PREVALENCE AND MOLECULAR CHARACTERIZATION OF CYSTIC ECHINOCOCCOSIS IN LIVESTOCK, ECHINOCOCCUS INFECTION AND THE RISK FACTORS IN DOGS IN KAJIADO-WEST SUB-COUNTY, KENYA

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Prevalence and Molecular Characterization of Cystic Echinococcosis in Livestock, *Echinococcus* Infection and the Risk Factors in Dogs in Kajiado-West Sub-County, Kenya

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DECLARATION

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

Signature……..…… ……Date……

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DEDICATION

I dedicate this work to my dear husband Dr. Samuel Gitau Mugo who has been my friend and mentor and more to that offered moral and financial support, and to my lovely children Teresia and Timothy.

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ACRONYMS AND ABBREVIATIONS

CE	Cystic Echinococcosis
CI	Confidence Intervals
ITROMID	Institute of Tropical Medicine and Infectious Diseases
JKUAT	Jomo Kenyatta University of Agriculture and Technology
KEMRI	Kenya Medical Research Institute
NTD	Neglected Tropical Diseases
PCR	Polymerase chain reaction
RFLP	Restriction fragment length Polymorphism
SERU	Scientific and Ethics Review Unit, KEMRI
SPSS	Statistical Package for Social Sciences
WHO	World Health Organization
PPEs	Personal Protective Equipment
ODK	Open Data Kit
PAIR	**P**uncture, **A**spiration of cyst fluid, **I**njection of 70% ethanol and **R**e-aspiration of ethanol

.

ABSTRACT

Cystic echinococcosis (CE) is a neglected zoonotic disease globally. In Africa CE is highly endemic in east Africa and northern Africa but rare or absent in central African and west African countries. CE is caused by the dog tapeworm *Echinococcus granulosus* sensu lato, and is more frequent in livestock rearing areas, where people live a nomadic lifestyle. The aim of this study was to establish the prevalence of cystic echinococcosis in cattle, sheep and goats and *Echinococcus* infection in dogs in Kajiado-West Sub-county. The study also identified *Echinococcus* species in livestock and dogs and assessed risk factors associated with *Echinococcus* infection in dogs. In total, 1,486 carcasses slaughtered in Kiserian and Keekonyokie slaughterhouses (388 cattle, 625 sheep and 473 goats) were examined for presence of hydatid cysts in various organs of the thoracic and abdominal cavities. Every cyst was separately preserved in 80% ethanol. Dog faecal samples were collected from the environment (ground) in three wards (Kiserian, Keekonyokie and Magadi) of Kajiado - West Sub county and preserved in 80% ethanol. Taeniid eggs were isolated using zinc chloride flotation-sieving technique and microscopically examined. Protoscoleces or tissue materials from cysts and eggs from faecal samples were picked under the microscope, lysed and genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and sequencing. The prevalence of CE was 15.2% (72/473) in goats, 14.9% (93/625) in sheep and 14.2% (55/388) in cattle). Out of the 421 cysts isolated, 389 were successfully characterized as *E. granulosus* sensu stricto (s. s.), 356/389 (91.5%), *E. canadensis* (G6/7), 26/389 (6.7%), or *E. ortleppi*, 7/389 (1.8%) respectively, while 32/421 (7.6%) could not be determined. Prevalence of taeniid infection (by microscopy) in dogs was 5.5% (19/345). However, by PCR taeniid eggs from only 8/19 faecal samples yielded PCR products. The prevalence of *Echinococcus* infection in dogs was 0.6% (2/345) by PCR. Two faecal samples contained a single egg of *E. equinus* (G4) and *E. felidis* each. Four *Taenia* species (1.2%) were identified in dogs; *T. multiceps* (3/345), *T. ovis* (2/345), *T. hydatigena* (1/345) and an unknown *Taenia* spp. (1/345) by sequencing. This is the first study to report *E. equinus* in dogs in Kenya. The detection of all the five species of *E. granulosus* s. l. in a single study is also reported for the first time in Kenya. This study confirms predominance of *E. granulosus* s. s. in livestock in Kajiado County. The importance of *E. ortleppi* and *E. canadensis* (G6/7) in our study was higher as compared to a previous study. More so, a high infection pressure for humans by *E. granulosus* s. s. based on its abundance could be speculated. The presence of *T. hydatigena*, *T. multiceps* and *T. ovis* in domestic dogs confirms the existence of ongoing transmission of cestodes from livestock to dogs even in absence of the major *Echinococcus* species (*E. granulosus* s. s., *E. canadensis* (G6/7) and *E. ortleppi* in dogs in this study. The presence of *E. felidis* and unknown *Taenia* spp. in faecal samples from dogs indicates a possibility that dogs are the link between the domestic and sylvatic cycles. Integrated control programs focusing on interrupting transmission from dogs to livestock and humans is recommended.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Echinococcosis is a zoonotic disease of public health significance with a global distribution. In Africa CE is endemic all African countries except in central Africa and some western African countries. In the eastern African countries, it is highly endemic in pastoral communities who are keeping large herds of livestock and the way of living favours the transmission of CE (Deplazes *et al*., 2017). At present 9 species of *Echinococcus* are recognized: *E. granulosus* sensu stricto, *E. equinus, E. ortleppi, E. canadensis* (G6 – G10), *E. felidis, E. multilocularis, E. vogeli, E. oligarthra and E. shiquicus* (Nakao *et al*., 2013b; Nakao *et al*., 2013c). Cystic echinococcosis is caused by the larval stage of the dog tapeworm *E. granulosus* sensu lato (s. l.) and the human infection is recognized by World Health Organization (WHO) as one of the neglected tropical diseases (WHO, 2013). CE is a common zoonotic disease of great public health significance globally due to its associated morbidity, mortality and economic losses (Budke *et al*., 2006). Globally, approximately US$ 3 billion are lost annually on treatment of CE in humans and losses incurred due to the condemnation of infected organs in livestock, (WHO, 2015). Dogs and to a lesser extent some wild canids and felids are the primary definitive hosts of *Echinococcus* species, while domestic, some wild herbivores and omnivores are acting as the intermediate hosts and the humans as aberrant intermediate hosts. The outcome of the infection in livestock and human is cyst development in the liver, lungs, or other organs (Thompson, 2017). Cysts in the liver or the lungs could be either fertile containing protoscoleces/daughter cysts or nonfertile. The non-fertile cysts can further be divided into calcified, degenerated, or sterile. These non-fertile cysts are non-infectious and, therefore, have no epidemiological significance in CE transmission to definitive hosts. *E. granulosus* s. l. consists of at least five species, namely, *E. granulosus* sensu stricto (s. s.), *E. equinus, E. ortleppi, E. canadensis* (G6–G10), and *E. felidis* (Nakao *et al*., 2013b; Nakao *et al*., 2013c).

The definitive hosts are infected by ingestion of offal containing hydatid cysts with viable protoscoleces. After ingestion, the protoscoleces evaginate, attach to the canine intestinal mucosa, and develop into adult tapeworms which release eggs or gravid proglottids in faeces. Following ingestion by the intermediate hosts a larva (oncosphere) is released from the egg. The oncosphere penetrates actively through the lamina propria and is transported passively through blood or lymph fluids to the liver, lungs, or other organs, where the oncosphere settles and develop into a hydatid cyst- (Thompson, 2017).

Echinococcus spp. infection in dogs does not cause significant pathology and is typically asymptomatic, even in animals with high parasite burdens. However data from dogs is important in accessing distribution, transmission dynamics, risks of infection for humans and other intermediate hosts (Carmena and Cardona, 2013). Diagnosis of *Echinococcus* and *Taenia* spp. infection in dogs is complicated by the inability to detect infection microscopically during prepatent period and periodic shedding of eggs and to distinguish *Echinococcus* from *Taenia* spp. *Echinococcus* infection in dogs can be detected by several techniques including necropsy, arecoline bromide purgation, copro-antigen ELISA and copro-PCR (Craig *et al*., 2015).

1.2 Statement of the problem

CE is a common zoonotic disease of great public health significance globally due to its associated morbidity, mortality and economic losses (Budke *et al*., 2006). Globally, approximately US$ 3 billion are lost annually on treatment of CE in humans and losses incurred due to the condemnation of infected organs in livestock, (WHO, 2015). East Africa and Kenya, in particular, have long been known to be one of the world's largest foci of CE in humans (Macpherson *et al*., 1987; Macpherson *et al*., 1989; Romig *et al*., 2011). Previous studies from Kenya have focused on CE situations mainly in Turkana and Maasailand but also in parts of other pastoralist areas (Romig *et al*., 2011). The prevalence of CE in livestock was 19.4% in cattle, 3.6% in sheep and 61.4% in camels in Turkana (Njoroge *et al*., 2002), in Maasailand 25.8% in cattle, 16.5% in sheep and 10.8% in goats (Addy *et al*., 2012) and 1.92% in cattle,

6.94% in camels, 0.37% in goats and 4.62% in sheep in Isiolo and Meru (Mbaya *et al*., 2014). These studies indicate the presence of all five *E. granulosus* s. l. and the recently discovered G_{omo} genotype (Wachira *et al*., 1993; Dinkel *et al*., 2004; Addy *et al*., 2012; Kagendo *et al*., 2014; Mbaya *et al*., 2014; Odongo *et al*., 2018). Six risk factors that included: free roaming dogs, feeding dogs with raw offal, failure to deworm and dogs aged ≤5 years, improper disposal of slaughter offal, lack of knowledge on CE transmission by dog owners were identified in Turkana and associated with Coproantigen-positivity in dogs (Buishi *et al*., 2006). To get a clearer CE situation in Kenya, epidemiological data from all endemic localities including Kajiado County is required.

