zoonosis caused by *Echinococcus granulosus* sensu lato. Reported prevalence of CE from the Maasai and Turkana pastoral communities ranges from 3.6% to 19.4%. *Echinococcus* taxa identified in hosts in these areas were; *E. granulosus* G1, *E. ortleppi* and *E. canadensis* G6. This study sought to find out the current prevalence, predilection site and genetic diversity of *Echinococcus* spp. in livestock from Maasailand and Turkana. A survey was carried out at four slaughter houses; one in Kitengela, one in Lomidat and two in Suswa to examine carcasses for *Echinococcus* cyst. PCR-RFLP and partial sequencing of the mitochondrial gene NADH dehydrogenase gene (*nad*-1) were used to differentiate 293 cyst isolates to the species level. Prevalence levels of CE were 25.8% (151/587) in cattle, 16.5% (71/430) in sheep and 10.8% (21/194) in goats from Maasailand and 12.4% (12/97) in cattle and 6.8% (5/73) in goats from Turkana. A total of 906 *Echinococcus* cysts were isolated from the liver (540 cysts), lungs (359 cysts), heart (3 cysts), kidney (2 cysts) and spleen (2 cysts). *Echinococcus granulosus* s.l. taxa identified were *E. granulosus* G1 in cattle, sheep and goats in Maasailand and Turkana, *E. ortleppi* in cattle and *E. canadensis* G6 in sheep and goats from Maasailand. The prevalence of livestock CE in Maasailand and Turkana ranges 6.8% to 25.8%. The liver of livestock is the main predilection site of *Echinococcus* cysts. *Echinococcus granulosus* G1 was the dominant taxon (287/293 isolates) in livestock from Maasailand and Turkana. Similar studies need to be carried out in dogs and humans in the Maasai and Turkana pastoral communities.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Hydatidosis or cystic echinococcosis (CE) is a global zoonotic disease caused by the metacestode of the dog tapeworm *Echinococcus granulosus* sensu lato (Dinkel *et al.*, 2004). This disease has been reported to occur widely in Africa including Kenya (Casulli *et al*., 2010; Hüttner *et al*., 2009; Maillard *et al.,* 2007; Dinkel *et al*., 2004 and Eckert *et al.,* 2001). Four species of *Echinococcus* have been recognised, namely, *E. granulosus*, *E. multilocularis*, *E. oligarthrus* and *E. vogeli* (Thompson and McManus, 2001). These species have wide intermediate host range but *E. granulosus* has ungulates as its prime intermediate host hence the apparent significance in livestock.

Echinococcus granulosus is transmitted in a synanthropic cycle involving canids and felids as definite hosts. Heterogeneity within *E. granulosus* is common resulting in a number of strains. This variability is reflected in the life cycle pattern, host specificity, development rate, pathogenicity, antigenicity, sensitivity to chemotherapeutic agents and transmission dynamics (Thompson and McManus, 2001). Through molecular analysis, some of the forms previously described as species or subspecies have currently been reclassified as distinct strains. A minimum of 11 strains denoted G1 to G10 and a lion strain (*E. felidis*) have been recognised globally (Hüttner *et al.,* 2009; Lavikainen *et al*., 2003; Eckert and Thompson, 1997 and Thompson *et al*., 1995).

Effort are being made to appropriately characterise locally occurring *Echinococcus* strains among livestock, humans, pets and wildlife using molecular approach. The approach targets mitochondrial, nuclear and/or RNA loci such as the mitochondrial cytochrome c oxidase subunit 1 (*cox* 1) and the Nicotiamide adenine dinucleotide dehydrogenase (*nad*-1). The nuclear Actin II (*Act* II), Homeobox 2 (*hbx 2*), 12S rRNA and Internal Transcribed Spacer (ITS 1 and ITS 2) genes are also used. Techniques used in strain typing include genotype specific PCR, RFLP-PCR, PCR-SSCP and DNA sequencing (Jabbar *et al.,* 2011; Hüttner *et al.,* 2009; Maillard *et al.,* 2007; Bart *et al.,* 2006; Dinkel *et al.,* 2004; Dinkel *et al.,* 1998). One or more of these techniques could be employed.

Previous studies in Eastern Africa revealed that different *Echinococcus* strains occur in the same geographical area. Strain have been reported to infect different hosts species (Romig *et al.,* 2011; Hüttner and Romig, 2009; Hüttner *et al.,* 2009; and Dinkel *et al.,* 2004). To design accurate and practical control programs of hydatidosis, throughput epidemiological data is needed. This study therefore sought to determine the prevalence and diversity of *Echinococcus* spp. in livestock from Maasailand and Turkana through molecular techniques.

1.2 Problem Statement

The impact of the zoonotic cystic echinococcosis (CE) in disadvantaged pastoral communities has long been recognised in Africa. Prevalence of CE (2.5% – 19.4%) has been reported in livestock and human patients in Maasailand and Turkana, Kenya (Magambo *et al.*, 2006; Njoroge *et al.*, 2002 and Macpherson, 1985). Cystic echinococcosis disease burden is close to African trypanosomosis and schistosomiasis. Globally, associated livestock production loss was estimated to be about US$ 2 billion/annum, due to organ condemnation, decreased carcass weight, decreased hide value, decreased milk production and decreased fecundity. Human hydatidosis manifests in systemic immunological reactions like urticaria, asthma, anaphylaxis or membranous nephropathy. It is estimated to cost about 9,314 DALYs (Disability Adjusted Life Years) and US$ 5 million economic loss to humans in sub-Saharan Africa (Budke *et al.*, 2006). Asymptomatic state of the disease is quite common and can persist for many years (Pawlowski *et al.*, 2001). Cystic echinococcosis disease burden in Kenya is not yet available but it is obvious that infected hosts and affected communities cannot evade the negative impact of the disease. To date, a suitable strategy to eliminate *Echinococcus* infestations is lacking partly due to diagnostic challenges that emanate from its poorly understood complex epidemiology in Africa.

1.3 Justification

Most data on cystic echinococcosis in sub-Saharan Africa have been gathered in the second half of the 20th century covering few countries (Hüttner and Romig, 2009). The scanty data from the few African countries indicate that the diversity of *Echinococcus granulosus* s.l. is greater than on any other continent (Romig, 2011). Sympatric taxa are shown to infect different hosts within and across geographical boundaries (Ibrahim *et al*., 2011; Casulli *et al*., 2010; Hüttner and Romig, 2009; Hüttner *et al.*, 2009; Maillard *et al.*, 2007 and Dinkel *et al*., 2004). These make identification of locally prevailing taxa, their distribution, host preference and pathogenicity an urgent requirement for cost-effective control effort (Romig, 2011). This work therefore sought to use molecular techniques to study the prevalence, distribution and genetic diversity of *Echinococcus* spp. in livestock in Maasailand and Turkana.

1.4 General Objective

To determine the prevalence, predilection site and genetic diversity of *Echinococcus* species infecting livestock in Maasailand and Turkana.

1.4.1 Specific Objectives

i. To determine the prevalence of *Echinococcus* species among livestock species in Maasailand and Turkana.
ii. To determine the predilection site of *Echinococcus* species in livestock species in Maasailand and Turkana.
iii. To determine the genetic diversity of *Echinococcus* species in livestock species in Maasailand and Turkana.

1.5 Hypotheses

i. The prevalence of *Echinococcus* species in livestock in Maasailand and Turkana is constant.
ii. The main predilection site of *Echinococcus* cysts in livestock in Maasailand and Turkana is the lungs.
iii. There is no genetic diversity among *Echinococcus* spp. isolates from livestock in Maasailand and Turkana.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Taxonomy of *Echinococcus*

The family *Taeniidae* is a medically important group of worms consisting of the two genera *Echinococcus* Rudolphi 1801 and *Taenia* Linnaeus 1758 (Nakao *et al.*, 2010a). *Echinococcus* Rudolphi 1801 is a small endoparasitic cyclophyllid cestode (a true tape worm) (Thompson *et al*., 1995). At present, *Echinococcus granulosus, Echinococcus multilocularis, Echinococcus oligarthrus* and *Echinococcus vogeli* are the four recognized species in the genus *Echinococcus* (Bowles and McManus, 1993) (Table 2.1). *Echinococcus granulosus* is a cosmopolitan species while *E. multilocularis* is known to occur in Central and Northern Eurasia and North America. *E. oligarthrus* and *E. vogeli* occur in central and South America (Thompson and McManus, 2001).

Adult *Echinococcus* is only a few millimetres long (rarely more than 7 mm) and usually has no more than six segments (Figure 2.1). It has flat segmented body with anterior scolex and posterior strobila. Adult are hermaphroditic, lacking gut and all metabolic interchange takes place across the syncytial outer covering, the tegument (Thompson and McManus, 2001). The metacestode stage of the parasites manifests itself in different forms. The metacestode of *E. granulosus* shows unilocular cysts referred to as cystic echinococcosis. *Echinococcus multilocularis* shows a multivesicular cyst referred to as alveolar echinococcosis while *E. oligarthrus* and *E. vogeli* manifest polycystic echinococcosis (Romig *et al*., 2011).

Table 2.1: Classification of *Echinococcus*

Classification level	Nomenclature
Kingdom	Animalia
Phylum	Platyhelminthes
Class	Cestodae
Order	Cyclophyllidea
Family	Taeniidae
Genus	*Echinococcus*
Species	*Echinococcus granulosus*, *Echinococcus multilocularis*, *Echinococcus oligarthrus* and *Echinococcus vogeli*

Source: The Taxonomicom [Last updated: 07.04.2012] [Date assessed: 18.10.2012].

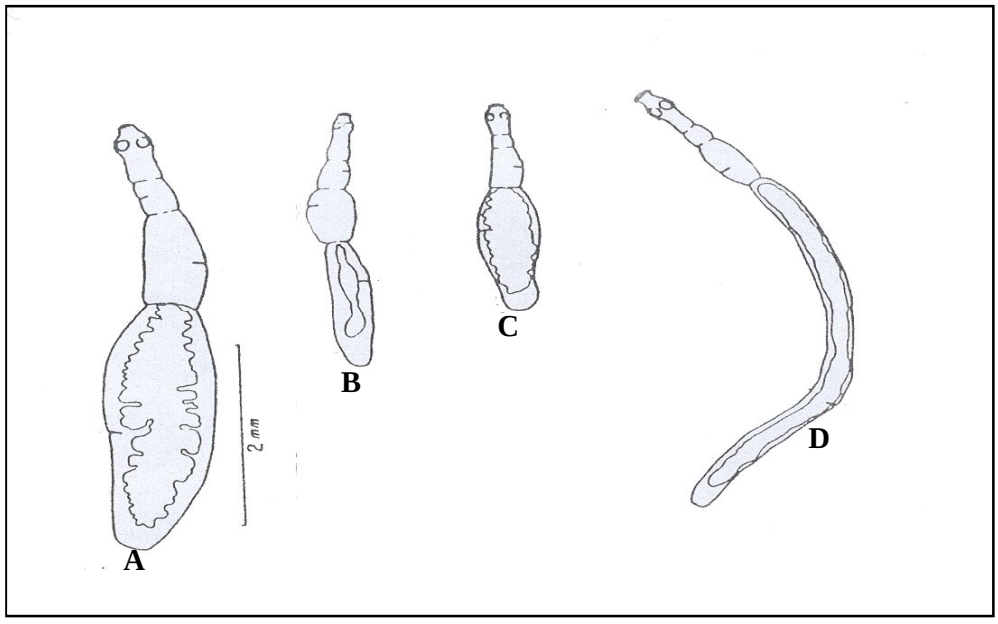

Figure 2.1: Adult *E. granulosus* (A), *E. multilocularis* (B), *E. oligarthrus* and *E. vogeli*. **Source:** Rausch, (1995)

2.2 Life Cycle and Cycle of Transmission of *Echinococcus* species

Understanding the aetiological agents of diseases demand appreciation of the life cycle of the agent and their transmission route. Comprehensive understanding of these two cycles is pivotal to the diagnosis and control/treatment of the parasite and the disease they cause. Studying the life cycle of parasites that present different forms of manifestation in different classes of hosts and whose life cycles are enhanced by transmission cycle is of scientific importance.

Echinococcus spp. is perpetuated in a life-cycle requiring two groups of mammals of predator-prey relationship (Nakao *et al*., 2010a) to complete a cycle as shown in Figure 2.1 (inner cycle). Carnivores such as the domestic dog serve as definitive hosts that harbour the hermaphroditic adult in the small intestine (stage 1 of outer cycle in Figure 2.1) while herbivorous and omnivorous animals play the intermediate host role. The definitive host passes on the parasite to the intermediate host by releasing through their faeces gravid proglottids containing embryonated eggs (stage 2) into the environment. Intermediate hosts such as livestock get infected with the released eggs via oral route during grazing or accidentally as in human (aberrant host) when living in close contact with the definite host.

After ingestion by a suitable intermediate host the egg hatches in the intestine and releases an oncosphere that gets attached to the intestinal mucosa (stage 3). The oncosphere penetrates the intestinal wall and enters the portal blood/lymph where they are transported passively throughout the body to major filtering organs mainly liver and/or lungs. After localizing in an organ, the parasite develops into larval hydatid cyst (stage 4) as unilocular fluid-filled bladder (Zhang *et al.,* 2003). These

consist of two parasite-derived layers; an inner nucleated multipotential germinal layer and an outer acellular laminated layer surrounded by a host-produced fibrous capsule. The hydatid cyst at this stage may contain numerous tiny tapeworm heads (called protoscolices) or brood capsules filling the cyst interior. Brood capsules and protoscolices evaginate from the germinal membrane. They increase in number over time via asexual or clonal reproduction. In addition, daughter cysts of variable sizes are often detected.

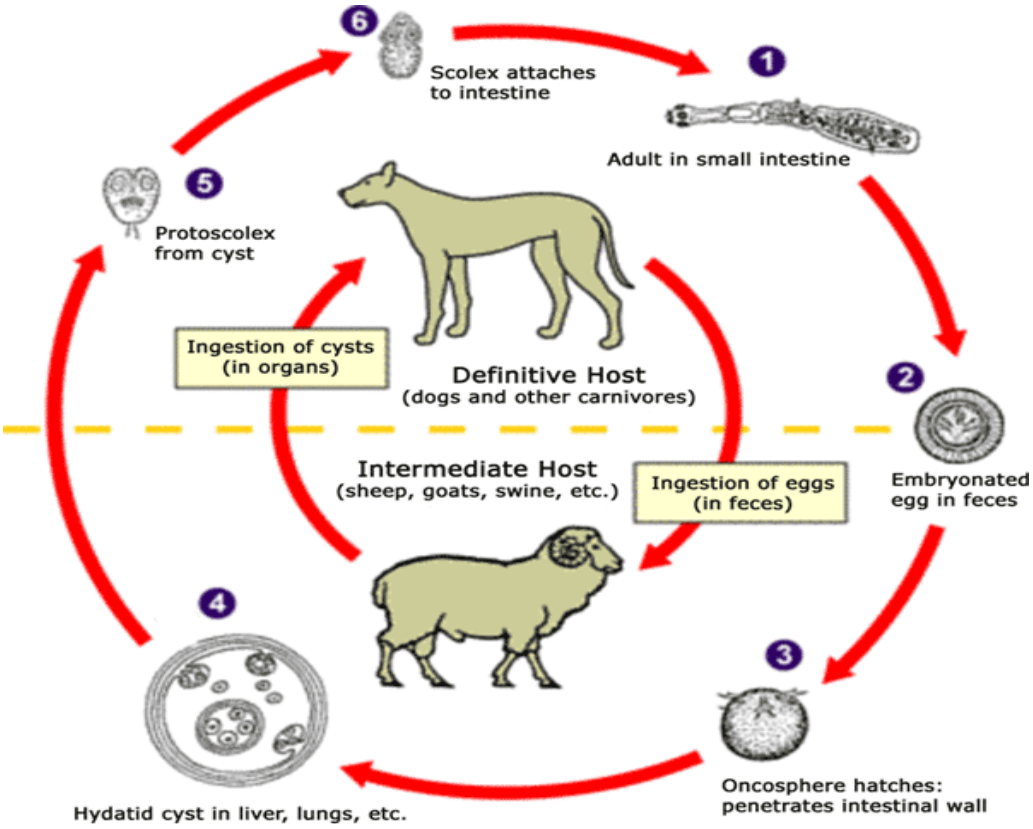

Figure 2.2: Life cycle and cycle of transmission of *Echinococcus* species. **Source:** www.dpd.cdc.gov/dpdx [Last updated: 20.07.2009] [Date accessed: 19.03.2011]

The growth rate of cysts is highly variable and may depend on strain differences (Eckert *et al*., 2001); however they all share the unique hermaphroditic and clonal reproduction systems (Casulli *et al*., 2012). The larvae of *Echinococcus granulosus* s.l. enlarge in size in connection with the asexual reproduction of scolices in the bladder-like cyst. Despite the primary infection route, a secondary echinococcosis can occur within an intermediate host. Secondary infection is caused by spontaneous trauma or during medical interventions where the larval tissue proliferates after being spread from the primary site of the metacestode (Thompson and McManus, 2001).

The life cycle is completed when infected intermediate hosts are eaten by definitive host(s). The ingested scolices (stage 5 and 6) attach to the intestinal mucosa and develop into egg-producing adult tapeworms consisting of a chain of proglottids with genital organs (Nakao *et al*., 2010a). Proglottids and/or eggs released from the adult worm initiate new life and transmission cycles.

2.3 Economic Importance of Cystic Echinococcosis

Global disease burden of CE in terms of disability adjusted life years (DALYs) in human compares favourably with other disease conditions such as onchocerciasis and the chagas disease and close to the African trypanosomosis (Budke *et al*., 2006). As a parasite, its infection of a host arouses immunological response. Cysts develop slowly but gradually get bigger causing considerable pressure and pain at affected part(s) (AMREF, 2007).

In human, CE is manifested in systemic immunological reactions like urticaria, asthma, anaphylaxis or membranous nephropathy. Asymptomatic CE is quite

common and may remain symptom-free for many years (Pawlowski *et al*., 2001). Consequent effect of cystic hydatid in livestock is shown in retarded growth rate, decreased milk yield and reduced resistance to harsh environmental conditions and consequent loss in market value of infected carcasses. Torgeson and Craig (2011) and Budke *et* al. (2006) estimated global annual livestock production losses to be about US$ 2 billion This high value stems from losses associated with carcasses and visceral condemnations, market value and reduced productivity. *Echinococcus* is known to infect all internal organs but with particular preference for liver and lungs (Varcasia *et al*., 2007). An extreme case was reported of the parasite found in the eyeball of human patient in Turkana, Kenya (AMREF, 2007).

Cystic echinococcosis is considered an emerging zoonotic disease in various regions including the Middle East, Central Asia, and Northern and Eastern Africa (Eckert *et al.*, 2001). In these areas, CE in humans is a significant public health problem among pastoralists; the Turkana and Maasai communities in Kenya are such examples. Three decades ago French and Nelson (1982) reported a prevalence of the disease in the Turkana district in Kenya as 220/100,000. It has been shown to be one of the places with highest incidences of CE in the world. Despite considerable management of the epidemic, hydatid disease has been persistent in this region. AMREF (2007) reported of a hydatid cyst measuring 26 litres removed from a patient's abdomen in Turkana. The disease burden had not been the focus of most studies done in Kenya in the past but it is obvious from reported prevalence that infected populations could not evade the burden of the disease. It poses substantial human health problem and significant negative economic effect on the livestock

industries in some of the most socioeconomically fragile countries (Budke *et al.*, 2006).

2.4 Diagnosis of Cystic Echinococcosis

Correct diagnosis of a disease is a significant step in its treatment and/or control efforts. However, this depends on understanding of the aetiological agent and the manifestation of the disease. Complex epidemiology of some disease conditions is a challenge that has befallen the effort to control diseases in animals and human.

Diagnoses of CE in living definitive hosts involve purgation, immunodiagnostics as well as necropsy approaches (Eckert *et al.*, 2001). The definitive host (dog) can be purged with arecoline hydrobromide (parasympathomimetic drug) that induces purgation which carries the worm with the faeces. There are two immunodiagnostic methods, namely; coproantigen which involves detection of parasite antigen in faeces using enzyme-linked immunosorbent assay (ELISA) and serum antibody detection using *E. granulosus* antigen preparations in ELISA. These methods have variable sensitivity and specificity. However, detection of specific antigen(s) in faecal samples from definitive hosts has the advantage over serum antibody detection in the high probability of correlation with current infection.

At necropsy, the main focus is the detection of the adult worm or the eggs in the small intestine of the host. For differential diagnosis the parasite is observed under microscope. It is about 2 mm-6 mm long, typically with 3 proglottids (up to 6). It has a genital pore usually posterior to the middle of the proglottids and a uterus with lateral sacculations. Alternatively DNA from small parasite materials obtained by the

above methods can be amplified to molecularly differentiate the aetiological agent (Wachira *et al.,* 1993).

The diagnosis of CE in intermediate hosts has been based mainly on necropsy findings (Eckert *et al.,* 2001). Clinical symptoms, usually mild manifestations, may be overlooked. Ultrasound examination for cystic structures in organs may be used for the diagnosis in smaller animals, such as sheep and goats (Sage *et al.,* 1998).

Immunological tests such as serum antibody detection and detection of circulating antigens for the diagnosis of *E. granulosus* metacestodes in animal intermediate hosts have been used. These diagnosis techniques are less sensitive and specific in animals than in human. Variation in the pathogenicity of strains/species of *Echinococcus* also influences the prognosis in animals (Eckert *et al.,* 2001).

To overcome the influence of strain and host animal factors on the diagnosis of cystic echinococcosis, recent approaches have been based on molecular analyses of genomic segments of the parasite. This enables differentiation of the various aetiological species and strains. Development of the polymerase chain reaction to amplify very minute genetic materials has fast driven the new molecular diagnostic approach. These include: PCR used in amplifying target sequences such as the 12S rRNA gene using specific primers (Bart *et al.,* 2006; Varcasia *et al.,* 2006; Dinkel *et al.,* 2004; Thompson and McManus, 2001 and Dinkel *et al.,* 1998). Polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) of ribosomal DNA (rDNA) such as the internally transcribed spacer (ITS 1 and ITS 2), mitochondrial DNA (mtDNA) such as *nad* 1 and *cox* 1 or other genomic regions have also been used (Hüttner *et al.,* 2009; Hüttner *et al.,* 2008 and Wachira *et al.,* 1993).

Single-strand conformation polymorphism (SSCP) has also been used. SSCP is a simple mutation scanning method with the potential to discriminate DNA sequence differing by a single nucleotide (Jabbar *et al.,* 2011 and Thompson and McManus, 2001). Sequencing of DNA in the above mentioned genomic segments are also useful (Casulli *et al.,* 2012; Hüttner *et al.,* 2009; Maillard *et al.,* 2007; Varcasia *et al.,* 2007; Bart *et al.,* 2006 and Dinkel *et al.,* 2004).

2.5 Prevalence of Cystic Echinococcosis

Compared with other parts of the world, the epidemiology of CE in sub-Saharan Africa is not fully understood despite the high incidence rates of human CE in some areas (Magambo *et al.*, 2006; Eckert *et al*., 2001 and Macpherson, 1983). Most data on *Echinococcosis* in sub-Saharan Africa have been gathered in the second half of the 20th century but there is no doubt that this parasite was present much earlier (Hüttner and Romig, 2009).

Global accounts show that *Echinococcus* spp. has varied distribution across all continents with varying prevalence or severity. 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.

In countries surrounding Kenya, Berhe (2009) recorded 32.1% prevalence of bovine hydatidosis in Ethiopia. Ibrahim *et al*. (2011) reported CE prevalence as 29.7% in camels, 2.6% in cattle and 0.6% in sheep in Sudan. In Tanzania, Ernest *et al*., 2004

recorded 48.7% in cattle, 63.8% in sheep and 34.7% in goats. Elsewhere, Varcasia *et al.* (2007) reported prevalence of *Echinococcus* in sheep and goats in Greece to be 30.4% and 14.7%, respectively. Higher prevalence was reported by Varcasia *et al.* (2006) in Sardinia to be 75.3% in sheep, 41.5% in cattle and 9.4% in pigs.

Hydatid cysts infect almost all internal organs of human beings and livestock, including liver, lungs, kidney, spleen and heart (Jabbar *et al*., 2011; Berhe, 2009; Varcasia *et al.,* 2007 and Varcasia *et al.,* 2006). It was also found in the eye ball (AMREF, 2007). The number of hydatid cysts in the various visceral organs in these studies however, varies between species, within species and even in an animal. The lungs appear to be the most preferred location of the parasite in infected animals (Berhe, 2009; Varcasia *et al.,* 2007 and Varcasia *et al.,* 2006). In high prevalent areas, average intensity of the parasite in livestock can be about 7.01 cysts Varcasia *et al.* (2006).

2.6 Diversity of *Echinococcus* species

Until recently, diversity of the parasite *Echinococcus* spp. has been studied using conventional means based on phenotypic properties (Brown *et al*., 1979). Multicellular parasites have traditionally been classified based on morphological properties which provide a prerequisite wealth of knowledge to differentiate aetiological agents isolated from humans and animals (Nakao *et al.,* 2010a). This approach served scientists well in fair understanding of parasites and their corresponding diseases. This approach did not provide enough informative insight to clearly define their genetic diversity. Nonetheless, attempts were made to classify the

parasite based on observed phenotypic differences (Rausch and Bernstein, 1972; Rausch, 1967; Verstr, 1965 and Williams and Sweatman, 1963). However, the delineation of sibling or cryptic species is a difficult challenge in morphological taxonomy (Nakao *et al.,* 2010a).

In addition to identifying species, exploring intraspecific variations has become a scientific imperative to characterize local populations of parasites. This is necessary to understand the different clinical manifestations and also enhance diagnoses and consequent control. As postulated by Nakao *et al.* (2010a) understanding of the biodiversity of parasitic organisms requires combination of their morphological taxonomy, molecular genetics and evolutionary ecology.

The techniques of choice in recent times are molecularly-based involving the study of the parasite at the genetic or molecular levels courtesy of the advances in the field of biochemistry and molecular biology. Recent advances in biochemical tools for DNA amplification and sequencing have provided a basis for the development of molecular taxonomy (Nakao *et al*., 2010a). This approach involves analyses of polymorphic regions of mitochondrial DNA such as the *nad*-1 and *cox* 1 and nuclear DNA like the ITS 1 and ITS 2. The 12S rRNA gene of the parasites genome has also been used (Simsek *et al.,* 2011; Casulli *et al.,* 2010; Hüttner and Romig, 2009; Maillard *et al.,* 2007; Schneider *et al.,* 2008; Varcasia *et al.,* 2007 and Dinkel *et al.,* 2004).

Mitochondrial DNA (mtDNA) has been shown to be the best informative target to differentiate closely related taxa compared to nuclear DNA. This is because mtDNA evolves rapidly, its haploid, has multicopy, it is non-recombining and it is maternally

inherited (Brown *et al*., 1979). For instance, Maillard *et al*. (2007) observed that the *act* II and *hbx* 2 nuclear genes were unable to differentiate between the common sheep and Tasmanian sheep strains but *nad*-1 and *cox* 1 gave clear strain differences.

Molecular analyses of *cox* 1 and *nad*-1 showed that *E. multilocularis, E. vogeli, E. oligarthrus* and *E. granulosus* maintain their genetic identities (Lavikainen *et al*., 2003; Scott *et al*., 1997; Bowles and McManus, 1993 and Bowles *et al*., 1992). *E.chinococcus granulosus* sensu lato is a complex of species/genotype whose biological diversity has been designated as strains. 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 mtDNA 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 species status of *E. canadensis* is still controversial while *E. granulosus* sensu stricto has been shown to have been over-simplified. In recent time, microvariants within the *E. granulosus* sensu stricto subspecies are being exploited and shown to have diversity (haplotypes) among species and populations in Europe and Asia.

Casulli *et al*. (2012) detected 24 and 7 haplotypes based on the *cox*-1 sequences in livestock and human populations in Eastern Europe and Italy, respectively. Earlier studies by Nakao *et al.* (2010b) in China reveal 43 haplotypes in the *E. granulosus* complex. In both studies, the G1 genotype was found to be the predominant hyplotype which they attribute to founder effect of the genotype.

It is obvious that scientists are striving to enumerate and appropriately characterise *Echinococcus* spp among livestock, human, pets and wildlife using molecular tools and techniques. This drive is worldwide with the African continent not left out. Table 2.1 summarises published accounts of cystic echinococcosis in Africa. There is evidence that there is rapid growth of scientific data on the epidemiology and genetic diversity of the parasite *Echinococcus* in Africa.

Table 2.2: Reported species/genotypes of *Echinococcus granulosus* s.l. from Africa

Country	Genotype	Host species	Source
Kenya	G1	Cattle, camel, goat, sheep, pig, human	Dinkel *et al*. (2004) and Wachira *et al*. (1993)
	G5	Pig, cattle	Mulinge *et al*. (2011) and Dinkel *et al*. (2004)
	G6	Cattle, camel, goat, pig, human	Casulli *et al.* (2010), Dinkel *et al*. (2004) and Wachira *et*
Algeria	G1	Cattle, sheep, camel, human	Maillard *et al*. (2007)
	G2	Sheep, camel, human	
	G6	Camel	
Mauritania	G6	Cattle, camel, human	Maillard *et al*. (2007)
Ethiopia	G1	Cattle, sheep	Maillard *et al*. (2007)
Libya	G1-G3	Cattle, human	Abushhewa *et al.* (2010)
	G6-G10	Cattle, camel	
Sudan	G5	Cattle	Dinkel *et al*. (2004)
	G6	Cattle, camel, sheep	Ibrahim *et al*. (2011), Omer *et al*. (2010) and Dinkel *et*
Uganda	*E. felidis*	Lion, spotted hyena, warthog	Hüttner *et al*. (2009)
	G1	Warthog	
Namibia	G5	Zebra	Obwaller *et al*. (2004)
South Africa	*E. felidis*	Lion	Hüttner *et al*. (2008)

2.7 Control of Cystic Echinococcosis

Control of CE in populations requires multiple strategies including chemotherapy, immunisation, surgery (in humans) and population control of dogs, sanitation and education (Pawlowski *et al.,* 2001 and Eckert *et al.*, 2001). In most communities, control policies employ more than one strategy for maximal effect but the core has been chemotherapy. Several groups of drugs including, cytostatics, antibiotics, sulphonamides, antiprotozoal compounds and several anthelmintic drugs have been tested for their efficacy against the metacestode of *Echinococcus*.

The most promising results were obtained with anthelmintics of the benzimidazole group. Benzimidazole (albendazole and mebendazole) can be used alone or with praziquantel in control of *Echinococcus* spp in livestock (Eckert *et al.,* 2001). Albendazole works by stopping the cysts from growing and eventually destroying it (AMREF, 2007). Mebendazole works by preventing the cyst from absorbing glucose which leads to loss of energy and subsequent death. Praziquantel induces severe spasms and paralysis of the worm.

It is now well known that the density-dependent constraint in the transmission cycle of *E. granulosus* is acquired immunity of the intermediate hosts. As a result, immunisation approaches are being sought. Recombinant technology is the technology of choice currently being applied to develop vaccine against *E. granulosus*. The recombinant *E. granulosus* (Eg95) vaccines gave promising results in sheep (up to 98% protection) (Eckert *et al.,* 2001 and Gemmell and Roberts, 1995).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Areas Description

The study covered Turkana and Maasailand. Turkana region (70 000 km^2), is located in North-Eastern Kenya (latitude 00°38′ 0.00′′N and longitude 33°29′ 0.00′′E); Turkana also borders Ethiopia, Sudan and Uganda (Figure 3.1). The region is characterised by a semi-arid ecology, mainly populated by the Turkana Nilotic pastoralists. They are nomads with herds of camels, cattle, goats and sheep. They have a livestock population of 848,780 cattle; 1,834,733 sheep and 3,148,113 goats (Kenya Open Data, 2011). Settlements among the Turkana are temporary *manyattas*. The people live in close proximity to their livestock and dogs (Casulli *et al.,* 2010).

Maasailand-Kenya occupies the South-Western section (40 000 km^2) of the Republic of Kenya. It is found at the Southern part of the vast Rift valley bordering Tanzania to the south. The Narok District (latitude 1°4′ 59.99′′S and longitude 35°52′ 0.12′′E) and the Kajiado District (latitude 2°0′ 0′′S and longitude 36°52′ 0.12′′) were selected to represent Maasailand, Kenya. Narok District has livestock population of 957,780 cattle; 1,465,249 sheep and 729,722 goats. Kajiado District also has livestock population as follows: 216,339 cattle; 326,299 sheep and 249,654 goats (Kenya Open Data, 2011). Average rainfall in the area varies from 500 mm to 1,800 mm a year. The basic economic and social unit is the *enkang*, a semi-permanent settlement of several families pasturing their stock together. There are 10 to 20 huts surrounded by a thorn or *leleshua* fence into which the livestock are driven at night. Livestock

supply the Maasai their main needs, milk, blood and meat for food, skin for leather items and dung for building materials (Evangelou, 1984 and Kaplan *et al*., 1976).

Figure 3.1: *Echinococcus* cyst sampling sites: Kajiado, Narok and Turkana Districts. **Source:** Adapted from the National Environment Management Authority (2011).

3.2 Ethical Considerations

The study was carried out in the consortium; Cystic Echinococcosis in sub-Saharan Africa Research initiative (CESSARi). It a collaborative research initiative between Kenyan institutions (KEMRI and AMREF) and German institutions (University of Hohenheim and University of Ulm). Proposal and ethical approval to carry out the study in Kenya was granted to the principal investigator of CESSARi (Dr. Peter Kern) by the Scientific Steering Committee (SSC) and the Ethics Review Committee (ERC) of KEMRI under the protocol number SSC-1684 (Appendix I, II and III).

3.3 Sample Size Estimation

The following formula was used to estimate the sample size of livestock to be sampled from Maasailand and Turkana study areas (Kasiulevičius *et al.*, 2006). Where $\boldsymbol{n}$ = sample size, $\boldsymbol{z}$ = Z statistic for 95% confidence interval (1.96), $\boldsymbol{p}$ = expected prevalence and $\boldsymbol{e}$ = precision or confidence or risk level (0.05).

$$\boldsymbol{n = \left[\left(\frac{z}{e}\right)^2 \ (p - p^2)\right]}$$

The calculated Maasailand sample size was 124 cattle, 114 sheep and 104 goats, based on the reported CE prevalence by Macpherson (1985): 8.9% in cattle, 8.1% in sheep and 7.1% in sheep. The calculated Turkana sample size was 240 cattle and 65 goats based on the reported CE prevalence by Njoroge *et al.* (2000): 19.4% in cattle and 4.5% in goats.