1.3 Justification of the study

There is limited data on CE in cattle, sheep and goats and *Echinococcus* infection in dogs within Kajiado County. The only available data from this area is nearly three decades old and did not distinguish the various species of *Echinococcus* spp. in livestock and showed CE prevalence of 8.9% in cattle, 8.1% in sheep and 7.1% in goats (Macpherson, 1985). Furthermore, the recent study by Addy *et al*. (2012) examined livestock originating mainly from Bissil area (Kajiado South) and recorded a total CE prevalence of 25.8%, 16.5% and 10.8% in cattle, sheep and goats respectively. The current study focused on the two main slaughterhouses in Kajiado - West Sub county receiving livestock from the wider Kajiado County. Up to date data on *Echinococcus* infection in dogs is missing in Kajiado, the previous study dates 40 years ago was done macroscopically by detection of adult worms (Eugester, 1978). CE causes notable economic losses. In Kenya, annual livestock associated losses globally through organ condemnation were reported to be (US$ 4,976) in Kisumu East/West district and (US$ 4,054) in Isiolo (Odero et al.,2015). The average annual losses due to surgical treatment was reported to be US$ 22,658. CE-associated monetary losses that were associated with lost economic opportunities was reported to be to US$ 4,414 for a herdsman and US$ 1,339 for a house wife. (Odero et al.,2014) This study sought to determine the prevalence of cystic echinococcosis (CE) in cattle, sheep and goats and *Echinococcus* infection in dogs, and determine the *Echinococcus* species in different hosts in Kajiado County.

1.4 Research Questions

2 What is the prevalence of cystic echinococcosis (CE) in cattle, sheep and goats and *Echinococcus* infection in dogs in Kajiado - West Sub-County?

3 What species of *Echinococcus* are prevalent in domestic animals in Kajiado - West Sub-County?

4 What risk factors are associated with *Echinococcus* infection in dogs in Kajiado - West Sub-County?

1.5 Study Objectives

1.5.1 General Objective

To determine the prevalence and molecular characterization of cystic echinococcosis in livestock and *Echinococcus* infection in dogs and assess the associated risk factors in Kajiado - West Sub-County.

1.5.2 Specific Objectives

2 To determine the prevalence of cystic echinococcosis (CE) in cattle, sheep and goats and *Echinococcus* spp. infection in dogs in Kajiado - West Sub County.

3 To determine *Echinococcus* species in cattle, sheep, goats and dogs in Kajiado - West Sub-County.

4 To determine the risk factors associated with *Echinococcus* infection in dogs in Kajiado - West Sub-County

CHAPTER TWO

LITERATURE REVIEW

2.1 Taxonomy of *Echinococcus* species

The tapeworm belongs to the family Taeniidae and genus *Echinococcus.* At present five morphologically distinct species of *Echinococcus* are recognized: *E. granulosus* sensu lato, *E. multilocularis, E. oligarthra, E. vogeli* and *E. shiquicus. E. granulosus* s. l. consists of at least five species, namely, *E. granulosus* sensu stricto (s. s.), *E. equinus*, *E. ortleppi*, *E. canadensis* (G6–G10), and *E. felidis* (Nakao *et al*., 2013b; Nakao *et al*., 2013c).

2.2 Epidemiology and prevalence of CE in Africa

Global data show that *Echinococcus* spp. have varied distribution across all continents with varying prevalence and severity (Deplazes *et al*., 2017). It is found in every continent except Antarctica (WHO 2015). Human rates for CE in endemic regions have been found to affect more than 50 per 100,000 persons per year with prevalence levels of 5-10% in parts of Argentina, Peru, East Africa, Central Asia and China. In slaughtered animals in hyper endemic areas of south America a varying prevalence of 20-95% was reported. (WHO 2015). CE is a widespread disease in Africa and poses great challenges in many countries where livestock rearing is the main livelihood (Romig *et al*., 2011). Update CE data is available from North African countries including Tunisia, Morocco, Libya, Algeria, Egypt, Mauritania and Sudan. However, little is known of CE in central and west African countries. In southern Africa data is available only from South Africa, Zambia and Namibia (Wahlers *et al*., 2012; Deplazes *et al*., 2017; Romig *et al*., 2017).

In Kenya, prevalence of CE in livestock varies between regions. Field surveys have been previously conducted on prevalence and diversity of cystic echinococcosis in Kenya (Romig *et al*., 2011). Aetiology of the disease was first confirmed by Nelson and Rausch (1963), who identified *E. granulosus* as the causative agent of the disease in Turkana, Kenya. Two studies in Narok and Kajiado counties reported high prevalence in livestock and sporadic cases in

humans (Macpherson, 1985; Macpherson *et al*., 1989). Similarly, Njoroge *et al*. (2002) conducted a study on prevalence of cystic echinococcosis in slaughter animals in three divisions of northern Turkana and showed that the prevalence was 19.4% in cattle, 3.6% in sheep and 61.4% in camels. In a study conducted in Eastern Kenya, specifically Meru and Isiolo counties, the prevalence of CE in cattle, camels, goats, and sheep was 1.9 %, 6.9%, 0.4% and 4.6% respectively (Mbaya *et al*., 2014). A survey conducted in Kitengela and Suswa slaughterhouses, examined livestock from Kajiado and Narok counties and reported CE prevalence of 25.8% in cattle, 16.5% in sheep and 10.8% in goats (Addy *et al*., 2012). Another study in Narok county revealed a similar prevalence of CE in sheep (16%) (Odongo *et al*., 2018). Elsewhere in Laikipia county, the prevalence of CE in livestock was 11.8% in cattle, 1.5% in sheep and 2.3% in goats (Gachengo et al., 2017).

All the species of *E. granulosus* s. l. have so far been identified in Africa (Romig *et al*., 2015; Romig *et al*., 2017) Similarly in Kenya, all *E. granulosus* s. l., species, have been reported, however *E. granulosus* s. s. and *E. canadensis* (G6/7) are the most common in livestock, humans and dogs (Romig *et al*., 2017; Mulinge *et al*., 2018). *E. ortleppi* and *E. equinus* are less frequent in livestock in Kenya (Romig *et al*., 2017). *E. felidis* has been identified in lions, hyena and for the first time in dogs in Kenya (Kagendo *et al*., 2014; Mulinge *et al*., 2018).

2.3 Genetic diversity of *Echinococcus granulosus*

Echinococcus granulosus is among a complex group of parasites that exhibit a wide range of intraspecies variation. This variation has been expounded by molecular typing techniques based on the mitochondrial, RNA loci or nuclear gene targets. For instance, analysis of NADH dehydrogenase 1and mitochondrial cytochrome C oxidase 1 discovered 10 different genotypes (Lavikainen et al., 2003). Other genes that may be exploited by molecular techniques include, nuclear actin II, internal transcribed spacer 1, homeobox 2 and 12SrRNA (Jabbar et al., 2011; Huttner et al., 2009). The existence of the different *Echinococcus granulosus* strains with different host preferences occurs based on geographical location. They include sheep strains (G1 and G2), Bovid strains (G3 and G5), horse strain (G4), camelid strain (G6), pig strain (G7), cervid strain (G8), human polish strain (G9) and fennoscandian cervid strain (G10) (Grosso et

al., 2012). Some strains have almost similar genetic characteristics and are considered to belong in the same taxon. For instance, GI to G3 are known as *Echinococcus granulosus sensu stricto* while G6 to G10 are termed as *Echinococcus Canadensis*. The other strains including *Echinococcus equinus*(G4) and *Echinococcus ortleppi*(G5) are genetically different.

Among the strains that infect humans *Echinococcus granulosus sensu stricto* is responsible for the highest number of human cystic echinococcosis, camel strains represent a smaller proportion while horse strains are considered not infectious to human (Alvarez et al., 2014). This observation has been attributed to uneven distribution of the prevalence of cystic echinococcosis in the human population based on the geographical location (Dinkel et al., 2004). al., 2011). For instance, a study in Isiolo, Kenya, revealed a very low prevalence of *Echinococcus granulosus sensu stricto* despite the high number of cattle in the area (Mbaya et al., 2014).

2.4 Life cycle of *Echinococcus* species

Echinococcus spp. is perpetuated in a life-cycle requiring two mammals in predator-prey relationship (Thompson, 2017) as shown in Figure 2.1 (inner cycle). Carnivores such as the domestic dog serve as definitive hosts harbouring 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 gravid proglottids containing embryonated eggs (stage 2) through their faeces into the environment. Intermediate hosts such as livestock get infected by ingesting eggs during grazing or accidentally as in human (dead-end 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 a larvae (oncosphere) that gets attached to the intestinal mucosa (stage 3) and penetrates actively through the intestinal wall and enters the portal blood system and is passively transported into organs mainly the liver and/or lungs. After settling in an organ, the oncosphere develops into a hydatid cyst (stage 4) as a unilocular fluid-filled bladder consisting of two parasite-derived layers; an inner nucleated multipotential germinal layer and an outer acellular laminated layer surrounded by a host-produced fibrous capsule (Zhang *et al*., 2003). Protoscoleces once

ingested by definitive hosts (once they access fertile cysts) evaginate in the small intestines and develop in to adult worms and the cycle is completed.

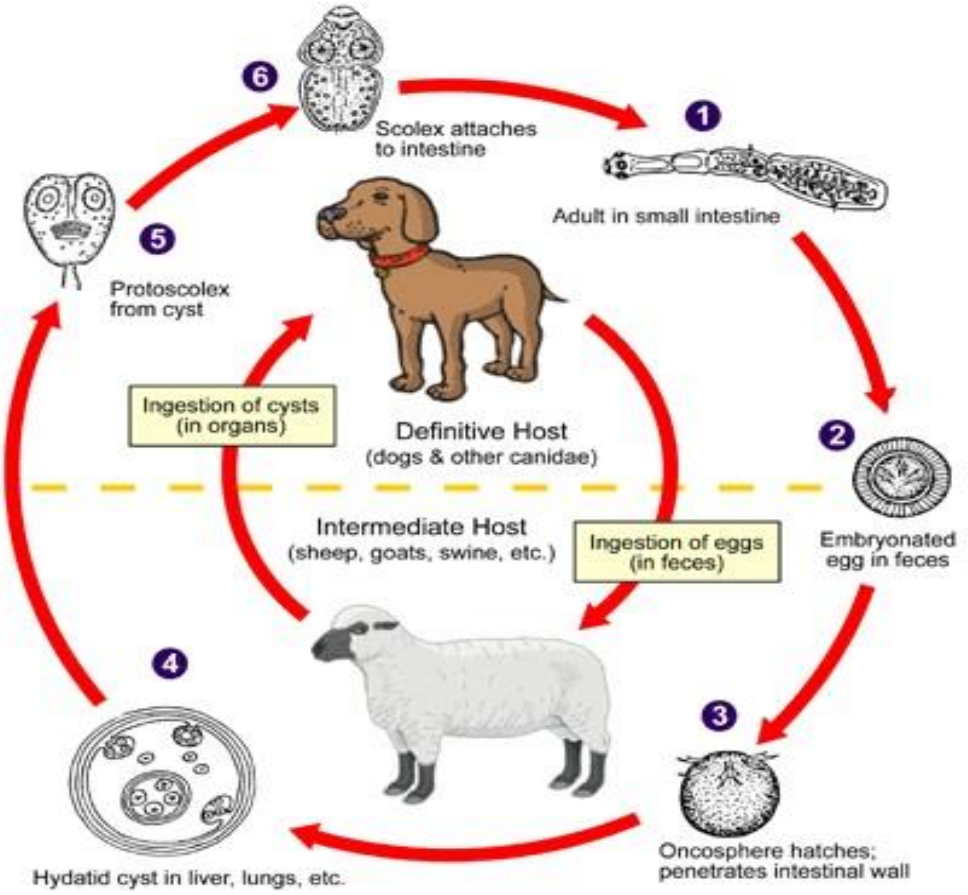

Figure 2.1: Life cycle of *Echinococcus* species adapted from www.dpd.cdc.govt/dpdx

2.5 Diagnosis of cystic echinococcosis and *Echinococcus* infection in dogs

Diagnosis of *Echinococcus* in definitive hosts involves necropsy, purgation, serum antibody detection, Copro-ELISA and copro-PCR (Craig *et al*., 2015). At necropsy, the main focus is the detection of worm and the burden in the small intestine of the definitive host. For differential diagnosis the parasite is observed under microscope. Necropsy and purgation allow differential diagnosis of adult and premature taeniid worms while the egg can be differentiated by use of molecular techniques (Craig *et al*., 2015). Necropsy is 100% specific in CE endemic areas and

offers a sensitivity rate of 97% but may fail to detect very low worm burdens (<6 worms). Purgation guarantees 99 – 100% specificity but has low sensitivity especially in low intensity infections and in situations where the full purge does not occur. The use of purgation is limited due to the environmental contamination and adverse side effects in dogs (Craig *et al*., 2015). Detection of specific antigen(s) in faecal samples from definitive hosts has the advantage over serum antibody detection, the latter reports past and current infections. Serum antibody detection has high specificity (90%), and a low sensitivity (35-40%) for natural infection in dogs. Copro-ELISA detects *Echinococcus* antigens in faecal samples and utilizes either polyclonal or monoclonal antibodies directed against either somatic or excretory/secretory (ES) antigens. Copro-ELISA tests for *E. granulosus* have shown good genus specificity (78 – 100%) and sensitivity of (85 – 95%) which is strongly associated with worm burden of the parasite (Allan *et al*., 1992; Buishi *et al*., 2005b). The diagnosis of CE in intermediate hosts (livestock) has been based mainly on necropsy findings mainly during meat inspection (Craig *et al*., 2015). Clinical symptoms, usually mild manifestations, may be overlooked. For diagnosis in humans, imaging techniques such as ultrasound, CT and MRI are commonly used (Figure 2.2). 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).

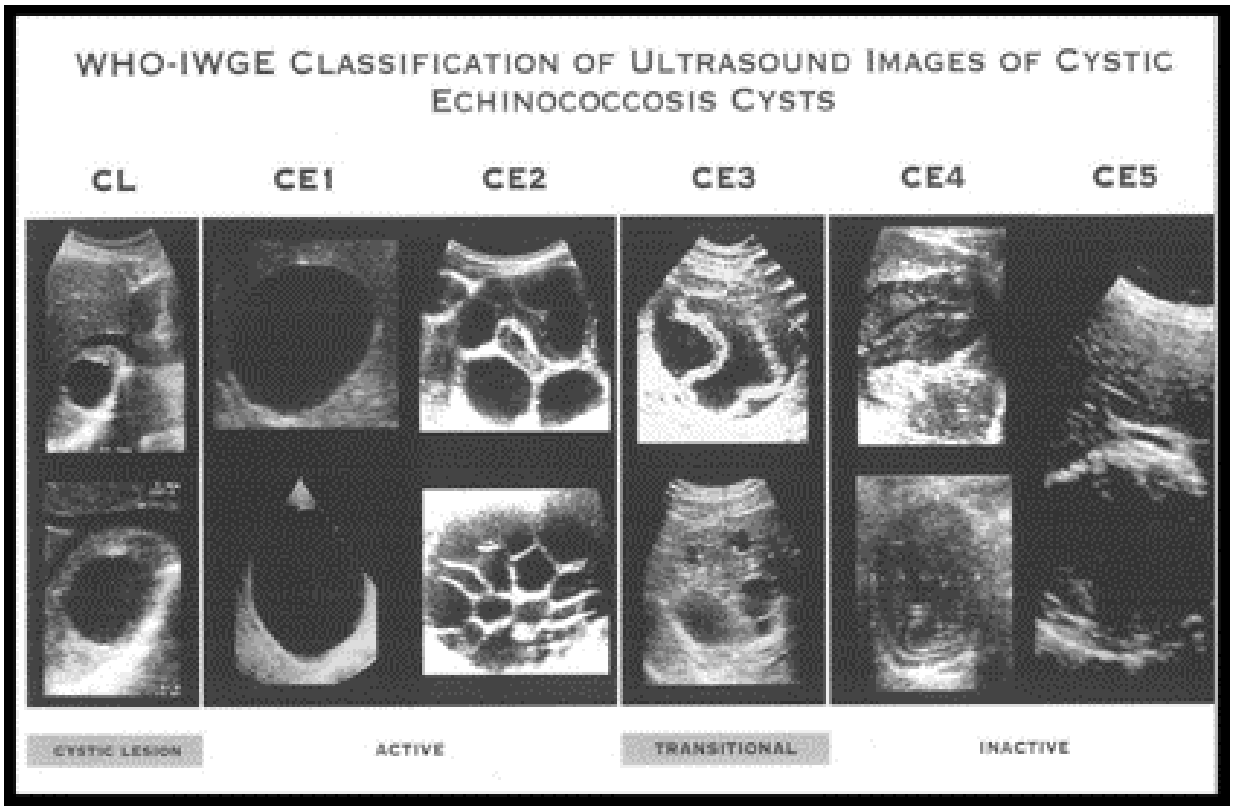

Figure 2.2: WHO-Informal Working Group on Echinococcosis standardized classification WHO/CDS/CSR/APH/2001.6)

CL-Undifferentiated 'cystic lesions' that requires further investigations before definitive diagnosis is made
CE1-Simple round or oval unilocular cyst with anechoic content and a visible double cystic wall.
CE2-Cysts completely filled with daughter cysts
CE3-Includes two stages, CE3a and CE3b which differ in terms of morphology, viability and clinical characteristics. CE3a is characterised by the 'water lily' sign, represented by floating membranes i.e. the endocyst detached from the cyst outer wall (pericyst). CE3b shows a predominantly echogenic structure with daughter cysts in all stages of degeneration
CE4-Cyst content is uniformly echogenic
CE5-Cysts are partially or completely calcified

Immunological tests such as serum antibody detection and detection of circulating antigens for the diagnosis of in human have been used. These diagnostic techniques are less sensitive and specific in animals than in human. Variation in the pathogenicity of species of *Echinococcus* also influences the prognosis in animals (Craig *et al*., 2015). Introduction of molecular

techniques has improved the diagnosis of CE up to species level (Abbasi *et al*., 2003; Dinkel *et al*., 2004; Hüttner *et al*., 2009).