3.4 Study Animals

The study covered all age categories of both male and female cattle, sheep and goat slaughtered at the visited slaughter houses. The survey was carried out at three slaughter houses in Maasailand (1 at Kitengela and 2 at Suswa) in October 2011 and at one slaughter house in Lomidat, Turkana, between February 2010 and February 2011. In Maasailand, 587 cattle, 430 sheep and 194 goats were sampled while in Turkana, 97 cattle and 73 goats were sampled. The number of cattle from Turkana was 143 less than the calculated sample size in section 3.3, due to lower slaughter volume and irregular slaughter routine at the slaughter house in Lomidat.

The Kitengela slaughter house sourced its animals from Kitengela Township, Bissili, Magadi, Isinya, Garisa, Athi River, Embakasi, Eldoret, Kakamega, Dagoretti and Kiserian whereas the slaughter houses in Suswa sourced their animals from Suswa Township, Ntulele, Bomet, Narok. Lomidat slaughter house sourced its animals from Mogila, Natamakaruo, Lokangae, Lokwanamur, Lokichogio, Abutungunan and Lopiding, Naporoto, Nakeikar, Songot, Loteteleit, Nabera, Loriemet, Lochereikope and Nakeruman. However, it must be acknowledged that true origin of animals investigated in all slaughter houses was difficult to trace since middlemen were the ones who sent animals to the slaughter house. They could only trace their animals to the last client from whom they purchased.

3.5 Sampling and Sample Storage

The data collected on animals prior to slaughter were livestock species, sex and estimated age based on their dentition and animal origin. Age estimation was done with the assistance of veterinarians at the slaughter houses. Animals were subsequently marked for easy traceability to their carcasses during meat inspection.

The liver, lungs, heart, kidney and spleen of carcasses were inspected by observation, palpation and systematic incision for the presence of *Echinococcus* cysts (Njoroge *et al.*, 2002). *Echinococcus* cysts identified were removed whole and collected in polythene bags. One polythene bag was used for hydatid cyst(s) obtained from each infected animal (243 in Maasailand and 17 in Turkana) and was labelled according to the carcass' mark. The cysts were transported to the AMREF-Nairobi, laboratory for microscopic examination.

Morphotype of cysts examined was determined as fertile (115 cysts) containing protoscolices or non-fertile (791 cysts) containing no protoscolices. The non-fertile cysts were further classified as sterile, degenerated or calcified (Varcasia *et al.*, 2006). Fertile and non-fertile cysts were further processed by extracting the cyst wall (germinal layer) and/or protoscolices. Extracted germinal layer was washed in normal saline and together with its protoscolices fixed in 70% (v/v) ethanol (Jabbar *et al.*, 2011).

3.6 Genetic Characterisation

The genetic characterisation was carried out partly at the laboratories of the Centre for Microbiology Research, Kenya Medical Research Institute, Kenya and the Fachgebiet Parasitologie, Universität Hohenheim, Stuttgart, Germany.

3.6.1 DNA Extraction

DNA was extracted from the ethanol preserved germinal layer or protoscolices using the method of Nakao *et al.* (2003). It involves pipetting protoscolices (1 to 3) or broken tissue pieces under dissecting microscope with ≤ 1µl volume into micro-tubes containing 10 µl 0.02 M NaOH. The mixture was then lysed at 95 ^{o}C for 10 min in the Applied Biosystems GeneAmp PCR System 9700 Thermal Cycler. The resultant crude DNA (lysate) was directly used as template in the polymerase chain reaction assay of the *nad*-1 gene as described in section 3.4.2.

Failure of the above method to give adequate DNA (in some calcified cysts) necessitated genomic DNA extraction from preserved tissues using the method of Dinkel *et al.* (2004). In this method, about 0.5 g cyst wall (germinal layers) were cut into small pieces and washed in distilled water to get rid of the ethanol. The clean minced tissues were digested at 56 ^{o}C for 3 h in the presence of 2 mg/ml proteinase K in 500 µl of 10 mM Tris –HCl (pH 7.5), 10 mM EDTA, 50 mM NaCl, 2% sodium dodecyl sulphate and 20 mM dithiothreitol. DNA was extracted using phenol–chloroform-isoamyl alcohol (25:24:1) with subsequent ethanol (-20 ^{o}C) precipitation. The DNA was washed in 70% ethanol, dried and dissolved in 100 µl nuclease free water. Dissolved DNA was used as template in the PCR assay described in 3.4.2.

Genomic DNA of characterised *Echinococcus granulosus* G1, *Echinococcus ortleppi* and *Echinococcus canadensis* G6 obtained from Kenya, Vietnam and Sudan, respectively were used as positive control.

3.6.2 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 (Table 3.1) previously used by Hüttner *et al*. (2009). In both reactions a 50 µl reaction mixture was made up of DNase/RNase free water, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2 mM $MgCl_2$, 200 µM of each dNTPs, 12.5 pmol of each primer and 1.25 U Taq polymerase.

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 GeneAmp PCR System 9700 Thermal Cycler. Reactions were run for 35 cycles with cycling conditions as follows: denaturation (94 ^{o}C for 30 s), annealing (55 ^{o}C for 30 s), elongation (72 ^{o}C for 1 min) and then post cycling final elongation (72 ^{o}C for 5 min).

Table 3.1: Primer pairs used in the PCR assay in this study

Target gene	Primers for PCR (5'- 3')
***nad*-1 mtDNA**	***F:**TGTTTTTGAGATCAGTTCGGTGTG
	***R:**CATAATCAAACGGAGTACGATTAG
	****F:**CAGTTCGGTGTGCTTTTGGGTCTG
	****R:**GAGTACGATTAGTCTCACACAGCA

Source: Hüttner *et al.* (2008). **F:** Forward primer; **R:** Reverse primer. Asterisks (*/**) are primer pairs for the primary and secondary PCR, respectively.

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.

3.6.3 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 ^{o}C for 3 h in the Applied Biosystems GeneAmp PCR System 9700 Thermal Cycler and enzyme inactivated for 20 min at 65 ^{o}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).

3.6.4 Partial DNA Sequencing of *nad*-1

Confounding banding patterns were resolved by partial sequencing of the *nad*-1 gene (Seqlab GmbH, Göttingen). The DNA was partially sequenced using the reverse internal primer in the Sanger dideoxy method. The DNA sequences were manually edited using the GENtle 1.9.4. program (Manske M. 2003, University of cologne, Germany). After editing, short clean DNA sequences obtained were compared with existing sequences in the GenBank databases using the BLAST (www.blast. ncbi.nlm.nih.gov/Blast.cgi).

3.7 Statistical Analysis

Statistical analysis was carried out using Epi Info 3.4.3. Data was first entered in Microsoft Office Excel spread sheet (2007) 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* cyst were then determined. Odds ratio (OR) and chi-square test (x^2) of male to female exposure risk to infection

by *Echinococcus* cysts were also determined. Tabulation of results and drawing of graph were done in Microsoft Office Excel

CHAPTER FOUR

4.0 RESULTS

4.1 Number, Sex and Age of Livestock Investigated

Three livestock species were investigated in this study, name: cattle, sheep and goat. A total of 1381 livestock were covered (Table 4.1). The Suswa slaughter houses had a sum record of 334 cattle (225 males and 109 females). The Kitengela slaughter recorded 253 cattle (135 males and 118 females), 430 sheep (280 males and 150 females) and 194 goats (119 males and 75 females). The Lomidat slaughter recorded 97 cattle (96 males and 1 female) and 73 goats (all males). Age range of cattle slaughtered at the Suswa and Kitengela (Maasailand) slaughter houses was 2 to 6 years. Slaughter sheep and goats at the Kitengela slaughter also had age range of 2 to 4 years. Cattle slaughter at the Lomidat slaughter house had age range of 5 to 6 years while goats were all 3 years old.

Mean and/or mode age of slaughtered cattle at the Suswa slaughter houses was 3 years in males and 4 years in females. Mean and/or mode age of slaughtered cattle at the Kitengela slaughter houses was 4 years in males and females. Among sheep slaughtered at the Kitengela slaughter house, mean age was 3 years in male and 2 years in females while the mode age was 3 years in males and 2 years in females. Mean and/or mode age of slaughtered goats at the Kitengela slaughter house was 2 in males and 2 years in females. The Mean and/or mode age of slaughtered male cattle at the Lomidat slaughter house was 5 years. The only female was also 5 years old.

Mean mode or median age of slaughtered goats at the Lomidat slaughter house was not assessed since they had same age (3 years).

Table 4.1: Number, sex and age of livestock species investigated

Slaughter house location	Livestock species	Sex (No.)	Age (years)				
			Range	Mean	Mode	Median	Standard deviation
Suswa*	Cattle	M (225)	2 - 6	3.25	3	3	0.99
(n = 334)	(n = 334)	F (109)	2 - 6	3.95	4	4	0.99
Kitengela*	Cattle	M (135)	2 - 6	4.01	4	4	0.85
(n = 877)	(n = 253)	F (118)	2 - 6	4.03	4	4	0.89
	Sheep	M (280)	2 - 4	2.60	3	3	0.59
	(n = 430)	F (150)	2 - 4	2.52	2	2	0.55
	Goat	M (119)	2 - 4	2.48	2	2	0.69
	(n = 194)	F (75)	2 - 4	2.36	2	2	0.48
Lomidat**	Cattle	M (96)	5 – 6	5.25	5	5	0.44
(n = 170)	(n = 97)	F (1)	5	5.00	5	5	-
	Goat	M (73)	3	3.00	3	3	-
	(n = 73)	F (0)	0.00	0.00	0.00	0.00	-

*Sampling sites in Maasailand. **Sampling site in Turkana. **M:** Male animal and **F:** Female animal.

4.2 Prevalence of Cystic Echinococcosis in Livestock from Maasailand

General prevalence levels were as follows: 25.8% (151/587) in cattle, 16.5% (71/430) in sheep and 10.8% (21/194) in goats (Table 4.2). Age specific prevalence levels among cattle were 22.4% (17/76) at 2 years, 21.7% (34/157) at 3 years, 22.9% (55/240) at 4 years, 38.9% (35/90) at 5 years and 41.7% (10/24) at 6 years. Age specific prevalence levels among sheep were 15.7% (32/204) at 2 years, 17.4% (36/207) and 15.8 (3/19) at 4 years. Age specific prevalence levels among goats were 8.1% (10/123) at 2 years, 17.2% (10/58) and 7.7 (1/13) at 4 years.

Table 4.2: Prevalence of cystic echinococcosis in livestock from Maasailand

Livestock species	**General prevalence (95% CI)**	**Age specific prevalence**		
		Age (yrs)	**Number**	**Prevalence (%)**
Cattle	25.8% (12.3 – 29.5)	2	76	22.7
(n = 587)		3	157	21.7
		4	240	22.9
		5	90	38.9
		6 or more	24	41.7
Sheep	16.5%	2	204	15.7
(n = 430)	(13.2 – 20.4)	3	207	17.4
		4	19	15.8
Goats	10.8%	2	123	8.1
(n = 194)	(6.8 – 16.1)	3	58	17.2
		4	13	7.7

CI: Confidence interval.

Chi-square (x^2) greater than 3.841 indicate that there is an association between sex of animals and rate of infection with *Echinococcus* cyst. Chi-square (x^2) less than or equal to 3.841 indicate that there is no association between sex of animals and rate of infection with *Echinococcus* cyst (Table 4.3). Odds ratio greater than 1 means sex of animal is a risk factor to infection by *Echinococcus* cyst. Odds ratio less than 1 means that sex of animal is a protective factor from infection with *Echinococcus* cyst. Odds ratio equals to 1 indicate there is no association between the sex of male and the rate of infection by *Echinococcus* cyst.

Male cattle (bulls) are about twice more often infected with *Echinococcus* cysts than their female (cows) counterparts. Male sheep (rams) are about three times often infected with *Echinococcus* cysts than the female sheep (ewes) (Table 4.3). Goats on the other hand had x^2 of 3.48 indicating no association between sex and *Echinococcus* cyst infection rate, despite the 1.63 odds ratio.

Table 4.3: Chi-square test and odds ratio of male to female exposure to infection by *Echinococcus* cysts in Maasailand

Livestock species	**Odds ratio (Male/female)**	**Chi-square test (x^2)**	**p-value**
Cattle	1.67	7.22	0.007
Sheep	2.91	5.37	0.021
Goats	1.63	3.48	0.062

Chi-Square value from probability table = 3.841 at df = 1 and p = 0.05

Total number of cysts isolated was 829: 614 from cattle, 164 from sheep and 51 from goats. Abundance of *Echinococcus* cysts (number of cysts per total number of livestock species sampled) in the animals' populations was noted as 1.05 (614/587) among cattle, 0.38 (164/430) among sheep and 0.26 (51/194) among goats. Amount of cyst harboured by animals varied from 1 to 16 cysts (Table 4.4). Among infected cattle: 75/151 harboured 1 cyst, 47/151 harboured 2-5 cysts, 21/51 harboured 6-10 cysts, 5/151 harboured 11-15 cysts and 3/151 harboured 16 cysts. Among infected sheep: 34/71 harboured 1 cyst, 28/71 harboured 2-5 cysts, 5/71 harboured 6-10 cysts, 2/71 harboured 11-15 cysts and 1/71 harboured 16 cysts and 1/71 harboured 34 cysts. Among infected goats: 10/21 harboured 1 cyst, 10/21 harboured 2-5 cysts while 1/21 harboured 19 cysts.

Table 4.4: *Echinococcus* cyst load in infected livestock from Maasailand

Livestock species	**Number of *Echinococcus* cysts**				
	1	**2 – 5**	**6 – 10**	**11 – 15**	**16 or more**
Cattle (n = 151)	75	47	21	5	3
Sheep (n = 71)	34	28	5	2	2
Goat (n = 21)	10	10	0	0	1
Total (243)	**119**	**85**	**26**	**7**	**6**

Mean infection intensity of *Echinococcus* cysts (number of cysts per number of infected livestock species) was 4.07 (614/151) cysts/cattle, 2.31 (164/71) cysts/sheep and 2.43 (51/21) cysts/goat. Stratifying *Echinococcus* cyst infection intensity among cattle and sheep revealed that older animals harboured more cysts than younger ones (Figure 4.1). On the contrary, infection intensity of *Echinococcus* decreased with age in goats. At age 2, the small ruminants had more infection than the cattle while at age 3 infected cattle harboured more *Echinococcus* cysts than sheep and much more than goats. At age 4, the infected sheep harboured more cysts than cattle and more than twice the amount the infected goat carried. Infected cattle at age 5 harboured more cysts than those in age 4, 3, and 2 but less than those in age 6. There were no records of sheep and goats at age 5 and 6 (Figure 4.1).

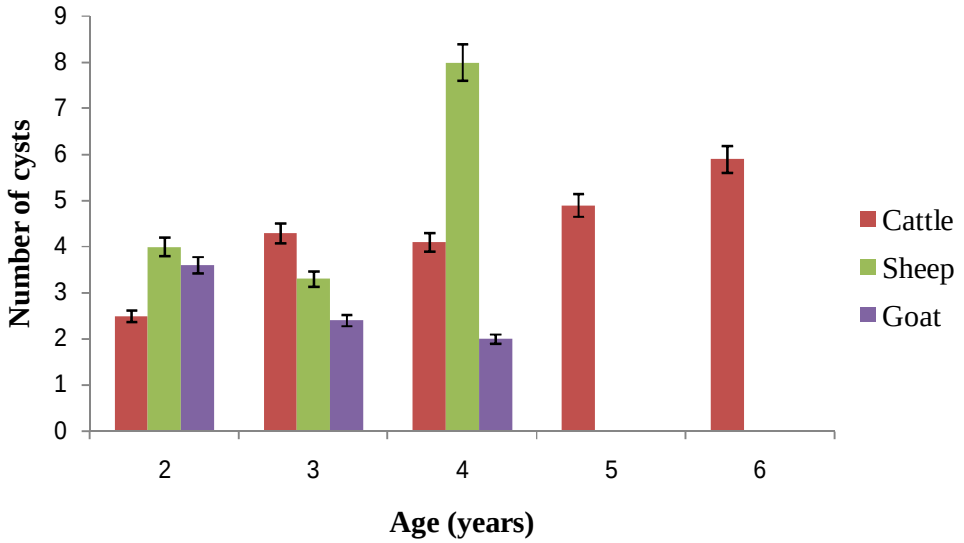

Figure 4.1: Mean infection intensity of *Echinococcus* species at different ages of cattle, sheep and goat from Maasailand. Error bar with 5% probability.

4.3 *Echinococcus* Cysts Predilection Site in Livestock from Maasailand

Echinococcus cysts occur in the liver, lungs, heart, kidney and spleen of livestock in Maasailand (Table 4.4). There were records of single and multiple organs infections with *Echinococcus* cysts. Among infected cattle, 59/151 had liver cysts, 43/151 had lungs cysts, 2/151 had heart cyst, while 45/151 had liver/lungs cysts, 1/151 had liver/spleen cysts and 1/151 had liver/lungs/kidney cysts. In the infected sheep: 42/71 had liver cysts, 14/71 had lungs cysts, 1/71 had heart cyst while 13/71 had liver/lungs cysts and 1/71 had liver/lungs/kidney/spleen cysts. Among the infected goats, 9/21 had liver cysts, 10/21 had lungs cysts and 2/21 had liver/lungs cysts. In total, animals with only liver cysts were 110/243 while those with only lungs cysts were 67/243 while animals who suffered both liver and lungs infections were 60/243. The liver was identified as main predilection site of *Echinococcus* spp. in livestock from Maasailand (Table 4.5 and 4.9).