2.6 Risk factors associated with *Echinococcus* infection in dogs

Dogs fed with raw offal were more likely to be Coproantigen positive (Moro *et al*., 1999; Buishi *et al*., 2005b; Moro *et al*., 2005; Buishi *et al*., 2006) while activities that can prevent dogs from accessing offal such as proper disposal of offal or not slaughtering at home were protective factors to acquisition of infection (Buishi *et al*., 2006; Acosta-Jamett *et al*., 2010). Keeping or ownership of sheep, yak and goats was associated with decreased risk of *Echinococcus* infection in dogs (Shaikenov *et al*., 2003; Budke *et al*., 2005; Mastin *et al*., 2011; Mastin *et al*., 2015). On the contrary a higher Coproantigen positivity was shown to correlate with the number of sheep kept in Argentina (Perez *et al*., 2006). Farm dogs and sheep-dogs were more likely to get infected with *Echinococcus* than village dogs (Moro *et al*., 1999; Shaikenov *et al*., 2003; Buishi *et al*., 2005b; Mastin *et al*., 2015), however low odds were shown for sheepdogs in Alay valley, Kyrgyzstan (Mastin *et al*., 2015). Free roaming dogs were likely to be infected with *Echinococcus* or tested positive on Coproantigen compared to restrained dogs (Parada *et al*., 1995; Budke *et al*., 2005; Buishi *et al*., 2005a; Buishi *et al*., 2006; Guzel *et al*., 2008; Huang *et al*., 2008; Ziadinov *et al*., 2008; Mastin *et al*., 2015). Furthermore, Inangolet *et al*. (2010) showed that stray dogs had higher intensity of worms than owned dogs in Moroto, Uganda. Young dogs aged 2 years or less were more likely to be Coproantigen positive compared to old dogs (Acosta-Jamett *et al*., 2010; Mastin *et al*., 2015). In other studies dogs older than 5 years were less likely to be *Echinococcus* positive and had lower parasite burden compared to younger dogs (Buishi *et al*., 2005b; Budke *et al*., 2006; Inangolet *et al*., 2010; Otero-Abad and Torgerson, 2013). Failure to deworm dogs or infrequent anthelmintic treatment were associated with increased odds of Coproantigen positivity in dogs (Buishi *et al*., 2005a; Buishi *et al*., 2005b; Acosta-Jamett *et al*., 2010). Dogs from rural communities where pastoralism is likely to be practiced presented a higher risk of infection than those living in urban settings (Parada *et al*., 1995; Buishi *et al*., 2005a; Buishi *et al*., 2005b; Elshazly *et al*., 2007; Acosta-Jamett *et al*., 2010). Lack of knowledge on CE and its transmission cycle has been shown to be a risk factor (Buishi *et al*., 2005b; Moro *et al*., 2005; Buishi *et al*., 2006; Mastin *et al*., 2015). Other

associated risk factors thought not to be significant include dog's sex and weight, with some studies identified male dogs to be more vulnerable to infection than females counterparts in China and Uganda (Budke *et al*., 2005; Inangolet *et al*., 2010) while the vice versa was reported in Uruguay and Peru (Parada *et al*., 1995; Moro *et al*., 2005).

2.7 Treatment and control of cystic echinococcosis

The treatment of CE in humans can be achieved by surgery, external drainage technique such as **PAIR** (**P**uncture – **A**spiration – **I**njection –**R**espiration), Chemotherapy and 'watch and wait' approach. The choice of treatment is based on the type of cysts (according to WHO-IGWE classification), cysts location and whether it is a complicated or non-complicated cysts (Kern *et al*., 2017). The use of recombinant EG95 vaccine in control of CE in the intermediates hosts (livestock mainly sheep) has been applied in several countries with varying degree of success. However, this approach is not sustainable in poor resources countries due to the short-lived immune protection (Lightowlers *et al*., 1996; Larrieu *et al*., 2013; Larrieu *et al*., 2015). The treatment of *Echinococcus* infection in dogs is achieved by regular deworming with Praziquantel and arecoline hydrobromide purgation (Craig *et al*., 2017). For successful prevention and control of CE, an integrated approach involving health education, dog management (dog population control and regular deworming), proper disposal of slaughter offal and strict slaughter management (Craig *et al*., 2017).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

This study was carried out in Kajiado West Sub-County (Figure 3.1**)**. The target site covers an area of 21,900.9 km^2 with an estimated population of 82,849 as per 2019 (KNBS, 2019). The county borders Nakuru, Nairobi and Kiambu counties to the north, Makueni and Machakos to the East, Narok County to the west, and Taita-Taveta County to the south. The county is divided into five sub-counties; Kajiado Central, Kajiado North, Kajiado East, Kajiado West, and Kajiado South. The county has a bi-modal rainfall pattern with the short rains between October and December and long rains are experienced between March and May. Temperatures vary with altitude and season in Kajiado - West Sub-County with the temperatures ranging between 10 ℃ and 34 ℃ and an average of 18.9 ℃. The cold months are between July and August, while the hottest months are January, February, September, and October. The rainfall amount ranges from as low as 500 mm to as high as 1250 mm (Bekure, de Leeuw, Grandin, & Neate, 1991). Sampling of hydatid cysts was done in two abattoirs in Kajiado West Sub-County namely, Kiserian and Keekonyokie, which are the two major abattoirs in the county (Figure 3.2). Dog faecal sampling was done in 3 wards namely: Kiserian, Keekonyokie and Magadi. These 3 sites were chosen due to their close proximity to the slaughter houses. The main use of land in the county is for livestock rearing through semi-nomadic pastoralism and crop production. According to the 2019 national census, it was reported that the main livestock species ranged from sheep (433,289) and goat (323,370) to beef and dairy cattle (169,873) in Kajiado-west sub - county. The county is, therefore, the leading producer of different livestock products such as beef, milk, skins, and hides.

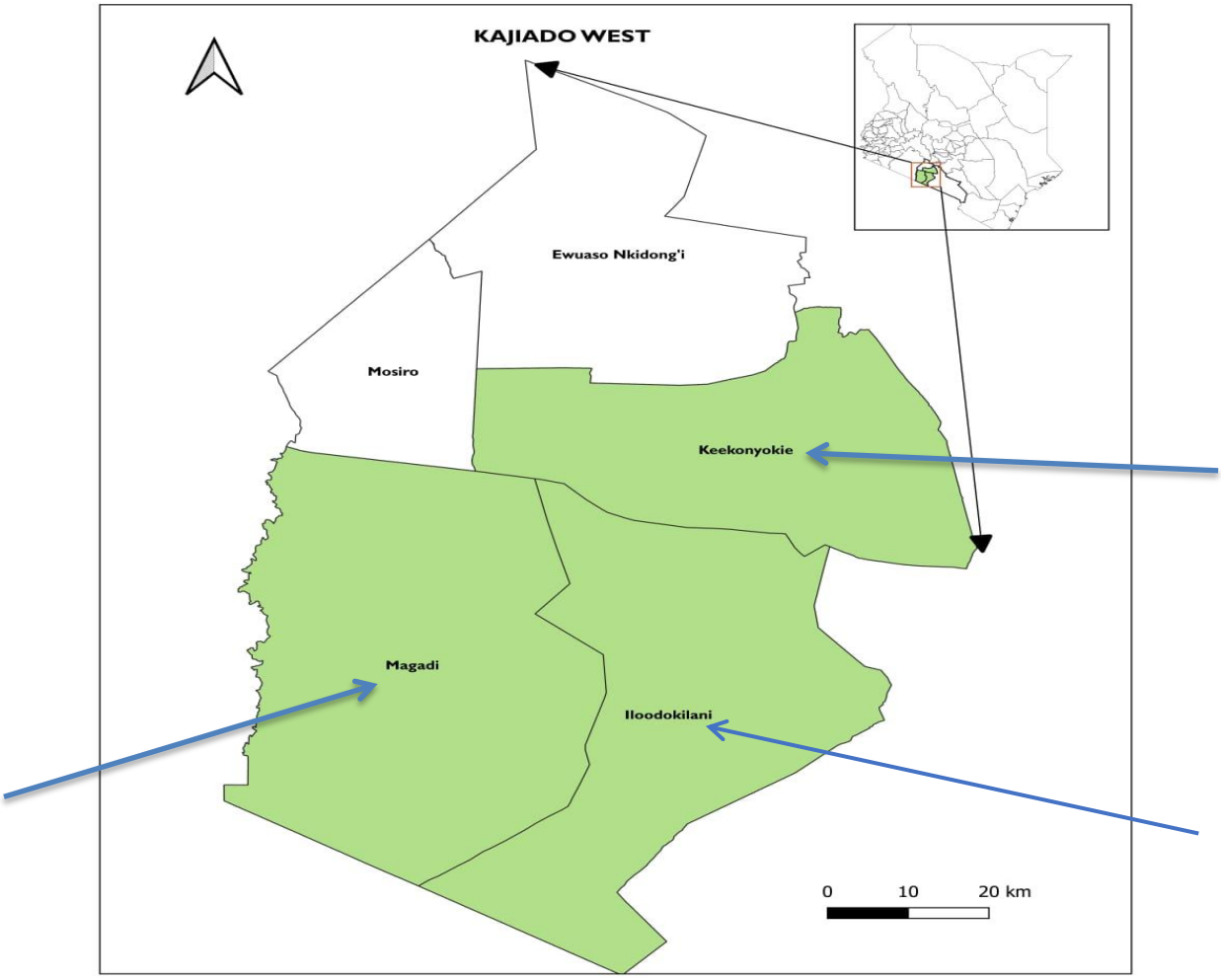

Figure 3.1: Map of the Kajiado West Sub-county. The three study sites (wards) are shown by arrows (Survey of Kenya)