Table 4.5: *Echinococcus* cysts predilection site in livestock from Maasailand

Diseased Livestock	**Organs infected**						
	Li	**Lu**	**Ht**	**Li /Lu**	**Li/ Sp**	**Li/Lu/Ki**	**Li/Lu/Ki/Sp**
Cattle (n = 151)	59	43	2	45	1	1	0
Sheep (n = 71)	42	14	1	13	0	0	1
Goat (n = 21)	9	10	0	2	0	0	0
Total (243)	**110**	**67**	**3**	**60**	**1**	**1**	**1**

Li: Liver; **Lu:** Lungs; **Ht:** Heart; **Ki:** Kidney; **Sp:** Spleen.

4.4 Prevalence of Cystic Echinococcosis in Livestock from Turkana

Prevalence of *Echinococcus* spp. in Turkana was 12.4% (12/97) in cattle and 6.8% (5/73) in goats (Table 4.6). In total, 54 and 23 cysts were isolated from cattle and goats, respectively. *Echinococcus* cysts abundance (number of cysts per total number of livestock species sampled) in the livestock's populations was 0.56 (54/97) among cattle and 0.32 (23/73) among goats. Mean infection intensity of *Echinococcus* cysts (number of cysts per number of infected livestock species) was 4.50 (54/12) cysts/cattle and 4.60 (23/5) cysts/goat.

Table 4.6: Prevalence, abundance and infection intensity of *Echinococcus* cyst in livestock from Turkana

Livestock species	Prevalence (95 % CI)	Abundance	Infection Intensity
Cattle (n = 97)	12.4% (6.6 -20.6)	54/97 = 0.6	54/12 = 4.5
Goat (n =73)	6.8% (1.3-15.3)	23/73 = 0.3	23/5 = 4.6

Li: Liver; **Lu:** Lungs; **CI:** Confidence interval.

The *Echinococcus* cyst load in livestock from Turkana varied from 1 to 17 cysts. Among infected cattle, 3/12 harboured 1 cyst each, 1/12 harboured 2 cysts, 3/12 harboured 3 cysts each, 2/12 harboured 4 cysts each, 1/12 harboured 5 cysts, 1/12 harboured 10 cysts and 1/12 harboured 17 cysts. Each of the five infected goats harboured 1, 4, 5, 6, 7 cysts each (Table 4.7).

Table 4.7: *Echinococcus* cyst load in infected livestock from Turkana

Livestock species	**Number of *Echinococcus* cysts**								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**10**	**17**
Cattle (n = 12)	3	1	3	2	1	0	0	1	1
Goats (n = 5)	1	0	0	1	1	1	1	0	0
Total (17)	**4**	**1**	**3**	**3**	**2**	**1**	**1**	**1**	**1**

4.5 *Echinococcus* Cysts Predilection Site in Livestock from Turkana

The study revealed that *Echinococcus* cysts occur in the liver and lungs of livestock in Turkana (Table 4.8). Among infected cattle, 3/12 had liver cysts, 2/12 had lungs cysts while 7/12 had liver/lungs cysts. Among the infected goats, 2/5 had liver cysts, 1/5 had lungs cysts, while 2/5 had liver/lungs cysts. In total, 7/17 infected livestock (cattle and goats) in Turkana had liver cysts, 3/17 had lungs cysts, while 9/17 had liver/lungs cysts. The liver was identified as main predilection site of *Echinococcus* spp. in livestock from Maasailand (Table 4.8 and 4.9).

Table 4.8: *Echinococcus* cysts predilection site in livestock from Turkana

Diseased Livestock	**Organs infected**		
	Liver	**Lungs**	**Liver /Lungs**
Cattle (n = 12)	3	2	7
Goat (n = 5)	2	1	2
Total (17)	**5**	**3**	**9**

4.6 States of *Echinococcus* Cysts in Livestock from Maasailand and Turkana

Echinococcus spp. infected organs had the parasite partly embedded in the organ tissue matrix and protruding to the surface of the organ (Figure 4.2, 4.3 and 4.4). Isolated individual cysts showed a bladder-like fluid filled appearance (Figure 4.5).

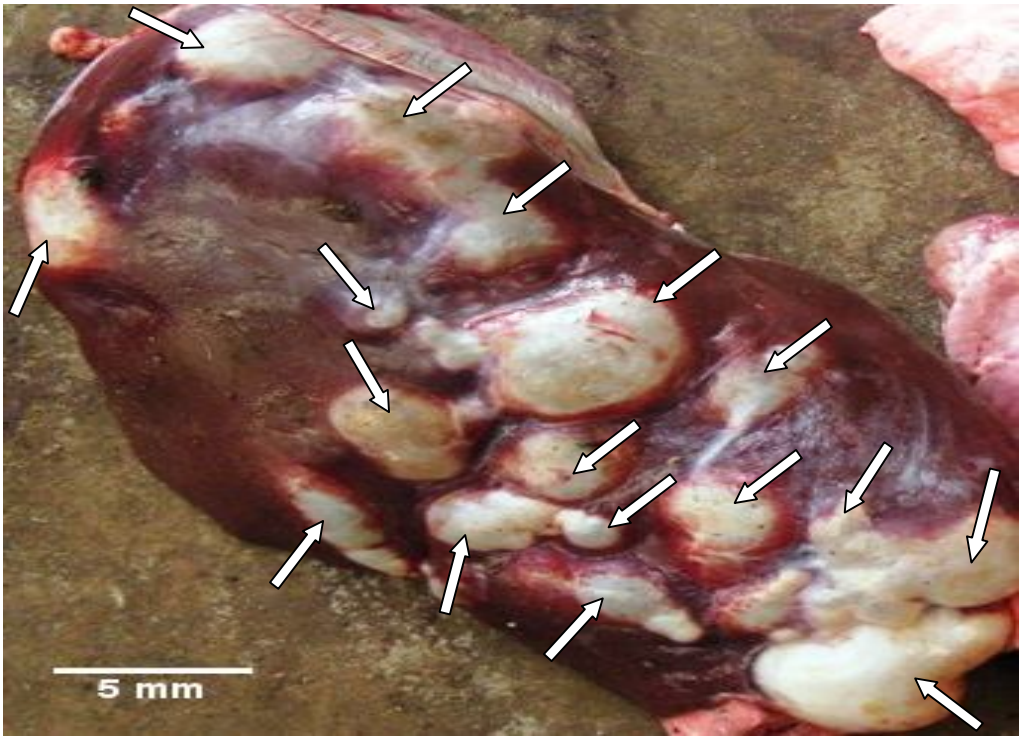

Figure 4.2: Outlook of liver of cattle from Maasailand infected with *Echinococcus* cysts. Arrows point to the positions of cysts.

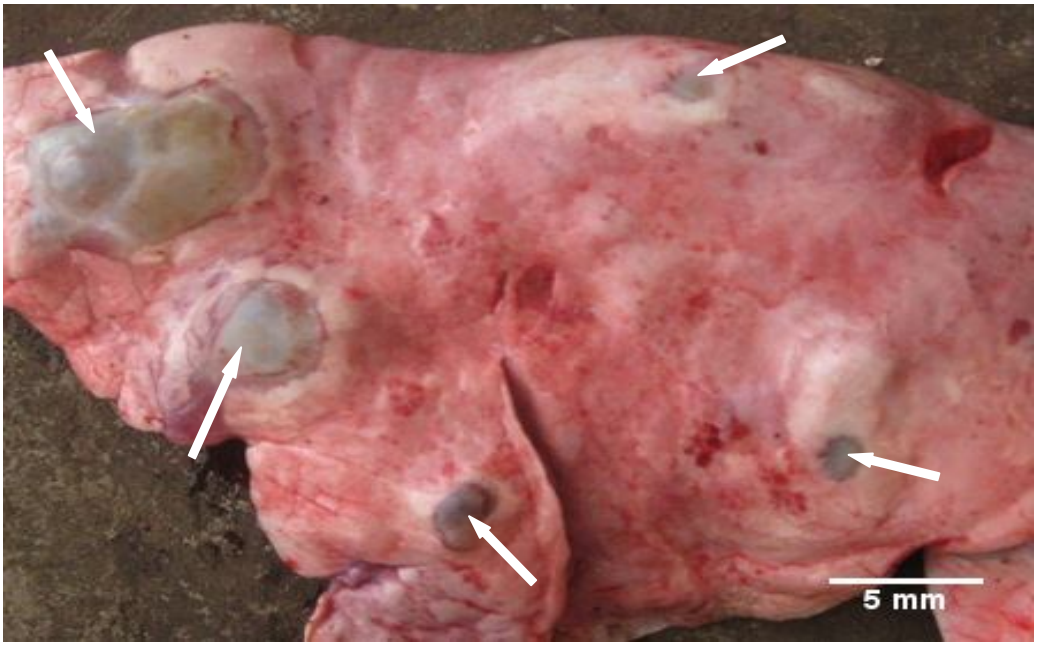

Figure 4.3: Outlook of lungs of cattle from Maasailand infected with *Echinococcus* cysts. Arrows point to the positions of cysts.

Figure 4.4: Outlook of spleen of cattle from Maasailand infected with *Echinococcus* cyst. Arrow points to the position of cyst.

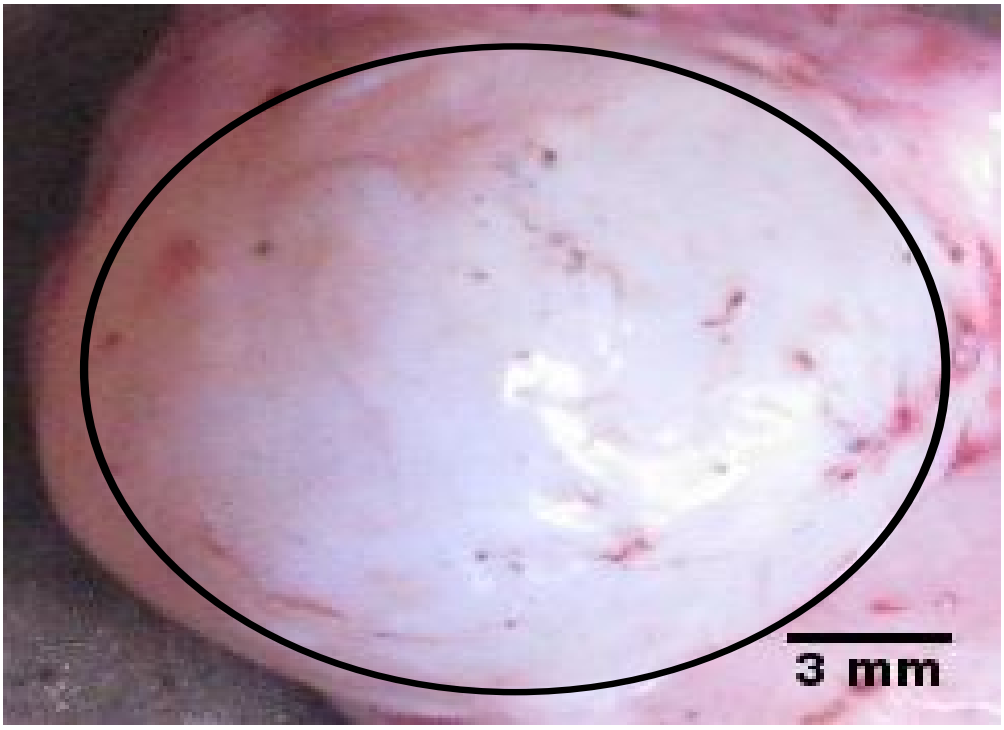

Figure 4.5: External view of an isolated *Echinococcus* cyst from lungs of cattle in Maasailand. Black outline shows estimated circumference of cyst.

Four main states of *Echinococcus* cysts were identified during the study. *Echinococcus* cysts were either fertile, sterile, calcified or degenerated (Figure 4.6 and Appendix IV and V). Cyst fertility (number of fertile per total number of cysts) in cattle from Maasailand was 6.51% (40/614) of which 30/40 were found in the lungs and 10/40 were found in the liver (Table 4.9). The non-fertile states of *Echinococcus* cyst in cattle were found in the liver (335/574), lungs (235/574), hear (2/574), kidney (1/574) and spleen (1/574). Among the infected sheep from Maasailand, cyst fertility was 25.61% (42/122) of which 26/42 were found in the liver and 16/42 were found in the lungs. The non-fertile states of *Echinococcus* cyst in sheep were found in the liver (89/122), lungs (30/122), hear (1/122), kidney

(1/122) and spleen (1/122). Cysts fertility among infected goats from Maasailand was 17.64% (9/51) all (9/9) were found in the lungs. The non-fertile cysts were found in the liver (33/42) and in the lungs (9/42).

Cysts fertility among the infected cattle from Turkana was 38.88% (21/54), of which 7/21 were found in the liver and 14/21 were found in the lungs (Table 4.9). The non-fertile cysts were found in the liver (20/33) and in the lungs (13/33). Among the infected goats from Turkana, cyst fertility was 13.04% (3/23), of which 2/3 were found in the liver and 1/3 was found in the lungs. The non-fertile cysts were found in the liver (18/20) and in the lungs (2/20).

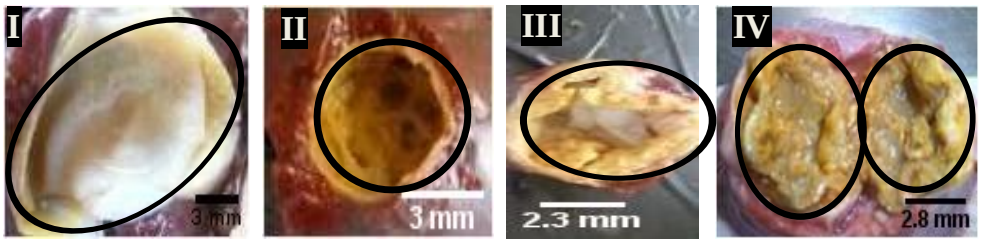

Figure 4.6: Interior look of different states of *Echinococcus* cysts (sectioned) isolated from livestock in Maasailand. Black outline shows wall of sectioned cysts. **I:** Fertile cyst; **II:** Sterile cyst; **III:** Fertile cyst with calcified endocyst; **IV:** Degenerated cyst.

Table 4.9: States of *Echinococcus* cyst isolates from livestock in Maasailand and Turkana

Livestock species	Cyst states	Maasailand						Turkana		
		Li	**Lu**	**Ht**	**Ki**	**Sp**	**Total**	**Li**	**Lu**	**Total**
Cattle	Fertile	10	30	0	0	0	**40**	7	14	**21**
	Non-fertile	335	235	2	1	1	**574**	20	13	**33**
Sheep	Fertile	26	16	0	0	0	**42**	0	0	**0**
	Non-fertile	89	30	1	1	1	**122**	0	0	**0**
Goat	Fertile	0	9	0	0	0	**9**	2	1	**3**
	Non-fertile	33	9	0	0	0	**42**	18	2	**20**

Li: Liver; **Lu:** Lungs; **Ht:** Heart; **Ki:** Kidney; **Sp:** Spleen

4.7 *Echinococcus* species from Maasailand and Turkana

Two hundred and ninety-three (293) selected *Echinococcus* cyst isolates from livestock in Maasailand (285/293) (Appendix IV) and Turkana (8/293) were differentiated to the species level. The primer pairs successfully amplified the *nad*-1 gene approximately 1073 bp to 1078 bp fragment (Figure 4.7). Digestion of the *nad*-1 amplicons with Hph I aided species differentiation except for four samples from Maasailand which were resolved by partial DNA sequencing of the *nad*-1 gene (Figure 4.8). Two out of the four samples were identified as *E. granulosus* G1 which showed single base-exchange at the Hph I binding site (Figure 4.8 – lane C; DNA sequences in Appendix VI and VII). Base-exchange was Adenine to Guanine at the nucleotide position 256 (numbered from the start codon) when compared to the *nad*-1 sequence (NC_008075.1). The two other samples were identified as *Taenia*

hydatigena from sheep and *Taenia saginata* from cattle (Figure 4.8 – lanes I and J, respectively, Appendix IV, VIII and IX).

Three taxa of *Echinococcus granulosus* s.l. were identified namely; *Echinococcus granulosus* G1, *Echinococcus ortleppi* and *Echinococcus canadensis* G6 (Figure 4.8, Table 4.10 and Appendix IV). *Echinococcus granulosus* G1 was the dominant species identified at a rate of 97.95% (287/293) in livestock from Maasailand and Turkana. The *E. ortleppi* (1/293) was identified in cattle from Maasailand. *Echinococcus canadensis* G6 taxon was identified in sheep (1/293) and goats (4/293) from Maasailand. One sheep from Maasailand (Kitengela) was co-infected with *Echinococcus granulosus* G1 and *Taenia hydatigena* (Figure 4.8 lane H and I, Appendix IV and VIII).

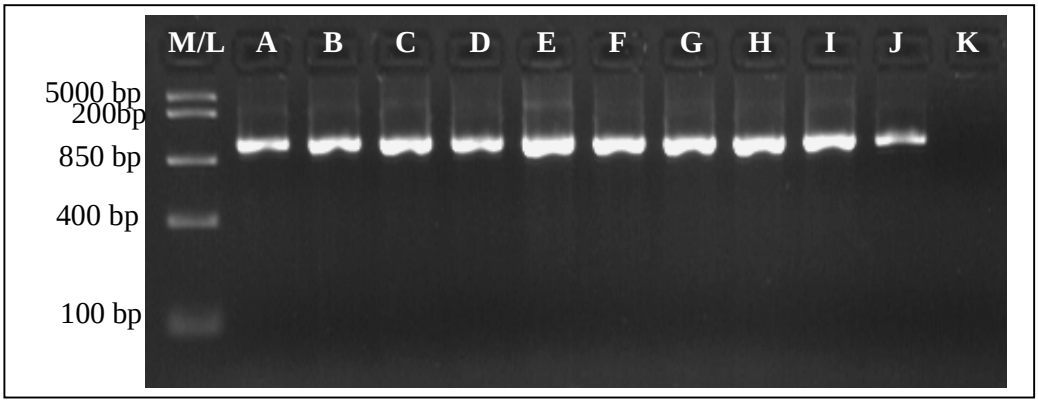

Figure 4.7: Agarose gel photo of nad-1 amplicons (~1073 – 1078 bp). **M/L:** Molecular ladder (FastRuler middle range), Lane **A – C:** Cattle samples; **D** and **E:** Sheep samples; **F** and **G:** Goat samples; **H** – **J:** Positive samples of *Echinococcus granulosus* G1, *Echinococcus ortleppi* and *Echinococcus canadensis* G6, respectively; **K:** Negative control (no DNA).

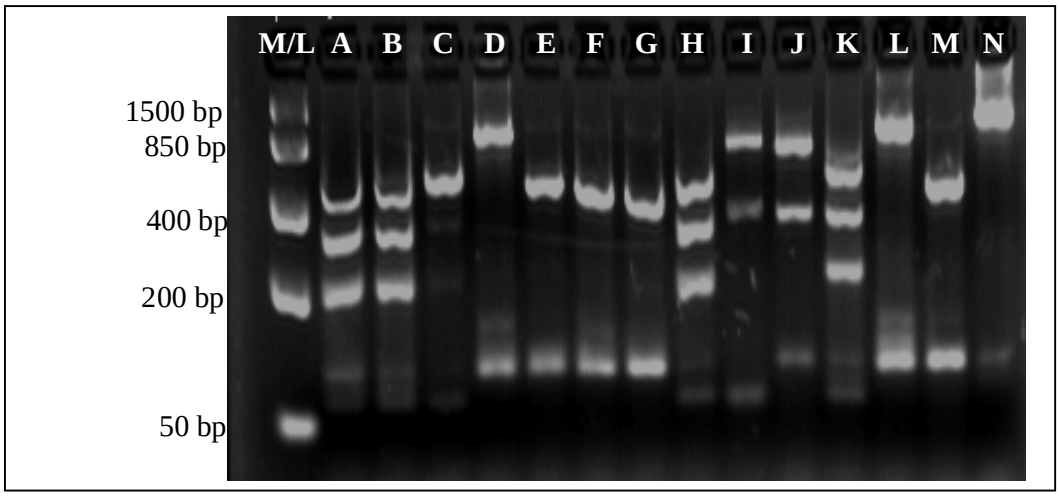

Figure 4.8: Agarose gel photo of PCR – RFLP of nad-1 using Hph I. Lane **M/L:** Molecular ladder (FastRuler, low range); **A, B** and **H:** *E. granulosus* G1; **C:** *E. granulosus* G1 with adenine to guanine substitution at the Hph I binding site; **D:** *E. ortleppi*; **E, F** and **G:** *E. canadensis* G6; **I:** *Taenia hydatigena*; **J:** *Taenia saginata*; **K, L** and **M:** References of *E. granulosus* G1 (485bp-320bp-204bp-64bp), *E. ortleppi* (867bp-107bp-102bp) and *E. canadensis* G6 (442bp-425bp-107bp-102bp); **N:** undigested *nad*-1 amplicon.

Table 4.10: *Echinococcus* species in livestock from Maasailand and Turkana

Livestock species	**Maasailand**			**Turkana**
	***E. granulosus* (G1)**	***E. ortleppi***	***E. canadensis* (G6)**	***E. granulosus* (G1)**
Cattle (n = 207)	200	1	0	6
Sheep (n = 69)	68	0	1	0
Goat (n = 17)	11	0	4	2
Total (293)	**279**	**1**	**5**	**8**

CHAPTER FIVE

5.0 DISCUSSION

5.1 Prevalence of Cystic Echinococcosis in Livestock from Maasailand and Turkana

Results from the present study corroborate previous reports on the occurrence of *Echinococcus* spp. in Maasailand and Turkana of Kenya (Casulli *et al*., 2010; Dinkel *et al*., 2004 and Wachira *et al*., 1993). In the present study, prevalence levels were shown to be similar to those reported by earlier studies (Macpherson, 1985 and Njoroge *et al*., 2002). The high prevalence coupled with the cysts fertility in livestock in this study supports the observation that *Echinococcus* spp. is endemic to nomadic pastoral tribes of East Africa (Magambo *et al*., 2006). The findings also reaffirm the public health significance of the disease among the Maasai and Turkana pastoral communities.

Sheep and goats in Maasailand and goats in Turkana were thought to be the most important contributors to the transmission of *Echinococcus* spp. This was partly due to the high cyst fertility and common practice of slaughtering of small ruminants at home without inspection and so could easily pass on cysts to dogs (Romig *et al*., 2011 and Macpherson, 1985). Contribution of cattle in the transmission cycle of *Echinococcus* spp. was thought to be less due to their low cyst fertility coupled with their inspective slaughter. The occurrence of *Echinococcus* spp. at different ages of livestock implies that the parasite is in endemic steady-state equilibrium which makes it amenable to control in Maasailand (Samia, 2011).

5.2 Predilection site of *Echinococcus* cysts in Livestock from Maasailand and Turkana

The account of *Echinococcus* spp. in the liver, lungs, hear, kidney and spleen of livestock in this study uphold previous observations by Jabbar *et al.* (2011), Omer *et al.* (2010), Berhe (2009) and Varcasia *et al*. (2007). However, the finding of the liver to be the main predilection site was in contrast to other studies that identifed the lungs to be the main predilection site in livestock (Ibrahim *et al.*, 2011; Ernest *et al.*, 2009; Berhe, 2009 and Njoroge *et al.*, 2002).

The observation of multiple organs infection of *Echinococcus* cyst coupled with the cyst load in this study implies higher economic lost due to condemnation of infected organs (Torgerson and Craig, 2011 and Budke *et al*., 2006). Wide organ range and multiple organ infection of *Echinococcus* spp. recorded in this study have meat inspection implications. Meat inspectors therefore have to concentrate effort on these internal organs of carcasses. Again, one has to be vigilant during routine meat inspection since in most cases *Echinococcus* spp. does not present its normal bladder-like fluid-filled structure but hard calcified solid appearance.

The observation of high infection in older cattle and sheep from Maasailand validates the observation by Eckert and Deplazes (2004) that intermediate hosts do not develop strong immunity under natural conditions. These older cattle and sheep might have had continuous infection of *Echinococcus* spp. from the environment. Alternatively, hosted parasites were only gradually reaching detectable stage (cyst) as host animal advanced in age. In either case, older cattle and sheep from Maasailand probably breeding stock were the most affected group.

5.3 Genetic Diversity of *Echinococcus* species from Livestock in Maasailand and Turkana

The identification of *E. granulosus* G1 (common sheep strain), *E. ortleppi* (cattle strain) and *E. canadensis* G6 (camel strain) in the present study validates earlier accounts on occurrence of taxa in Kenya (Casulli *et al.*, 2010; Dinkel *et al*., 2004 and Wachira *et al*., 1993). The identification of the common sheep strain (G1) as the dominant taxon among cattle, sheep and goat elucidates the observation that, it is the most pathogenic taxon causing livestock cystic echinococcosis (Eckert and Deplazes, 2004; Dinkel *et al*., 2004 and Wachira *et al*., 1993). These findings are however in contrast to the pattern in neighbouring Sudan where *E. canadensis* G6 was reported as the predominant taxon in livestock (Ibrahim *et al*., 2011; Omer *et al*., 2010 and Dinkel *et al*., 2004).

The record of *E. ortleppi* in cattle from Maasailand is the first account of it in South-Western Kenya and has added to the limited account of this taxon in the country (Mulinge *et al.*, 2011 and Dinkel *et al*., 2004). This shows that *E. ortleppi* is present in Kenya but occurs rarely.

The account of *E. canadensis* G6 in sheep and goats in Maasailand where camels are mostly absent supports previous findings (Wachira *et al*., 1993). In earlier accounts of *E. canadensis* G6 in Kenya, it was thought to be restricted to areas where camels were abundant. Findings in this studies and other recent studies have shown that the taxon may be more wide spread than previously thought (Casulli *et al*., 2010 and Dinkel *et al*., 2004). The identification of *E. canadensis* G6 in goats from Maasailand coupled with the cyst fertility shows that it has adapted to infecting goats. This is in

agreement with the observation of goats as suitable hosts of the closely related 'pig strain' *E. canadensis* G7, (Thompson and McManus, 2001). Goat could be good intermediate host in maintaining the perpetuation of the G6 taxon in places where camels are rare like Maasailand.

The *E. granulosus* G1 isolates showing base-exchange at the Hph I binding site indicate heterogeneity within this taxon. This substantiates the observation of micro-variance elsewhere within the taxon (Casulli *et al*., 2012; Nakao *et al*., 2010b and Maillard *et al*., 2007). It is therefore apparent that the diversity of *Echinococcus* spp. is still yet to be fully understood.

The amplification of the *nad*-1 of *Taenia saginata* from cattle in Maasailand has broadened the scope of *Taenia* parasite the primer pairs can amplify including *Taenia taeniaeformis, Taenia hydatigena, Taenia pisiformis* and *Taenia regis* (Hüttner *et al*., 2009). This means that the primer pairs can be used to screen for wide range of *Taenia* spp. as well as *Echinococcus* spp. based on the *nad*-1 gene. However, the unique restriction fragments of *T. hydatigena* and *T. saginata* makes it easier to differentiate from *E. granulosus* sensu lato taxa when digested with Hph I.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

i. Prevalence of cystic echinococcosis among livestock in Maasailand and Turkana is between 6.8 and 25.8%.

ii. *Echinococcus* cysts occur in the liver, lungs, heart, kidney and spleen of livestock but the main predilection site is the liver.

iii. Prevailing *Echinococcus granulosus* sensu lato taxa in Maasailand are *E. granulosus* G1, *E. ortleppi* and *E. canadensis* G6.

iv. *Echinococcus granulosus* G1 is present in Turkana.

6.2 Recommendation

i. Similar studies need to be carried out in dogs and humans in the Maasai and Turkana pastoral communities where people live in close interaction with their animals.

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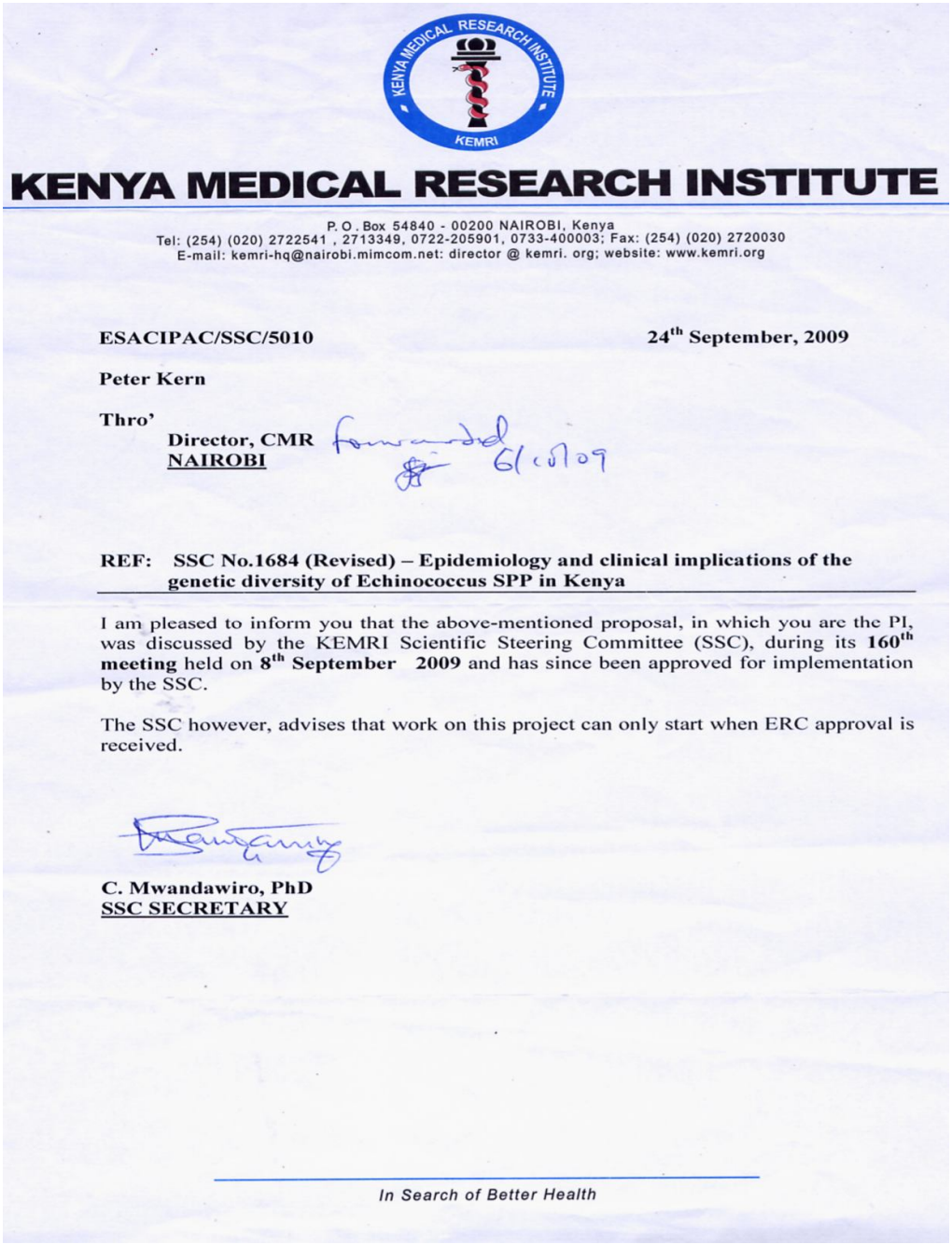
APPENDICES

Appendix I: Proposal approval by the Scientific Steering Committee of the Kenya Medical Research Institute



KENYA MEDICAL RESEARCH INSTITUTE

P. O. Box 54840 - 00200 NAIROBI, Kenya
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E-mail: kemri-hq@nairobi.mimcom.net: director @ kemri. org; website: www.kemri.org

ESACIPAC/SSC/5010 **24th September, 2009**

Peter Kern

Thro'
Director, CMR
NAIROBI

forwarded 6/10/09

REF: SSC No.1684 (Revised) – Epidemiology and clinical implications of the genetic diversity of Echinococcus SPP in Kenya

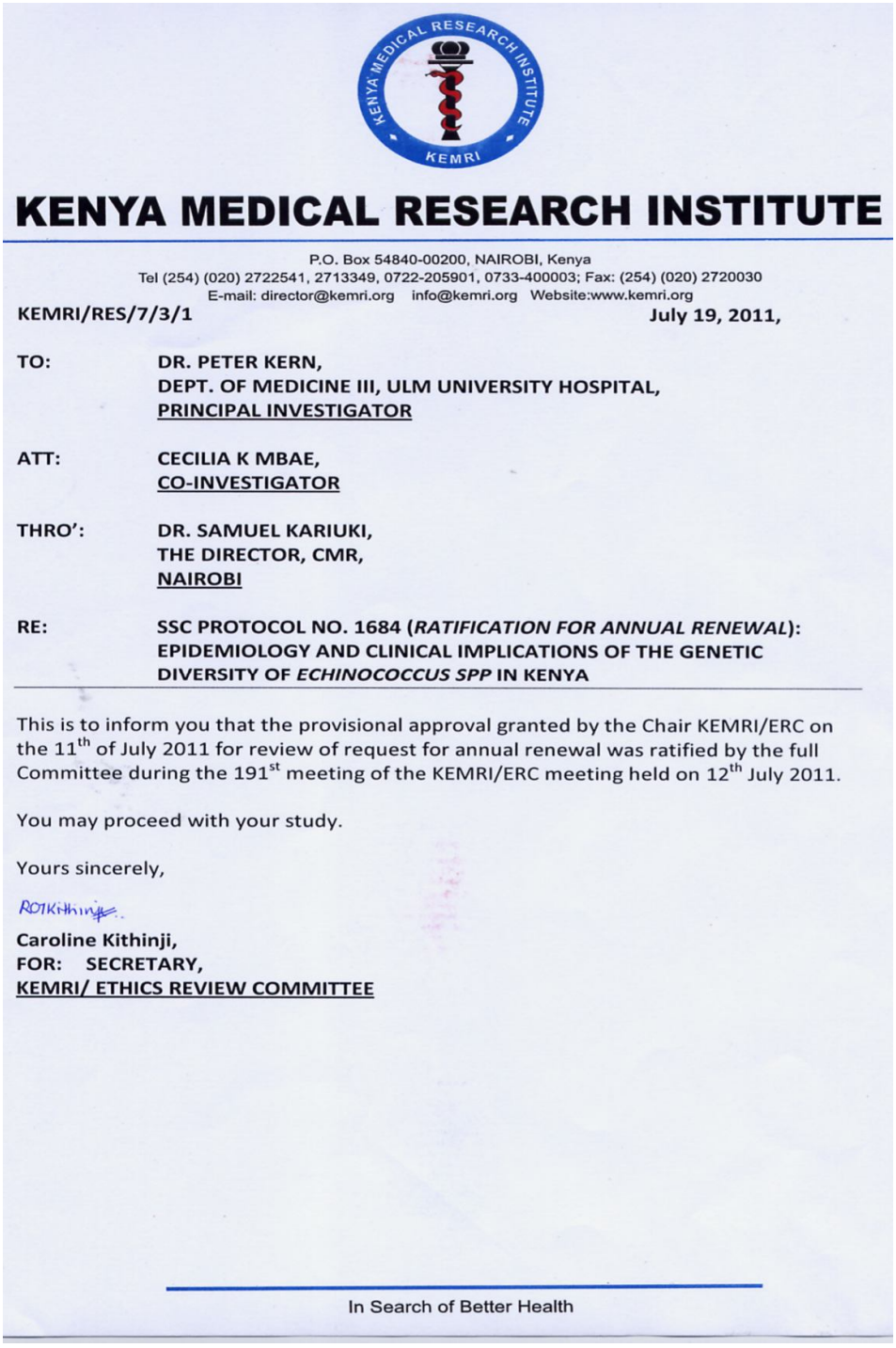
I am pleased to inform you that the above-mentioned proposal, in which you are the PI, was discussed by the KEMRI Scientific Steering Committee (SSC), during its **160th meeting** held on **8th September 2009** and has since been approved for implementation by the SSC.