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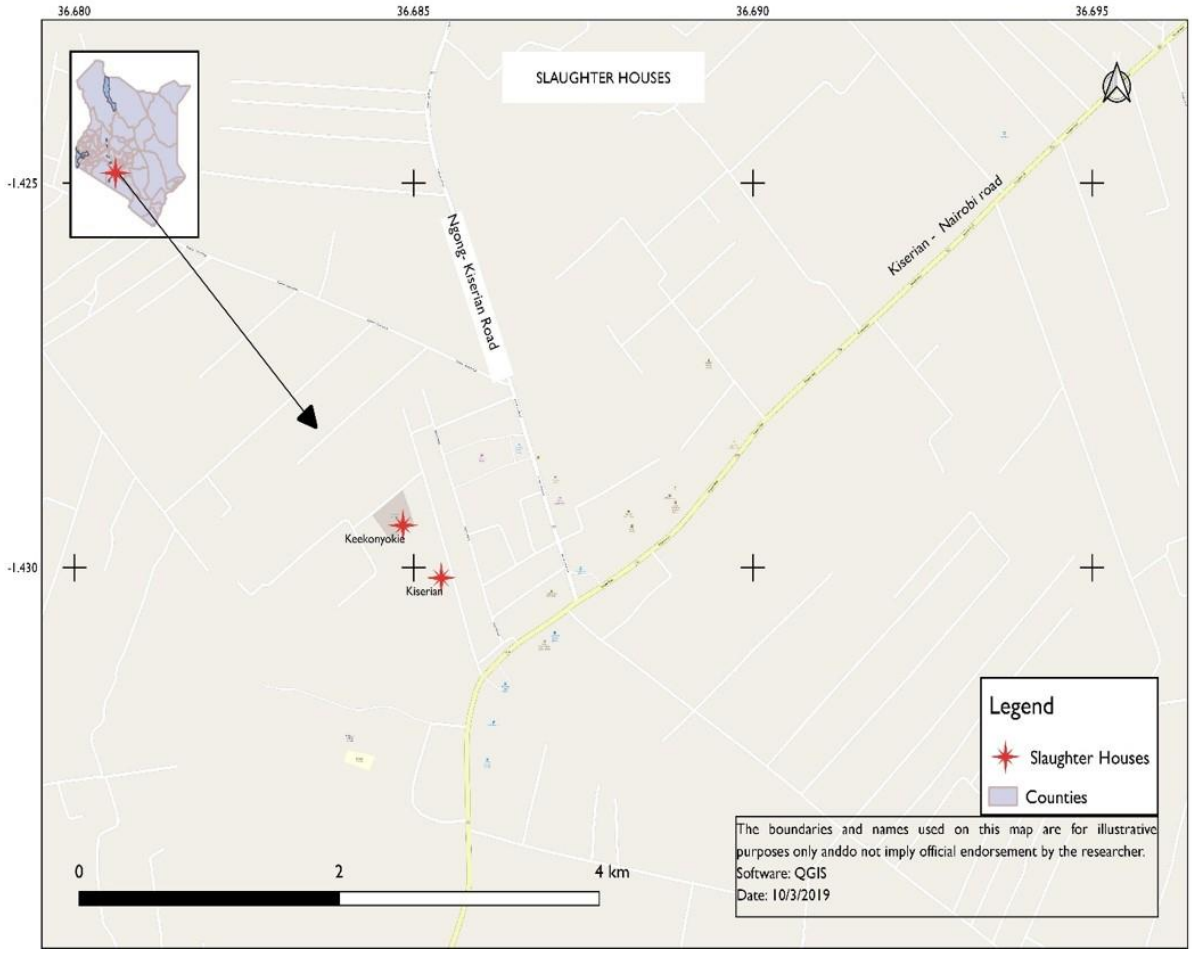


Figure 3.2: A map showing the location of the two abattoirs located in Kiserian and Keekonyokie (Source: QGIS)

3.2Study design

This study used cross-sectional design to collect data from a total of 1,486 animals comprising 388 cattle, 625 sheep, 473 goats and 345 dogs at a single point in time. Livestock data collection forms (Appendix 1) were used to document the observations while in the slaughter houses and a questionnaire was administered to assess the risk factors for *Echinococcus* infection in dogs in Kajiado West Sub-county.

3.3 Study Animals

Cysts samples were collected from different livestock species including 388 cattle, 625 sheep and 473 goats slaughtered at Kiserian and Keekonyokie slaughterhouses, between December 2016 and February 2017. Dog faecal samples (316) were collected in randomly selected households in Kiserian, Keekonyokie and Magadi wards, while 29 were collected around the slaughter houses. Whereas it was easier to identify the true origin of dogs based on the households selected, it was however difficult to trace the origin of livestock animals since they were sent to the slaughter house by middlemen who in turn bought them from clients. However, majority of the slaughtered livestock originated from the entire Kajiado County

3.4Selection criteria

3.4.1 Inclusion criteria

1. All carcasses of cattle, sheep and goats sampled from Kiserian and Keekonyokie slaughterhouses of Kajiado -West Sub-county
2. All dog owners from Kiserian, Keekonyokie and Magadi wards who consented to participate.
3. Only the fresh faecal samples in the selected homesteads

3.4.2 Exclusion criteria

1 All dog owners who did not consent for the study.

3.5 Sampling technique

All carcasses from cattle, sheep and goats in Kiserian and Keekonyokie slaughterhouses were examined for cysts and fresh dog faecal samples from selected households were collected in Kiserian, Keekonyokie and Magadi wards in Kajiado - West Sub-county.

3.6 Sample size determination

The sample size was calculated based on Fischer *et al*., (1998) formula below and previous reported prevalence rates for each species of animals in the country (Table 3.1) at 95% confidence interval and 5% desired absolute precision. That is:

$$n = \frac{Z^2\alpha/2pq}{d^2}$$

Where n = sample size, z = Z statistic for 95% confidence interval (1.96), p = the target proportion of CE, q =1-p and d (0.05) = Permissible error in the estimate of P.

Considering the previous prevalence rates of CE to be used are 25.8% in cattle, 16.5% in sheep, 10.8% in goats (Addy *et al*., 2012) and 25% in dogs (Zeyhle, pers. com), the calculated sample size is as tabulated below (Table 3.1).

Table 3.1: Calculated sample size of the study animals based on prevalence of previous studies

Animal	Prevalence of CE (%)	Sample size
Cattle	25.8[a]	294
Sheep	16.5[a]	212
Goats	10.8[a]	148
Dogs	28[b]	310
Total		964

a – (Addy *et al*., 2012) and b - (Zeyhle, pers. comm.)

3.7 Data collection procedures

3.7.1 Questionnaire

This study used a closed structured questionnaire with multiple choices (Appendix 2) to determine possible risk factors for canine *Echinococcus* infection. The questionnaire was

written in English and translated in Swahili and Maasai languages and then used to collect data from dog owners and thereafter, faecal samples were collected on the ground at the homesteads. Data collected included information about the occupation of the owner of the dog, the name of the research site and date of sampling, demographic data relating to the age, colour, sex, breed, and use (pet, guard, hunting or shepherd) of each dog, information on dog keeping practices, dog behaviour such as types and sources of food/access of the dog to slaughter offal and carcasses of dead animals carcasses of slaughtered animals, free roaming or restrained, contact with humans and association with livestock. The dog owners were also asked whether the dogs are treated with anthelmintic and the deworming strategy employed (frequency of treatments and kind of anthelmintic used). The questionnaire also focused on periodical livestock home slaughter by the dog owner, disposal of carcasses of slaughtered animals and the owner's knowledge of *Echinococcus* infection in dogs. A detailed questionnaire is inserted in Appendix 2.

3.8 Hydatid cyst collection from cattle, sheep and goats

A total of 388 carcasses of cattle, 625 sheep, and 473 goats were randomly selected for inspection of hydatid cysts and any other cysts in all organs of the thoracic and abdominal cavities after slaughter. All carcasses (lungs, liver, heart as well as kidneys) were carefully examined by visual inspection, palpation plus incision according to Eckert (2001). Livestock data collection form was used to collect other animal data (Appendix 1). The cysts were excised from the organ and each cyst packed in a labeled separate bag and transported in a cool box to KEMRI, Center for Microbiology Research (CMR) parasitology lab for examination and further analysis.

In the laboratory, the cysts were dissected using a sterile scalpel blade and hydatid fluid aspiration followed with examination of individual cyst for fertility and/or sterility according to Capuano *et al.*, (2006) and Scala *et al*., (2006). To determine the fertility of cysts, aspirated fluid was examined under a light microscope (Olympus CHBS, Tokyo, Japan) (40x) in a Petri dish by demonstrating presence of protoscoleces. The viability of the cysts was assessed based on the morphology of protoscoleces as well as their flame cells motility. Cysts were classified

as fertile (with protoscoleces) sterile (fluid-filled without protoscoleces), degenerated (collapsed cyst walls with caseated protoscoleces and soft cheesy debris without calcification), and calcified (hard solid appearance of the ectocyst). Cyst material (pieces of endo cyst or protoscoleces from the individual cysts were fixed in 80% ethanol for further processing All the cysts from the same organ were examined individually to confirm mixed infections.

Plate 3.1: Examining carcasses for CE. The arrow points at a cyst in lungs of a sheep (source: Nungari, 2020)

3.9 Faecal sample collection

Fresh faecal samples were collected from the environment (ground) in the homesteads and along the pathways leading to the homes. The samples were identified following a procedure reported previously by Mulinge et al. (2018) and by the help of the dog owners. To reduce instances of collecting faecal samples originating from the same dog, care was taken to collect

only the samples that were at most one-day old. The collected faecal samples were preserved in 80% ethanol in clean tightly closed containers, which were then packed in boxes for transportation to parasitology laboratory of CMR, KEMRI.