The SSC however, advises that work on this project can only start when ERC approval is received.

C. Mwandawiro, PhD
SSC SECRETARY

In Search of Better Health

Appendix II: Ethical approval/ratification of annual renewal I



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E-mail: director@kemri.org info@kemri.org Website:www.kemri.org

KEMRI/RES/7/3/1 **July 19, 2011,**

TO: **DR. PETER KERN,**
DEPT. OF MEDICINE III, ULM UNIVERSITY HOSPITAL,
PRINCIPAL INVESTIGATOR

ATT: **CECILIA K MBAE,**
CO-INVESTIGATOR

THRO': **DR. SAMUEL KARIUKI,**
THE DIRECTOR, CMR,
NAIROBI

RE: **SSC PROTOCOL NO. 1684 (*RATIFICATION FOR ANNUAL RENEWAL*): EPIDEMIOLOGY AND CLINICAL IMPLICATIONS OF THE GENETIC DIVERSITY OF *ECHINOCOCCUS SPP* IN KENYA**

This is to inform you that the provisional approval granted by the Chair KEMRI/ERC on the 11th of July 2011 for review of request for annual renewal was ratified by the full Committee during the 191st meeting of the KEMRI/ERC meeting held on 12th July 2011.

You may proceed with your study.

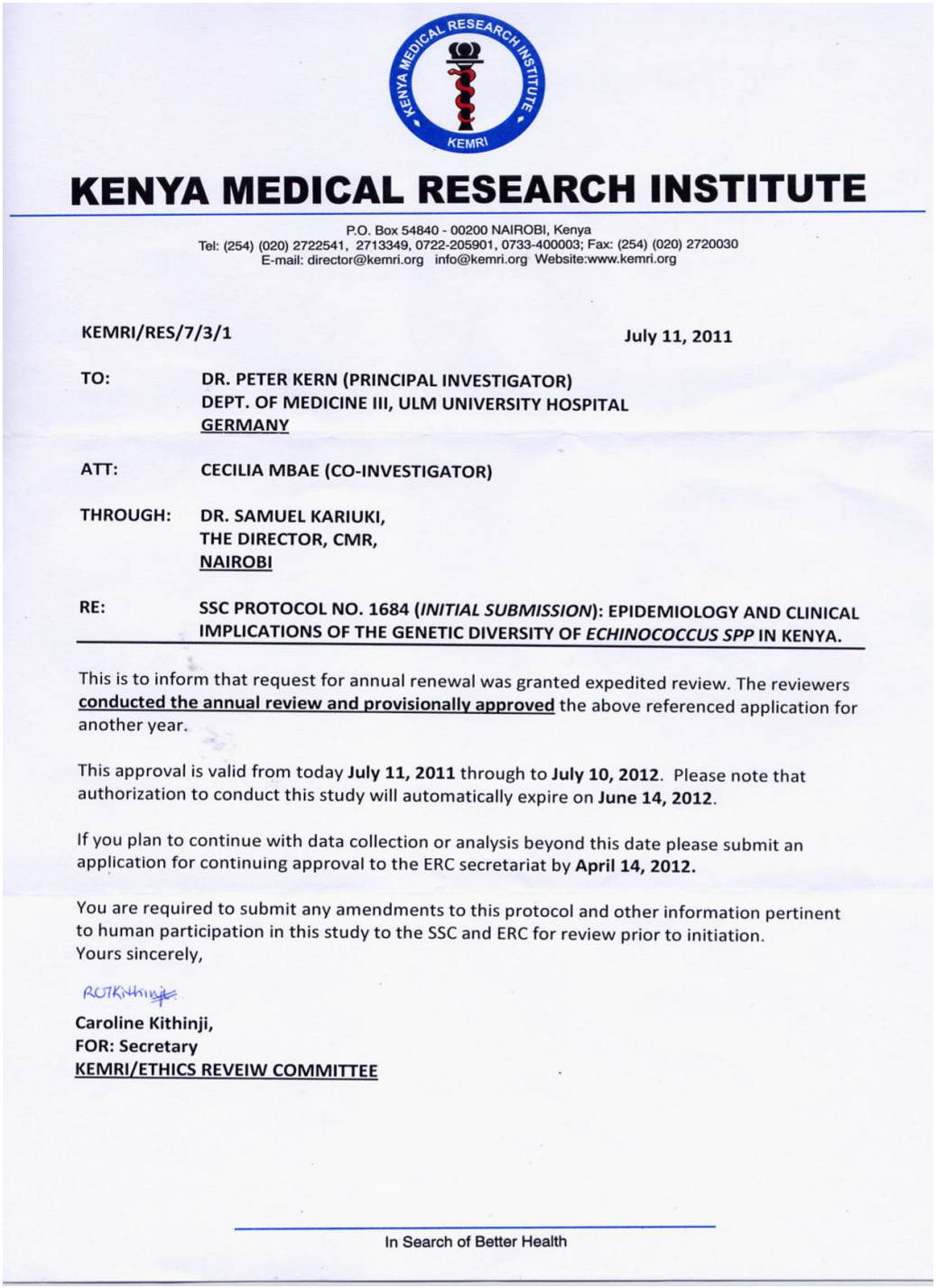
Yours sincerely,

RDTKithinji

Caroline Kithinji,
FOR: SECRETARY,
KEMRI/ ETHICS REVIEW COMMITTEE

In Search of Better Health

Appendix III: Ethical approval/ratification of annual renewal II



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KEMRI/RES/7/3/1 **July 11, 2011**

TO: **DR. PETER KERN (PRINCIPAL INVESTIGATOR)**
DEPT. OF MEDICINE III, ULM UNIVERSITY HOSPITAL
GERMANY

ATT: **CECILIA MBAE (CO-INVESTIGATOR)**

THROUGH: **DR. SAMUEL KARIUKI,**
THE DIRECTOR, CMR,
NAIROBI

RE: **SSC PROTOCOL NO. 1684 (*INITIAL SUBMISSION*): EPIDEMIOLOGY AND CLINICAL IMPLICATIONS OF THE GENETIC DIVERSITY OF *ECHINOCOCCUS SPP* IN KENYA.**

This is to inform that request for annual renewal was granted expedited review. The reviewers **conducted the annual review and provisionally approved** the above referenced application for another year.

This approval is valid from today **July 11, 2011** through to **July 10, 2012**. Please note that authorization to conduct this study will automatically expire on **June 14, 2012**.

If you plan to continue with data collection or analysis beyond this date please submit an application for continuing approval to the ERC secretariat by **April 14, 2012.**

You are required to submit any amendments to this protocol and other information pertinent to human participation in this study to the SSC and ERC for review prior to initiation.
Yours sincerely,

RCTKithinji

Caroline Kithinji,
FOR: Secretary
KEMRI/ETHICS REVEIW COMMITTEE

In Search of Better Health

Appendix IV: Supplementary data sheet for Maasailand samples

S/N∘	Slaughter house	Live-stock spp	Age (yrs)	Sex	Origin	Organ infected	Cyst condition	Genotype
1	Kitengela	Goat	2	M	Kitengela	Lungs	Sterile	G6
2	Kitengela	Goat	3	M	Bissili	Lungs	Chambered	G1
3	Kitengela	Goat	3	F	Kitengela	Liver	Calcified	G1
4	Kitengela	Goat	3	F	Bissili	Lungs	Fertile	G6
5	Kitengela	Goat	3	F	Bissili	Lungs	Fertile	G6
6	Kitengela	Goat	3	F	Bissili	Lungs	Fertile	G6
7	Kitengela	Goat	2	F	Kitengela	Lungs	Fertile	G1
8	Kitengela	Goat	2	F	Kitengela	Lungs	Fertile	G1
9	Kitengela	Goat	2	F	Kitengela	Lungs	Fertile	G1
10	Kitengela	Goat	2	F	Kitengela	Lungs	Fertile	G1
11	Kitengela	Goat	2	F	Bissili	Lungs	Fertile	G1
12	Kitengela	Goat	2	m	Kitengela	Lungs	Sterile	G1
13	Kitengela	Goat	3	F	Bissili	Lungs	Sterile	G1
14	Kitengela	Goat	3	F	Bissili	Lungs	Sterile	G1
15	Kitengela	Goat	3	F	Bissili	Lungs	Sterile	G1
16	Kitengela	Sheep	3	M		Lungs	Calcified	G1
17	Kitengela	Sheep	3	M		Liver	Calcified	G1
18	Kitengela	Sheep	3	M		Lungs	Sterile	G1
19	Kitengela	Sheep	3	F		Liver	Sterile	G1
20	Kitengela	Sheep	2	F		Liver	Sterile	G1
21	Kitengela	Sheep	2	F		Liver	Sterile	G1
22	Kitengela	Sheep	2	F		Liver	Sterile	G1
23	Kitengela	Sheep	2	F		Liver	Sterile	G1
24	Kitengela	Sheep	4	M		Liver	Fertile	G1

25	Kitengela	Sheep	4	M		Liver	Fertile	G1
26	Kitengela	Sheep	4	M		Liver	Fertile	GI
27	Kitengela	Sheep	4	M		Liver	Fertile	G1
28	Kitengela	Sheep	4	M		Liver	Fertile	G1
29	Kitengela	Sheep	4	M		Liver	Fertile	G1
30	Kitengela	Sheep	4	M		Liver	Fertile	G1
31	Kitengela	Sheep	4	M		Liver	Fertile	G1
32	Kitengela	Sheep	4	M		Lungs	Fertile	G1
33	Kitengela	Sheep	4	M		Lungs	Fertile	G1
34	Kitengela	Sheep	3	M	Bissili	Heart	Sterile	G1
35	Kitengela	Sheep	3	M		Lungs	Sterile	G1
36	Kitengela	Sheep	2	M	Kitengela	Liver	Chambered	G1
37	Kitengela	Sheep	3	M	Kitengela	Liver	Calcified	G1
38	Kitengela	Sheep	3	F	Kitengela	Liver	Fertile	G1
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50	Kitengela	Sheep	3	F	Kitengela	Lungs	Fertile	G1
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52	Kitengela	Sheep	2	F	Kitengela	Liver	Sterile	G1
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58	Kitengela	Sheep	2	F	Kitengela	Lungs	Sterile	G1
59	Kitengela	Sheep	2	F	Kitengela	Lungs	Sterile	G1
60	Kitengela	Sheep	2	F	Kitengela	Lungs	Sterile	G1
61*****	Kitengela	Sheep	2	M	Kitengela	Liver	Sterile	G1
62*****	Kitengela	Sheep	2	M	Kitengela	Liver	Calcified	*Taenia hydatigena*
63	Kitengela	Sheep	3	F	Kitengela	Liver	Sterile	G1
64	Kitengela	Sheep	3	F	Kitengela	Liver	Sterile	G1
65	Kitengela	Sheep	3	F	Bissili	Liver	Sterile	G1
66	Kitengela	Sheep	2	F	Bissili	Lungs	Sterile	G6
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112	Kitengela	Cattle	5	F	Bissili	Kidney	Sterile	G1
113	Kitengela	Cattle	5	F	Bissili	Liver	Sterile	G1
114	Kitengela	Cattle	5	F	Bissili	Lungs	Sterile	G1
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154	Suswa	Cattle	4	F	Narok	Lungs	Fertile	*E. ortleppi*
155	Suswa	Cattle	3	F	Narok	Lungs	Sterile	G1
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160	Suswa	Cattle	6	F	Narok	Lungs	Fertile	G1
161	Suswa	Cattle	6	F	Narok	Lungs	Chambered	G1
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163	Suswa	Cattle	5	F	Narok	Heart	Sterile	G1
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172	Suswa	Cattle	3	F	Ntulele	Lungs	Sterile	G1
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174	Suswa	Cattle	4	F	Ntulele	Liver	Sterile	G1
175	Suswa	Cattle	3	M	Suswa	Lungs	Sterile	G1
176	Suswa	Cattle	3	M	Suswa	Lungs	Sterile	G1
177	Suswa	Cattle	5	F	Suswa	Liver	Sterile	G1
178	Suswa	Cattle	5	F	Suswa	Liver	Sterile	G1
179	Suswa	Cattle	5	F	Suswa	Lungs	Sterile	G1
180	Suswa	Cattle	5	F	Suswa	Lungs	Sterile	G1
181	Suswa	Cattle	5	F	Suswa	Liver	Calcified	G1
182	Suswa	Cattle	5	F	Suswa	Lungs	Sterile	G1
183	Suswa	Cattle	4	F	Suswa	Liver	Sterile	G1
184	Suswa	Cattle	4	F	Suswa	Lungs	Sterile	G1
185	Suswa	Cattle	4	F	Suswa	Liver	Calcified	G1
186	Suswa	Cattle	5	F	Suswa	Liver	Fertile	G1
187	Suswa	Cattle	6	F	Suswa	Liver	Sterile	G1
188	Suswa	Cattle	6	F	Suswa	Lungs	Sterile	G1
189	Suswa	Cattle	6	F	Suswa	Lungs	Sterile	G1
190	Suswa	Cattle	6	F	Suswa	Lungs	Sterile	G1

191	Suswa	Cattle	6	F	Suswa	Lungs	Sterile	G1
192	Suswa	Cattle	6	F	Suswa	Lungs	Sterile	G1
193	Suswa	Cattle	3	M	Suswa	Lungs	Sterile	G1
194	Suswa	Cattle	2	M	Suswa	Liver	Sterile	G1
195	Suswa	Cattle	4	F	Suswa	Liver	Fertile	G1
196	Suswa	Cattle	6	F	Suswa	Lungs	Sterile	G1
197	Suswa	Cattle	7	F	Suswa	Lungs	Sterile	G1
198	Suswa	Cattle	6	M	Suswa	Liver	Sterile	G1
199	Suswa	Cattle	5	M	Suswa	Lungs	Sterile	G1
200	Suswa	Cattle	2	M	Suswa	Lungs	Sterile	G1
201	Suswa	Cattle	4	M	Suswa	Lungs	Sterile	G1
202	Suswa	Cattle	3	M	Suswa	Liver	Sterile	G1
203	Suswa	Cattle	3	M	Suswa	Liver	Sterile	G1
204	Suswa	Cattle	4	F	Bomet	Lungs	Chambered	G1
205	Suswa	Cattle	4	F	Bomet	Liver	Sterile	G1
206	Suswa	Cattle	4	F	Bomet	Liver	Chambered	G1
207	Suswa	Cattle	3	M	Ntulele	Lungs	Sterile	G1
208	Suswa	Cattle	3	M	Ntulele	Liver	Calcified	G1
209	Suswa	Cattle	4	F	Ntulele	Lungs	Fertile	G1
210	Suswa	Cattle	4	F	Ntulele	Lungs	Sterile	G1
211	Suswa	Cattle	4	F	Ntulele	Lungs	Sterile	G1
212	Suswa	Cattle	4	F	Ntulele	Lungs	Sterile	G1
213	Suswa	Cattle	4	M	Ntulele	Lungs	Sterile	G1
214	Suswa	Cattle	4	M	Ntulele	Liver	Sterile	G1
215	Suswa	Cattle	4	F	Ntulele	Liver	Fertile	G1
216	Suswa	Cattle	4	F	Ntulele	Liver	Fertile	G1
217	Suswa	Cattle	4	F	Ntulele	Liver	Fertile	G1
218	Suswa	Cattle	4	F	Ntulele	Liver	Fertile	G1

219	Suswa	Cattle	3	M	Ntulele	Lungs	Sterile	G1
220	Suswa	Cattle	4	F	Ntulele	Lungs	Sterile	G1
221	Suswa	Cattle	5	F	Ntulele	Lungs	Fertile	G1
222	Suswa	Cattle	5	F	Ntulele	Lungs	Fertile	G1
223	Suswa	Cattle	5	F	Ntulele	Lungs	Fertile	G1
224	Suswa	Cattle	5	F	Ntulele	Lungs	Fertile	G1
225	Suswa	Cattle	5	F	Ntulele	Lungs	Fertile	G1
226	Suswa	Cattle	5	F	Ntulele	Liver	Calcified	G1
227	Suswa	Cattle	5	F	Ntulele	Lungs	Sterile	G1
228	Suswa	Cattle	5	F	Ntulele	Lungs	Sterile	G1
229	Suswa	Cattle	3	M	Ntulele	Lungs	Sterile	G1
230	Suswa	Cattle	5	F	Ntulele	Lungs	Sterile	G1
231	Suswa	Cattle	5	F	Ntulele	Lungs	Sterile	G1
232	Suswa	Cattle	5	F	Ntulele	Lungs	Sterile	G1
233	Suswa	Cattle	5	F	Ntulele	Lungs	Sterile	G1
234	Suswa	Cattle	2	M	Ntulele	Lungs	Sterile	G1
235	Suswa	Cattle	3	F	Ntulele	Lungs	Sterile	G1
236	Suswa	Cattle	3	F	Ntulele	Lungs	Sterile	G1
237	Suswa	Cattle	3	F	Ntulele	Lungs	Sterile	G1
238	Suswa	Cattle	3	F	Ntulele	Liver	Sterile	G1
239	Suswa	Cattle	4	M	Ntulele	Lungs	Sterile	G1
240	Suswa	Cattle	4	M	Ntulele	Lungs	Sterile	G1
241	Suswa	Cattle	4	M	Ntulele	Liver	Calcified	G1
242	Suswa	Cattle	3	M	Ntulele	Lungs	Sterile	G1
243	Suswa	Cattle	3	M	Ntulele	Lungs	Sterile	G1
244	Suswa	Cattle	2	M	Ntulele	Lungs	Calcified	G1
245	Suswa	Cattle	3	F	Ntulele	Liver	Calcified	G1
246	Suswa	Cattle	3	M	Ntulele	Lungs	Chambered	G1

247	Suswa	Cattle	4	M	Ntulele	Liver	Calcified	G1
248	Suswa	Cattle	5	M	Suswa	Lungs	Sterile	G1
249	Suswa	Cattle	5	M	Suswa	Lungs	Sterile	G1
250	Suswa	Cattle	4	M	Suswa	Lungs	Sterile	G1
251	Suswa	Cattle	3	M	Suswa	Lungs	Sterile	G1
252	Suswa	Cattle	4	M	Suswa	Liver	Sterile	G1
253	Suswa	Cattle	4	M	Suswa	Liver	Sterile	G1
254	Suswa	Cattle	4	M	Suswa	Lungs	Sterile	G1
255	Suswa	Cattle	4	M	Suswa	Liver	Sterile	G1
256	Suswa	Cattle	3	M	Suswa	Lungs	Sterile	G1
257	Suswa	Cattle	3	M	Suswa	Lungs	Sterile	G1
258	Suswa	Cattle	4	F	Suswa	Lungs	Sterile	G1
259	Suswa	Cattle	4	F	Suswa	Lungs	Sterile	G1
260	Suswa	Cattle	4	F	Suswa	Lungs	Chambered	G1
261	Suswa	Cattle	5	M	Suswa	Lungs	Sterile	G1
262	Suswa	Cattle	5	M	Suswa	Liver	Sterile	G1
263	Suswa	Cattle	3	M	Suswa	Lungs	Sterile	G1
264	Suswa	Cattle	3	M	Suswa	Lungs	Sterile	G1
265	Suswa	Cattle	3	M	Suswa	Lungs	Sterile	G1
266	Suswa	Cattle	2	M	Suswa	Lungs	Sterile	G1
267	Suswa	Cattle	4	M	Suswa	Lungs	Sterile	G1
268	Suswa	Cattle	5	F	Suswa	Lungs	Sterile	G1
269	Suswa	Cattle	5	F	Suswa	Lungs	Sterile	G1
270	Suswa	Cattle	5	F	Suswa	Liver	Sterile	G1
271	Suswa	Cattle	5	F	Suswa	Liver	Sterile	G1
272	Suswa	Cattle	5	F	Suswa	Lungs	Fertile	G1
273	Suswa	Cattle	5	F	Suswa	Lungs	Sterile	G1
274	Suswa	Cattle	3	F	Suswa	Liver	Sterile	G1

275	Suswa	Cattle	3	F	Suswa	Liver	Sterile	*Taenia saginata*
276	Suswa	Cattle	4	M	Suswa	Liver	Fertile	G1
277	Suswa	Cattle	4	M	Suswa	Lungs	Sterile	G1
278	Suswa	Cattle	4	M	Suswa	Lungs	Sterile	G1
279	Suswa	Cattle	3	F	Suswa	Lungs	Sterile	G1
280	Suswa	Cattle	5	M	Suswa	Liver	Sterile	G1
281	Suswa	Cattle	5	M	Suswa	Liver	Sterile	G1
282	Suswa	Cattle	5	M	Suswa	Liver	Chambered	G1
283	Suswa	Cattle	5	M	Suswa	Liver	Calcified	G1
284	Suswa	Cattle	4	F	Suswa	Liver	Calcified	G1
285	Suswa	Cattle	5	F	Suswa	Lungs	Sterile	G1
286	Suswa	Cattle	5	M	Suswa	Liver	Calcified	G1
287	Suswa	Cattle	4	F	Suswa	Liver	Calcified	G1

61* and **62*** identified as *E. granulosus* G1 and *Taenia hydatigena*, respectively were found in one sheep

Appendix V: Supplementary data sheet for Turkana samples

S/No.	Slaughter house	Species	Age (yrs)	Sex	Origin	Organ infested	Cysts condition
1	Lomidat	cattle	5	m	Loriemet	Liver and Lungs	Fertile, sterile and calcified
2	Lomidat	cattle	5	m	Mogila	Liver and Lungs	Fertile and calcified
3	Lomidat	cattle	6	m	Mogila	Liver	Calcified
4	Lomidat	cattle	6	m	Mogila	Liver and Lungs	Calcified
5	Lomidat	cattle	6	m	Lochereikope	Liver and Lungs	Calcified
6	Lomidat	cattle	6	m	Songot	Liver and Lungs	Fertile and sterile
7	Lomidat	cattle	6	m	Songot	Lungs	Fertile
8	Lomidat	cattle	6	m	Lotetelei t	Liver	Sterile
9	Lomidat	cattle	5	m	Lopiding	Liver	Sterile and calcified
10	Lomidat	cattle	6	m	Songot	Liver and Lungs	Fertile, sterile & calcified
11	Lomidat	cattle	6	m	Songot	Lungs	Calcified
12	Lomidat	cattle	6	m	Songot	Liver and Lungs	Fertile, sterile & calcified
13	Lomidat	goat	3	m	Natamakaruo	Liver	Sterile and calcified
14	Lomidat	goat	3	m	Abutungunan	Liver and Lungs	Calcified
15	Lomidat	goat	3	m	Lokichokio	Liver and Lungs	Sterile
16	Lomidat	goat	3	m	Lopiding	Liver	Sterile and calcified
17	Lomidat	goat	3	m	Lopiding	Lungs	Fertile

Appendix VI: Nucleotide sequence (565 bp) of *nad*-1 of *Echinococcus granulosus* G1 isolated from cattle in Maasailand. Sample's DNA sequence showed single base-exchange of Guanine for Adenine at Hph I binding site compared with the *nad*-1 sequence NC_008075.1 (nucleotide position 265 – counting from the start codon)

AATTAACAATAGCAAACAGCCCTAAAATCAACATACATAAACAAACTTC
TCAAAAAAATTTTACAAAACAATCATAACGAACACGTGGTAATGTCGCC
CGAGCCCACATAAAAAATAATAAATTAAACACTAACACCAACATACCGA
TAAAACCGCCACCAAACATCAACACAACTCCCAACCATGAAAACACATA
TATAATAATATACTCACAAGCAAATAAACACGTAAAATAAATACCACTA
TACTCAACTTTAAACCCACTGACCAACTCTCTTTCAGCCTCTCCATAATCA
AATGGCGTACGATTAGTTTCACACAATATACATATTAAAAATAACACATA
AATTAATGGAAATAATAACAAACTTAATCAACAATTATAATAAAAATCA
ATTAAATTATACCTACAACTACACAAAGCACAAAAAATCACCACACACA
TAAAACAAGCCTCAAACCTAACAGATCCAAAAGCACATCGAACCGACCT
TAAAAATGAATAATTGTTGTAACCACCCCAACCAGTACACAACAAAGAA
TACCTAGATGTTCTGGCGGCAGC

Appendix VII: Nucleotide sequence (645 bp) of *nad*-1 of *Echinococcus granulosus* G1 isolated from cattle in Maasailand. Sample's DNA sequence showed single base-exchange of Guanine for Adenine at Hph I binding site compared with the *nad*-1 sequence NC_008075.1 (nucleotide position 265 – counting from the start codon)

AATTTTACAAAACAATCATAACGAACACGTGGTAATGTCGCCCGAGCCC
ACATAAAAAATAATAAATTAAACACTAACACCAACATACCGATAAAACC
GCCACCAAACATCAACACAACTCCCAACCATGAAAACACATATATAATA
ATATACTCACAAGCAAATAAACACGTAAAATAAATACCACTATACTCAA
CTTTAAACCCACTGACCAACTCTCTTTCAGCCTCTCCATAATCAAATGGC
GTACGATTAGTTTCACACAATATACATATTAAAAATAACACATAAATTAA
TGGAAATAATAACAAACTTAATCAACAATTATAATAAAAATCAATTAAA
TTATACCTACAACTACACAAAGCACAAAAAATCACCACACACATAAAAC
AAGCCTCAAACCTAACAGATCCAAAAGCACATCGAACCGACCTTAAAAA
TGAATAATTGTTGTAACCACCCCAACCAGTACACAACAAAGAATACCTA
GATGTTCTGGCGGCAGCCAAAAACCACAACACGGAGAGGCCTCTATAAC
TAGCTCTATAATATCTACCATAAATAAATGAATAAACAACCACCAAAGCC
ATTAATAACACAACACCAAACAAACCAACATACCTACGACTTTGGAAGT
AAAAA

Appendix VIII: Nucleotide sequence (887 bp) of *nad*-1 of *Taenia hydatigena* isolated from sheep in Maasailand. Maximum identity 99% (GQ228819.1)

GTAAAAAGACTTAATATAAGCAAACACAAACACACATCCCAAAAAAATT
TAACAAAGTAATCATAACGAACACGTGGCAATGTAGCACGAGCTCACAT
TATGAATAATAAAATAAATGACAAAACAAAACCCCCCCAAAGTCCACCA
TTAAACATTAATATAGACCCTAACCAAGAAAATATATATATAACAATATA
TTCACATGCAAATAAACAAGTGAAAAAGATACCACTATACTCAACATTA
AATCCACTAACCAATTCCCTCTCAGCTTCACCGTAATCAAATGGTGTACG
ATTAGTCTCACATAACACACATATCAAATAAAGTAAAAACACCAATGGA
AACAATAATACAGAAAACCAATCACTTAAAAAATAATCAACCAAATTAT
AACTAAAATACCTCAACGACGAAAAAATAACAACACACATAAAACAAGC
CTCAAACCTTATAGAACCAAAAGCACAACGCACCGAACTTAAAAAAGAA
TAATTTTTATAACTACCTCATCCAACACACATTATAGAATAACTACAAAA
TCTAGTTATTACCAAAAATCACAACAATGAAAGTGAATTAAAGCTACAA
CTATGATAAGACCCATAAACAAAAGTATAAAATACCACCAAACAGACTA
GTAAAAAAACACCAAACAGACCAACTCATCTACGAGTCTGAAAAAAATA
ATTCTTAAACTTAACAACCAACTTCAATAAATCTGAAAATCTTTGTAACA
AACCCATTATACCAACCTTATTTGGCCCCTTACGAAATTGAGAATAACCC
AAAATCTTACGTTCGCCCAATATAAAAAATGCTATAACTAGCAAACTAAT
TAATAAACCAAAAACCCCAGACAATAAGCCAAAAATAATCATGATAAA

Appendix IX: Nucleotide sequence (513 bp) of *nad*-1 of *Taenia saginata* isolated from cattle in Maasailand. Maximum identity 99% (AY684274.1)

ACCAATAAACCCACCACCAAACATTAAAATCATCCCTAATCATGAAAAA
ATAAATATAATTATATACTCACATGCAAATAAACATGTAAAATATATACC
ACTATACTCCACTTTAAAACCACTAACTAACTCACTTTCAGCTTCACCATA
ATCAAATGGAGTACGATTAGTTTCACATAAAACACATATTATATACAAAA
ATAACAAACAAGGAAAAATCACAACTGACAACCAATCACTTAAAAAAAA
ATCCACCAAACTATATCTACAATAACACAATGCAGAAAATATAATAATA
CACATAAAACAAGCCTCAAACCTTATAGATCCAAAAGCACAACGAATTG
AACTTAAAAACGAATAACTTTTATAACTACCCCAACCTGTACACAATATA
GAATAACTACAAAATCTGGTAATAACCAAAAACCATAATAGAGACAATG
AATTAAAACTATACCTATAATAACCACCATACACGAACGAATAATAAAT
AACTAAACCAACCAATAA

Appendix X: Part of results published as abstract in the Book of Abstracts of the 25th Annual Meeting of the German Society for Parasitology (DGP)

DGP Jahrestagung - Annual Meeting 2012

29 Genetic diversity of cystic echinococcosis in Maasailand, Kenya

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Cystic echinococcosis, caused by a number of taxa within the species complex Echinococcus granulosus sensu lato, is a public health concern in countries with extensive livestock economies worldwide, and has a particular medical and economic impact on pastoralist communities. In East Africa, previous studies showed several species or strains of the parasite occurring in different or the same livestock species within the same areas, and also showed considerable differences in species / strain composition between different regions. Here, we characterized a panel of samples obtained from livestock carcasses in the Maasailand of Kenya. 96 cyst isolates were collected from cattle, 33 from sheep and eight from goats. Protoscolices or endocysts were fixed in 70% ethanol until DNA extraction. DNA was extracted by lysis in 0.02N NaOH at 99oC for 10min and the lysate used as template for PCR. A nested PCR was performed to amplify a fragment including the mitochondrial gene NAD hydrogenase gene (nad 1). Amplicons were subjected to restriction fragments length polymorphism (RFLP) analysis using the Hph I endonuclease and the banding patterns resolved on 3% agarose gel. Genotypes were determined by comparing a sample's banding pattern with defined banding patterns of G1, G5 and G6 strains. Out of the 96 isolates from cattle, 95 were the G1 genotype of E. granulosus s.s., and one isolate was E. ortleppi (G5). Of the sheep isolates, 32 were G1 and one conformed to the E. canadensis G6 banding pattern. Of the goat isolates, four were G1 and four G6. Our results confirm previous data with smaller numbers of samples concerning the predominance of G1 in cattle and sheep. The predominance of G1 ('common sheep strain') in Maasailand is a partial explanation for the large number of human patients in this area, as G1 is known the world over as the main genotype found in humans. For the first time, E. ortleppi was found in southern Kenya in its main host, cattle. Interestingly, half of the isolates from goats were found to be the 'camel strain' (G6) of E. canadensis, rare in other hosts in southern Kenya, which confirms the adaptation of this parasite to goats. Goats are apparently suited to maintain the transmission of G6, a strain normally infecting camels, in a region where camels are rare or absent. The findings of this survey are put in perspective regarding results from other regions of East Africa.

References: BodyROMIG, T., OMER, R. A., ZEYHLE, E., HÜTTNER, M., DINKEL, A., SIEFERT, L., ELMAHDI, I. E., MAGAMBO, J., OCAIDO, M., MENEZES, C. N., AHMED, M. E., MBAE, C.,

Appendix XI: Part of results published in the journal, Parasitology Research

Parasitol Res
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ORIGINAL PAPER

Prevalence and diversity of cystic echinococcosis in livestock in Maasailand, Kenya

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Abstract Cystic echinococcosis (CE) is a zoonotic disease caused by several members of the *Echinococcus granulosus* species complex. In East Africa, several species/strains are known to occur in livestock and humans, but host preferences, relative frequencies and spatial distribution of these taxa are poorly known. Here, we contribute livestock data for Maasailand of southern Kenya. Total CE prevalence was 25.8 % in cattle (151/587), 16.5 % in sheep (71/430) and 10.8 % in goats (21/194), which is a significant increase compared to surveys done about three decades ago. The majority of cysts occurred in the liver (56 % in cattle, 70 % in sheep and 65 % in goats). Molecular characterization by PCR–RFLP and sequencing of parts of the mitochondrial *nad*-1 gene was done for a subsample of 285 cysts. *E. granulosus* G1 was dominant in all host species (200 of 201 cysts from cattle, 68 of 69 from sheep and 11 of 15 from goats); the remaining taxa were *Echinococcus canadensis* G6 (one cyst from sheep, four from goats) and *Echinococcus ortleppi* (one cyst from cattle). Considering cyst fertility, sheep appear to be the most important hosts for *E. granulosus* G1, while goats were found to be suitable hosts for *E. canadensis* G6 (three of four cysts were fertile). For the first time, *E. ortleppi* was found in cattle from southern Kenya. Our data show an intense and possibly increasing level of CE transmission in southern Kenya, and the predominance of *E. granulosus* G1, which appears to be particularly pathogenic to humans, calls for urgent control measures.

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Introduction

Cystic echinococcosis (CE) is a zoonotic disease caused by the larval stage of *Echinococcus granulosus* sensu lato. The various species in this cluster were previously considered as strains (Nakao et al. 2007). They use canids and/or felids as definitive hosts and a wide range of ungulates as intermediate hosts, where cysts develop in the liver, lungs and other organs. The disease occurs worldwide and has a particular economic and medical impact on rural pastoral societies (Eckert et al. 2001). It is widespread in Africa, posing a public health concern in most countries with extensive livestock economy (Wachira et al. 1993; Dinkel et al. 2004; Magambo et al. 2006; Maillard et al. 2007, 2009; Hüttner et al. 2008, 2009; Omer et al. 2010; Romig et al. 2011). With exception of western and central Africa, from where only sporadic cases are known, CE is endemic among humans and livestock populations in all countries of the continent (Magambo et al. 2006; Romig et al. 2011). According to the currently available information on species, strains and genotypes of CE agents, Africa is arguably the continent with the highest diversity of these parasites (Romig et al. 2011).