3.9.1 Isolation of taeniid eggs from faecal contents

The preservative was washed off by adding 8 ml of distilled water and the taeniid eggs were concentrated by zinc chloride floatation-sieving method (Mathis *et al*., 1996). The ethanol was first drained from the samples and the samples were rinsed with 8 ml of distilled water. The rinsing water was drained off to obtain faecal pellets. One part of the obtained pellets was mixed with four parts of the zinc chloride and after floatation, the top layer, which contained the taeniid eggs was collected and filtered using 50 µm sieve followed by a 22 µm sieve (Franz Eckert GmbH, Germany) as shown in Figure 3.2. The eggs were then washed off from the 22 µm sieve using distilled water into a 15 ml falcon tube. The samples were centrifuged at 400 rpm and the eggs were collected using Pasteur pipette and stored in 2 ml microcentrifuge tubes at 4°C.

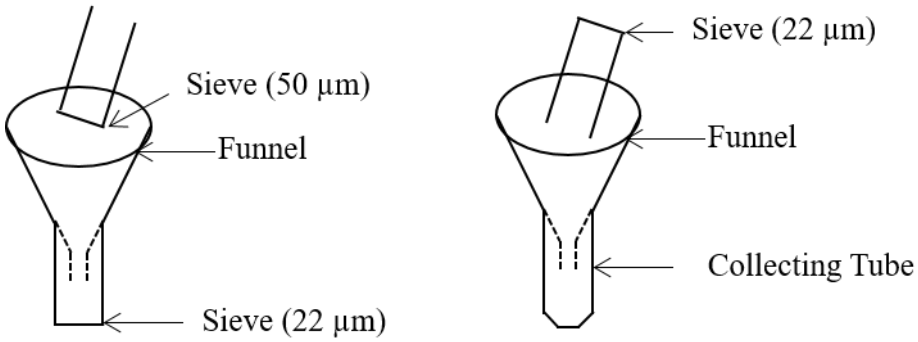


Figure 3.2: An illustration showing zinc chloride flotation-sieving technique for taeniid eggs

3.9.2 DNA extraction from cyst material and protoscoleces

DNA was obtained from endocyst material and protoscoleces by lysing in 0.02 M NaOH at 95 °C for 10 minutes (Nakao *et al*., 2003) In a few instances where the above process failed to yield adequate DNA, genomic DNA was extracted using DNeasy Blood &Tissue Kit (Qiagen, Hilden, Germany). The germinal layers or cyst walls were cut into small pieces and lysed in ATL lysis buffer (180 μl) and proteinase K (20 μl), and DNA was subsequently extracted using the manufacturer's protocol. Extracted DNA was eluted in 50 μL of elution buffer.

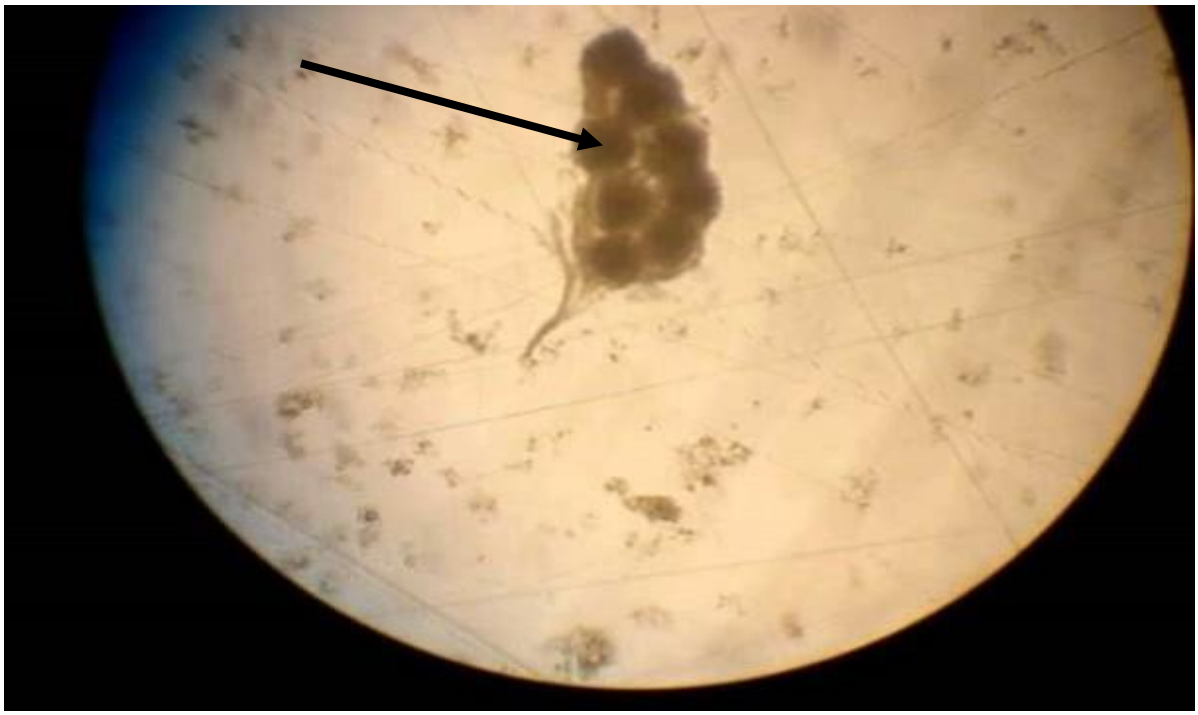


Plate 3.2: Protoscoleces in a brood capsule – light microscope magnification X10

3.9.3 DNA extraction from taeniid eggs

To isolate DNA from the taeniid eggs, individual eggs were picked under a microscope. Up to 20 eggs were picked per faecal sample using magnification X4 and placed into 0.2 ml thin wall PCR tubes containing 10 µl of 0.02M NaOH. The eggs were lysed at 99 °C for 10 minutes to get crude DNA (lysate) (Mulinge *et al*., 2018).

3.10 Polymerase chain reaction

Two nested PCR assays targeting part or the entire NADH dehydrogenase subunit 1 gene (*nad1*) were used for genotyping of cyst materials/protoscoleces and eggs. The first nested PCR (entire *nad1* gene) was performed as described by Hüttner *et al*. (2009). The cyst materials negative using the first PCR assay and taeniid eggs were genotyped using a second nested PCR as described by Mulinge *et al*. (2018) which amplifies part of the *nad1* gene (545-552 bp). In both PCR assays, the reaction mixture contained 2 μl of the DNA, 1 × DreamTaq Green Buffer (20

mM Tris-HCl (pH 8.0), 1 mM DTT, 0.1 mM EDTA, 100 mM KCl, 0.5 % (v/v) Nonidet P40, 0.5 % (v/v) Tween 20) (Thermo Scientific), 0.2 mM dNTPs, 0.25 μM of forward and reverse primers, 2 mM $MgCl_2$, and 0.625 units of DreamTaq Green DNA Polymerase (Thermo Scientific) in 25 μl final volume. The PCR cycling conditions were 5 min. for initial denaturation at 94 °C, 40 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 60 s, and a final extension at 72 °C for 5 min. The reaction and cycling conditions that were used for the primary and secondary PCRs were the same except that 2 µl of primary PCR product was used as template in the secondary PCR. For the NAD 1 PCR, the primers NAD A and NAD C were used in the primary PCR while NAD B and NAD D were used for the secondary PCR. For the Nadnest PCR, the primers Nadnest A and NAD C were used in the primary PCR while Nadnest B and NAD D were used for the secondary PCR (Table 3.2).

Table 3.2: NAD 1 and Nadnest gene primers (Hüttner *et al.*, 2009)

Name of the primer	Nucleotide sequence (5'- 3')
NAD A	TCG AAC TCA GTT TGA GCT TTA CTA
NAD C	ATA TCA AAG TAA CCT GCT ATG CAG
NAD B	TAT TAA AAA TAT TGA GTT TGC GTC
NAD D	TCT TGA AGT TAA CAG CAT CAC GAT
Nadnest A	TGT TTT TGA GAT CAG TTC GGT GTG
Nadnest B	CAG TTC GGT GTG CTT TTG GGT CTG

3.11 Agarose gel electrophoresis

3.11.1 Procedure

Agarose (2.0 g) was suspended in 100 ml of 1× TBE buffer and heated in a microwave oven until it dissolved completely. The volume was adjusted with dH_2O to 100 ml after minimal

volume loss due to evaporation. The secondary PCR product (10 µl) was separated in 2% agarose gel stained with ethidium bromide and photographed using a digital camera.

3.11.2 Identification of *Echinococcus* and *Taenia* species by RFLP

Ten (10 µl) of the NAD1 PCR products were digested using *Hph*I restriction enzyme for species identification (Hüttner *et al*., 2009). For identification of *Echinococcus* and differentiation of *Taenia* species, Nadnest secondary PCR products were subjected to a restriction digestion using the enzyme *Hph*I (Mulinge *et al*., 2018). Briefly, 20 µl of reaction mixture was constituted from a mixture of 10 µl *nad*-1 PCR amplicons 7.5 µl nuclease free water, 2 µl digestion buffer (supplied with enzyme) and 0.5 µl of the *Hph*I endonuclease. Restriction digestions were performed overnight at 37 ^{o}C incubator. The restriction digests were separated on 3.0% agarose gel stained with ethidium bromide and photographed using a digital camera. Genotyping of samples was done by comparing each sample's banding patterns with the defined reference patterns of *E. granulosus* s. s, *E. ortleppi* (G5) and *E. canadensis* (G6/7). The RFLP tool identified and differentiated taeniid eggs of *Echinococcus* species from those of *Taenia* species, but could not identify the *Taenia* species and therefore they were sequenced as described by (Mulinge *et al*., 2018).