To date, five species of *Echinococcus granulosus* s. l. have been identified in Africa: *E. granulosus* sensu stricto

(common sheep strain G1), *Echinococcus equinus*, *Echinococcus ortleppi*, *Echinococcus canadensis* (camel/pig strain G6/G7) and *Echinococcus felidis* (Wachira et al. 1993; Thompson and McManus 2001; Hüttner et al. 2008; Maillard et al. 2009; Casulli et al. 2010; Omer et al. 2010). In East Africa, the parasite presents a complex pattern of infectivity and prevalence, with several species or strains occurring sympatrically in different or the same livestock species. Based on the limited number of studies so far, there seem to be considerable differences in species/strain composition among different regions (Romig et al. 2011). This scenario depicts a complex epidemiology of the disease which is not fully understood. Regions populated by pastoral communities (e.g. Maasai and Turkana) in Kenya and others in some neighbouring countries seem to be transmission foci of these parasites.

The main life cycles involve domestic dogs and various livestock species (cattle, camels, sheep and goats) (Wachira et al. 1993; Dinkel et al. 2004). *Echinococcus* taxa from wildlife may also contribute to the infection of livestock and humans (Hüttner et al. 2009). Surveys for CE done in Maasailand about 30 years ago established that the region was a hyperendemic focus with high prevalence levels in livestock and frequent occurrence of human cases (Macpherson 1985; Macpherson et al. 1989). In the meantime, several cyst isolates from southern Kenya have been genotyped (Wachira et al. 1993; Dinkel et al. 2004). However, the relative contribution of the identified species to the total CE burden is not known because the collection of samples had not been done in the context of systematic surveys. Here, we provide an update on the CE prevalence in all livestock species of economic relevance in southern Kenya and quantify the relative impact of different *Echinococcus* species on their hosts. In addition, we attempt to estimate the relative importance of different livestock species for the transmission of *Echinococcus* spp. in Maasailand.

Materials and methods

Study area

The current study was done in three abattoirs located in Kitengela town (approximately 30 km south of Nairobi) and Suswa (approximately 50 km west of Nairobi), which are key slaughter facilities for Maasai livestock, supplying the meat markets of Nairobi and environs. Maasailand occupies the southern part of the East African Rift valley in southern Kenya and northern Tanzania. The communities known as Maasai continue to practise traditional semi-nomadic pastoralism. People live in semi-permanent settlements of several families pasturing their stock together, 10–20 huts surrounded by a thorn fence into which the livestock is driven at night. The landscape is dominated by grazing land and large-scale cereal production; some sections of Maasailand have been converted into National Parks and game reserves. The area receives low to moderate rainfall (average 500–1,800 mm/a) and is known for recurrent years of drought when a considerable number of livestock perishes (Evangelou 1984 and Kaplan et al. 1976).

Isolation of cysts

During the month of October 2011, a total of 1,211 carcasses of cattle (587), sheep (430) and goats (194) were inspected for cysts in all organs of the pleural and abdominal cavities. The age of each slaughtered animal was estimated. Eight hundred twenty-nine cysts were obtained. Cyst contents were microscopically inspected for the presence of protoscolices. Cysts with viable protoscolices were considered fertile; cysts without protoscolices and calcified cysts were considered non-fertile. Protoscolices and pieces of cyst wall (germinal layer) intended for molecular characterization were fixed and stored in 70 % ethanol. Characterised isolates of *E. granulosus* G1, *E. ortleppi* and *E. canadensis* G6 from Kenya, Vietnam and Sudan, respectively, were used as positive controls during species differentiation.

DNA extraction

DNA was extracted from protoscolices or tissue pieces by lysing in 0.02 M NaOH at 95 °C for 10 min as previously described by Nakao et al. (2003). In a few instances where the above process failed to yield adequate DNA, genomic DNA was extracted as described elsewhere (Dinkel et al. 2004). About 0.5-g cyst wall (germinal layer) was cut into small pieces and digested in the presence of 2 mg/ml proteinase K in 500 µl of 10 mM Tris–HCl (pH 7.5), 10 mM EDTA, 50 mM NaCl, 2 % sodium dodecyl sulphate and 20 mM dithiothreitol. DNA was extracted using phenol–chloroform–isoamyl alcohol (25:24:1) with subsequent ethanol precipitation. After drying, the DNA was dissolved in 100-µl nuclease-free water.

Polymerase chain reaction

A nested PCR assay was conducted to amplify the NADH dehydrogenase subunit 1 (*nad*-1) gene using the following primer pair: *for.*TGT TTT TGA GAT CAG TTC GGT GTG/*rev.*CAT AAT CAA ACG GAG TAC GAT TAG, for the primary reaction and internal primer pair: *for.*CAG TTC GGT GTG CTT TTG GGT CTG/*rev.*GAG TAC GAT TAG TCT CAC ACA GCA, for the nested reaction (Hüttner et al. 2008). In both reactions, a 50-µl reaction mixture was made up of DNase/RNase-free water, 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 2 mM $MgCl_2$, 200 µM of each dNTPs, 12.5 pmol of each primer and 1.25 U Taq polymerase.

Amplification conditions were as follows: start denaturation 95 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, elongation at 72 °C for 1 min and final elongation 72 °C for 5 min post cycling. Amplification results were detected on 1.5 % (*w/v*) agarose gel stained with Gel Red® (Biotium, Inc.).

Restriction fragment length polymorphism of *nad*-1

The *nad*-1 amplicons were digested as described previously (Hüttner et al. 2009) with the restriction enzyme Hph I (Fermentas GmbH, Germany). A total reaction mixture of 30.5 µl constituted of 15 µl PCR amplicons, 2 µl buffer B (supplied with enzyme), DNase/RNase-free water and 0.5 µl enzyme. Reaction mixture was incubated at 37 °C for 3 h, followed by deactivation of the enzyme at 65 °C for 20 min. Banding patterns were detected on 3 % (*w/v*) agarose stained with Gel Red® (Biotium, Inc.). Genotypes of samples were determined by comparing their banding patterns to defined patterns of *E. granulosus* G1, *E. ortleppi* and *E. canadensis* G6. The *nad*-1 PCR product of samples with different or unclear banding patterns were analysed by partial DNA sequencing (Seqlab GmbH, Göttingen). DNA sequences were compared with existing sequences in the GenBank databases using the BLAST (www.blast.ncbi.nlm.nih.gov/Blast.cgi).

Results

Prevalence and distribution

Results of the prevalence survey are shown in Table 1. The highest prevalence of CE was found in cattle (25.8 %) followed by sheep (16.5 %) and goats (10.8 %). The average number of cysts per surveyed animal was 1.0, 0.4 and 0.3 for cattle, sheep and goats, respectively. The number of cysts varied widely from one to over 16 cysts per infected animal. About half of the infected livestock harboured more than one cyst (Table 2). In some extreme cases, one sheep and goat each harboured 34 and 19 cysts, respectively. Prevalence was correlated with age in cattle (Fig. 1a), and infection intensity showed a trend towards an increase with age in cattle and sheep (Fig. 1b).

Table 2 Load of *Echinococcus* spp. in infected livestock

Infected livestock species	Number of cysts				
	1	2–5	6–10	11–15	16 or more
Cattle (n=151)	75	47	21	5	3
Sheep (n=71)	34	28	5	2	2
Goat (n=21)	10	10	0	0	1

Despite the high prevalence and infection intensity of *Echinococcus* spp. among cattle, cyst fertility in cattle was low (6.5 %) compared to sheep (25.6 %) and goats (17.6 %) (Table 3). The liver was found to be the most frequently involved organ (Table 3), but liver cysts were less frequently fertile compared to lung cysts in all species: in cattle (2.9 against 11.3 %), in sheep (22.6 against 34.8 %) and in goats (0 against 50 %). Multiple organ involvement was common in all three livestock species (Tables 1 and 3). Other organs aside the liver and lungs were the heart, kidney and spleen, but these organs only harboured non-fertile cysts in our survey.

Genetic characterisation

A total of 285 cyst isolates from cattle (201), sheep (69) and goats (15) recovered during the survey were characterised to the species/genotype level by PCR/RFLP and confirmatory partial mtDNA sequencing of the selected samples (Table 3). Three species of *Echinococcus* were identified: *E. granulosus* G1, *E. ortleppi* and *E. canadensis* G6. *E. granulosus* G1 was, by far, the dominant species in our survey (279 of 285 isolates) which often reached fertility in sheep (approx. 25 %) and goats (45.5 %) but rarely in cattle (approx. 6 %) (Table 3). In addition, one fertile lung cyst from cattle belonged to *E. ortleppi*, and the lungs of one sheep and four goats were infected with cysts of *E. canadensis* G6; three of the four goat cysts were fertile.

Discussion

Our results demonstrate the persistence of cystic echinococcosis in Maasailand, Kenya. The prevalence levels of

Table 1 Prevalence of cystic echinococcosis and cyst location in livestock

Livestock	Prevalence (95 % CI)	Liver (n)	Lungs (n)	Heart (n)	Li/Lu (n)	Li/Sp (n)	Li/Lu/Ki (n)	Li/Lu/Ki/Sp (n)
Cattle (n=587)	25.8 (12.3–29.5)	59	43	2	45	1	1	0
Sheep (n=430)	16.5 (13.2–20.4)	42	14	1	13	0	0	1
Goat (n=194)	10.8 (6.8–16.1)	9	10	0	2	0	0	0

Prevalence $p<0.001$

CI confidence interval, *Li* liver, *Lu* lungs, *Ki* kidney, *Sp* spleen, *n* number of animals

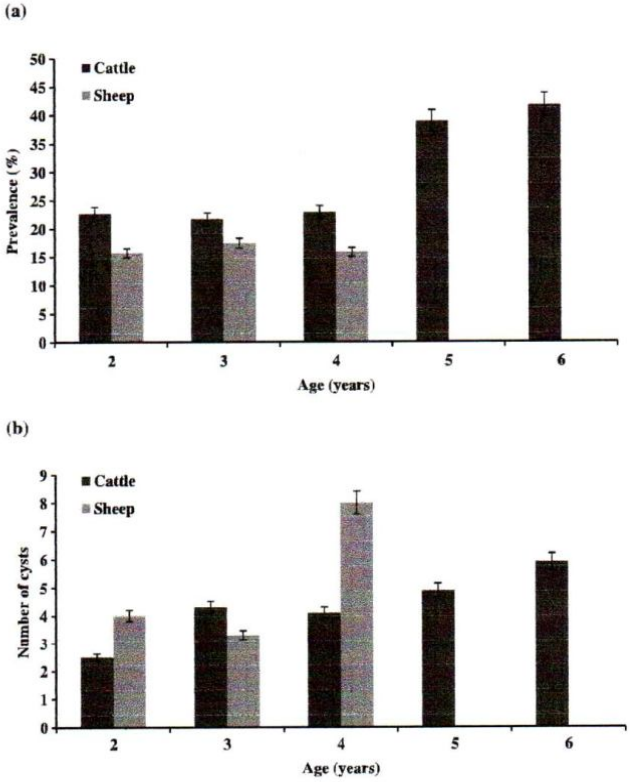

Fig. 1 Prevalence of cystic echinococcosis (**a**) and mean infection intensity of *Echinococcus* species (**b**) in cattle and sheep at different ages. *Error bar* with 5 % probability; *n* at age 2: 76 cattle, 204 sheep; at age 3: 157 cattle, 207 sheep; at age 4: 240 cattle, 19 sheep; at age 5:90 cattle; and at age 6: 24 cattle. No sheep were recorded at ages 5 and 6. Goats were not included due to the smaller number of infected animals (21/194)

Echinococcus spp. among livestock in the present study are significantly higher than those reported from the last comprehensive survey done in Maasailand three decades ago, at least in cattle and sheep (25.8 vs. 8.9 % and 16.5 vs. 8.1 %, respectively) (Macpherson 1985). They are also higher than those reported from other parts of Kenya (Njoroge et al. 2002) and neighbouring Sudan (Omer et al. 2010; Ibrahim et al. 2011), which confirm this region in southern Kenya as one of the hot spots of CE in Africa. Whether our data reflect a true increase of prevalence is difficult to conclude, as various factors which could influence the outcome of surveys (pre-selection of livestock to be slaughtered, requirements of abattoirs) may have changed between the two survey periods. Importantly, no figures on animal age were given in

Table 3 Conditions and species/genotypes of *Echinococcus* cyst isolates

Livestock species	Cyst condition	Liver		Lungs		Heart		Kidney		Spleen	
		n	*E.* spp.	*n*	*E.* spp.	*n*	*E.* spp.	*n*	*E.* spp.	*n*	*E.* spp.
Cattle	Fertile (*n*=40)	10	8 G1	30	14 G1/1 G5	0	0	0	0	0	0
	Non-fertile (*n*=574)	335	78 G1	235	98 G1	2	1 G1	1	1 G1	1	0
Sheep	Fertile (*n*=42)	26	14 G1	16	10 G1	0	0	0	0	0	0
	Non-fertile (*n*=122)	89	22 G1	30	20 G1/1 G6	1	1 G1	1	0	1	1 G1
Goat	Fertile (*n*=9)	0	0	9	5 G1/3 G6	0	0	0	0	0	0
	Non-fertile (*n*=42)	33	1 G1	9	5 G1/1 G6	0	0	0	0	0	0

G1 E. granulosus G1; *G5 E. ortleppi*; *G6 E. canadensis* G6

the previous survey, and prevalence is known to be strongly correlated with the age of the host animal. Both surveys agree on the fact that cattle, although showing the highest prevalence, play a minor role in transmission due to the low cyst fertility in this host. Moreover, cattle are clearly over-represented in our survey as they are sold to abattoirs more often than small stock which is usually slaughtered at home.

When considering the high fertility rate of cysts in sheep, it becomes clear that this species, together with goats, possibly is the most important intermediate host that maintains transmission of the disease in the Maasai population. Sheep slaughtered at home without inspection will easily pass on the parasites to dogs and consequently perpetuate the disease in the population (Macpherson 1985). High infection intensity in older animals may be caused by a continuous acquisition of new infections over time or may reflect the time needed for small, inconspicuous cysts to reach a detectable size. In either case, older animals, e.g. breeding stock, are the most affected animals in a population. This is important especially in the case of sheep and goats because such animals have low market value and are more likely to be slaughtered at home without supervision.

Echinococcus spp. indeed has wide organ infection range (Varcasia et al. 2006, 2007; Berhe 2009; Omer et al. 2010; Abdul et al. 2010) which has implications for meat inspection practises. Also, multiple organ infection translates into greater economic loss due to condemnation of affected organs as well as reduction in market value of entire carcasses.

The identification of *E. granulosus* G1, *E. ortleppi* and *E. canadensis* G6 is in support of earlier accounts of these species/genotypes isolated from African livestock (Wachira et al. 1993; Dinkel et al. 2004; Maillard et al. 2009; Omer et al. 2010; Ibrahim et al. 2011). However, the finding of G1 as the dominant taxon among cattle, sheep and goats (Wachira et al. 1993; Dinkel et al. 2004) is in contrast to the situation in neighbouring Sudan where the camel strain of *E. canadensis* is the dominant taxon (Dinkel et al. 2004; Omer et al. 2010; Ibrahim et al. 2011). A frequent presence of G1 was also reported from the North and Northeast of Africa (Maillard et al. 2007). Considering the predominance of *E. granulosus* G1 in the survey area, the above-discussed characteristics of CE in Maasailand (prevalence, fertility in hosts, organ involvement) are representative for this taxon. The dominance of G1 could also explain the high prevalence of human CE among the Maasai people, as this taxon has been shown to be the most pathogenic form of CE in humans (Wachira et al. 1993; Dinkel et al. 2004).

The account of *E. canadensis* G6 (camel strain) in sheep and goat in Maasailand where camels are mostly absent supports the previous findings (Wachira et al. 1993), where G6 was isolated from a cow and goat in Maasailand. In recent studies, the taxon was found in various livestock species including sheep and goats (Dinkel et al. 2004). The high fertility rate of G6 cysts in goats (three fertile cysts out of four) shows that this parasite is well adapted to goats which seem to be able to maintain the lifecycle in places where camels are absent. This is in agreement with the suitability of goats as hosts of the closely related 'pig strain' *E. canadensis* G7, as was shown in southern Europe (Varcasia et al. 2007).

Prior to our finding of *E. ortleppi* in its typical host, cattle, this taxon in Kenya had only been reported from pigs in the South (Dinkel et al. 2004) and from cattle in the north-central part of the country (Mulinge et al. 2011). The data available so far suggest that *E. ortleppi* is widespread but rare in eastern Africa, as in most other parts of the world. This appears difficult to explain, given the ubiquity of cattle in the area. However, cattle (in contrast to sheep) are rarely slaughtered at home, so local dogs rarely have access to cysts from cattle and may therefore not be able to pass the parasite to the local cattle population.

None of the examined isolates belonged to *E. felidis*, which was recently recorded from lions and warthogs in the Queen Elizabeth National Park, Uganda (Hüttner et al. 2009). No information on its range of intermediate hosts is available, and it may be present in livestock from Maasailand where animals graze around or in the vicinity of game reserves such as Maasai Mara or Amboseli.

In a pending further investigation of the diversity of CE in Maasailand, the disease was confirmed to be highly endemic with sympatric taxa coexisting in the same livestock population. Further evaluation of the current situation, especially in dogs, humans and wildlife will improve our understanding on the epidemiology of the disease in Maasailand and provide the background for the design of cost-efficient control strategies.

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Ethical standards The study complies with the current laws of Kenya.

Conflict of interests The authors declare that they have no conflict of interests.

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