3.12 DNA sequencing for taeniid eggs and identification of *Taenia* species

The PCR products from taeniid eggs were purified using High Pure PCR Product Purification Kit (Roche, Germany) following the manufacturer's instructions. The products were sequenced at Inqaba biotec, South Africa. The reverse primer (NAD D) for the nested PCR was used for sequencing. The sequences were viewed and edited using GENtle v. 1.9.4 http://gentle.magnusmanske.de and *Taenia* species identified by the Basic Local Alignment Search Tool (BLAST) (http://www.ncbi.nlm.nih.gov/BLAST/).

3.13 Biosafety measures

Biosafety training and education of study team about potential hazards and safe work practices was essential for creating a safe work environment. Personal Protective Equipment (PPEs), for

example gloves for handling potentially contaminated materials, containers, equipment or surfaces, were required. Decontamination of materials, containers, equipment and surfaces was done using 10% sodium hypochlorite.

3.14 Disposal of materials and samples

All the samples used in the study were disposed according to KEMRI waste disposal protocols as outlined on the KEMRI Health Safety and Environment Policy. The cysts and the faecal samples were put in biosafety bags and incinerated at the KEMRI incinerator.

3.15 Data analysis

Collected data was entered in Microsoft Office Excel spread sheet (2016) and statistically analyzed using SPSS Version 20 software. Descriptive statistics were presented using mean, mode, median and standard deviation. The exact binomial 95% confidence intervals (CI) for prevalence values were calculated using the SPSS Version 20 Tools. A p-value of <0.05 was considered statistically significant. The results were presented using narrative texts, tables and graphs.

3.16 Ethical consideration

Institutional approval was sought from Institute of Tropical Medicine and Infectious Diseases (ITROMID) at Jomo Kenyatta University of Agriculture and Technology Board of Post graduate (JKUAT-BPS). (Appendix 14) Ethical clearance was sought from KEMRI Scientific and Ethics Review Unit (SERU) KEMRI/SERU/CMR/P00048/3395. (Appendix13) KEMRI Animal Care and Use Committee (ACUC) (Appendix 12), and Ministry of Agriculture, Livestock and Fisheries Department of Veterinary Services-Kajiado West Sub-county (Appendix 15). At the community and household level, the relevant local authorities and household heads were informed about the survey. Finally, voluntary participation was sought by informed consent from all dog owners (Appendix 3). Data collection emphasized on issues of confidentiality and privacy by restricted access to the information collected and coding of questionnaires. Each participant was informed about their right to decline or withdraw at any

time from participating in the study without feeling constrained before consenting to participate in the study. In addition, potential benefits of the study to the individual and the population were also explained to the subjects. Research assistants were duly trained on how to handle confidential information. The data collected was kept under lock and restricted only to authorized study team. Documents linking households and sample results were safely kept awaiting destruction after the study.

CHAPTER FOUR

RESULTS

4.1 Prevalence of cystic echinococcosis in livestock in Kajiado West

A total of 1486 livestock at slaughter were screened during the survey and they included cattle 388, sheep 625 and goats 473. The general prevalence of CE in the current study area was 14.8% (220/1486). Goats had the highest prevalence at 15.2% (72/473) followed by sheep 14.9% (93/625) and 14.2% (55/388) for cattle (Table 4. 1).

Table 4.1: Prevalence of cystic echinococcosis in livestock in Kajiado - West Sub-county

Livestock	Number	Prevalence
Cattle (n=388)	55	14.2%
Goat (n=473)	72	15.2%
Sheep (n=625)	93	14.9%
Total (N=1486)	210	14.8%

4.2 Cysts location

In all the infected livestock, liver and lungs were the only organs harbouring cysts, and the liver (45.5%) was the most infected organ. The number of cattle that had either cyst in the liver or lungs was 45.5% and 29.1% respectively. Infections in both liver and lungs in cattle were 25.5%. In goats, the liver was the most infected organ with 62.5% and in the lungs 29.2%, while infection in both liver and lungs organs was 8.3%. In sheep, 72.0% had liver infection, 19.4% had lung infection while only 8.6% had both liver and lungs infection. Cattle recorded the highest number of animals with infections in both liver and lungs (Table 4.2). CE infected liver and lungs are shown in Plate 4.1.

Table 4.2: Cyst organ location in livestock in Kajiado West Sub-county

Livestock	Liver only (n)	Lungs only (n)	Both organs (n)
Cattle (n=55)	25 (45.5%)	16 (29.1%)	14 (25.5%)
Goat (n=72)	45 (62.5%)	21 (29.2%)	6 (8.3%)
Sheep (n=93)	67 (72.0%)	18 (19.4%)	8 (8.6%)

a b

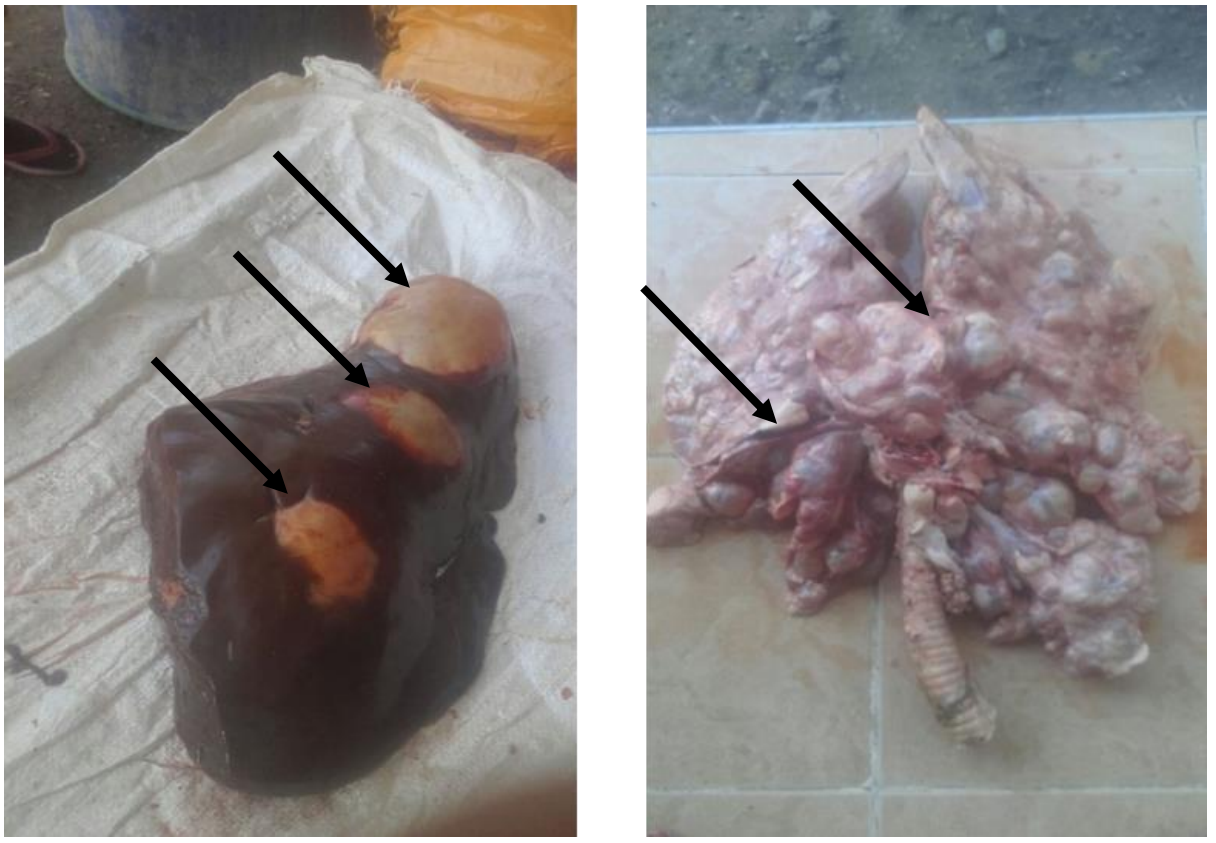


Plate 4.1: Hydatid cysts in the (a) liver and (b) lungs of cattle. Arrows pointing at the cysts.

4.3 Cysts load in livestock

Table 4.3 shows cattle had the highest number of cysts with an average of 2.6 cysts per infected animal, followed by sheep (1.8) and goats (1.6). There was a wide range in the number of cysts per infected animal. In some instances, as many as 18 cysts were isolated from one cattle and 17 cysts from a goat. Sheep recorded the least number of cysts per animal with 7 cysts being the highest number of cysts isolated from a single sheep (Table 4.3).

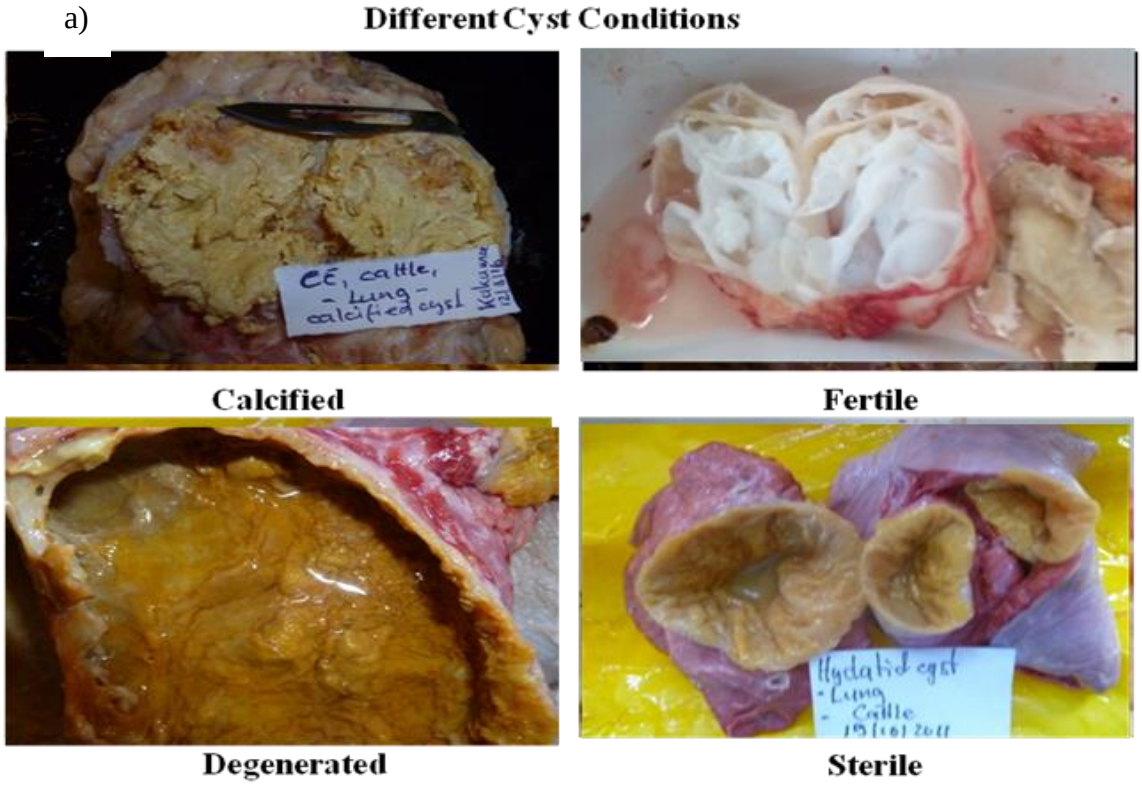
Table 4.3: Cyst load in the infected livestock in Kajiado West Sub-county

Livestock species	Number of cysts											
	1	2	3	4	5	6	7	9	13	17	18	Total
Cattle	26	14	2	8	1	1	0	1	1	0	1	55
Goat	51	10	6	3	0	0	2	0	0	0	0	72
Sheep	64	17	5	1	2	2	1	0	0	1	0	93

4.4 Classification of the isolated cysts

The cysts were classified into four different conditions namely fertile, sterile, calcified and degenerated. Cysts were classified as fertile (with protoscoleces) sterile (fluid-filled without protoscoleces), degenerated (collapsed cyst walls with caseated protoscoleces and soft cheesy debris without calcification), and calcified (hard solid appearance of the ectocyst) (Figure 4.1). Majority of cysts of cattle origin were sterile (41.9%), while most of the calcified cysts were found in sheep (48.5%) and goats (52.2%). On average, sheep had the highest number of fertile cysts at 40.5%, followed at 25.9% in cattle and 18.3% in goats (Table 4.4).

a)



b)

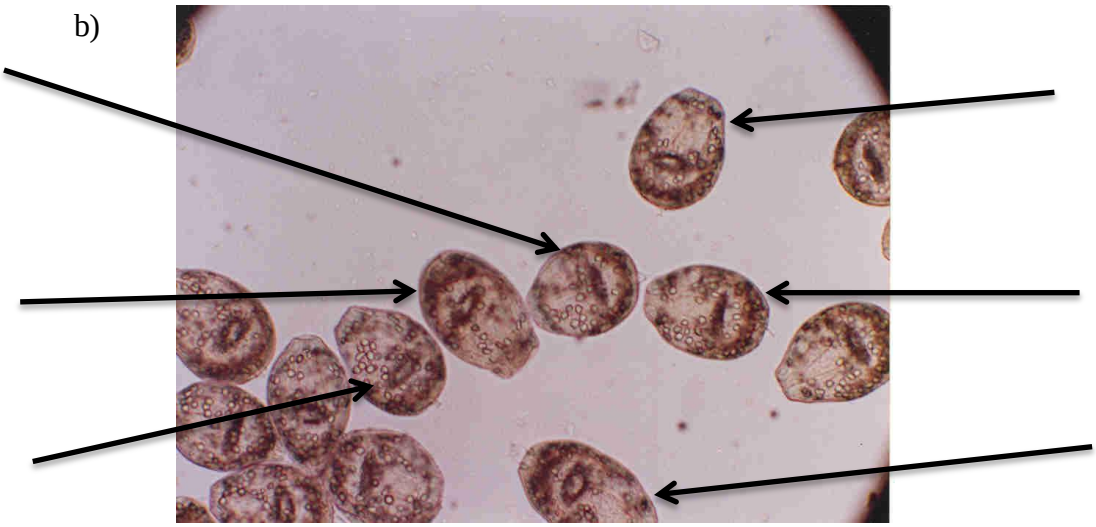


Plate 4.2: a) Pictures of liver and lungs from infected livestock showing the various cyst conditions. **b)** A picture showing single protoscoleces as pointed (A gift from a colleague Eberhard Zeyhle).

Table 4.4: Classification of the isolated cysts in Kajiado -West Sub-county

		Conditions of the cysts					
Livestock		Fertile	Sterile	Calcified	Degenerated	Total	Fertility Rate
Cattle	Liver	7	34	22	14	77	
	Lungs	30	26	4	6	66	
	Total	**37**	**60**	**26**	**20**	**143**	**25.9%**
Goat	Liver	14	0	51	10	75	
	Lungs	7	3	9	21	40	
	Total	**21**	**3**	**60**	**31**	**115**	**18.3%**
Sheep	Liver	25	4	71	8	108	
	Lungs	41	1	8	5	55	
	Total	**66**	**5**	**79**	**13**	**163**	**40.5%**

4.5 Genotyping of the Cyst Material from livestock by PCR-RFLP

Out of the 421 cysts material isolated, 389 were successfully characterized to be either *E. granulosus* sensu stricto (s. s.), 356/389 (91.5%), *E. canadensis* (G6/7), 26/389 (6.7%), or *E. ortleppi*, 7/389 (1. 8%).The genotype of 32/421 (7.6%) could not be determined thus, 6, 12, and 14 from cattle, goats, and sheep, respectively. Results of gel electrophoresis after PCR amplification of NAD1 are shown in Figure 4.2. Identification of *Echinococcus* species through PCR-RFLP of NAD1 is shown in Figure 4.3.

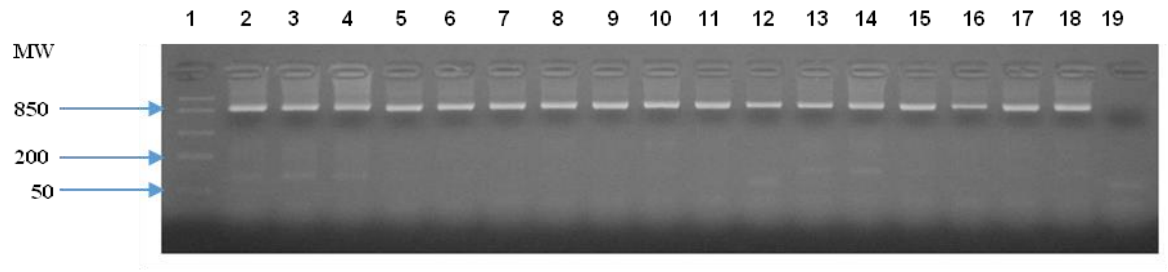


Figure 4.1: A gel showing results of NAD1 PCR

NAD1 PCR products were separated on 2% agarose gel and stained in ethidium bromide. Lane 1 represent Molecular weight marker; lanes 2-17 represent PCR positive samples lane 18 represent the positive control and lane 19 represent the negative control.

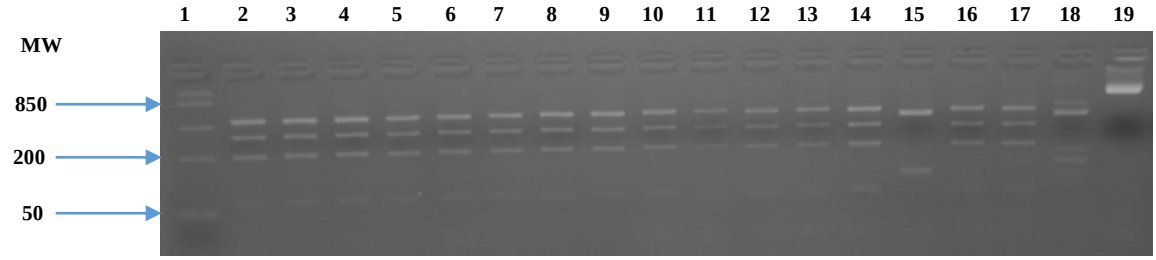


Figure 4.2: A gel showing results of RFLP of NAD1 PCR digested with *Hph*I.

Digests were separated on 3% agarose gel and stained in ethidium bromide. Lane 1 represent molecular weight marker; lanes 2 – 14 and 16 represent *E. granulosus* s. s., lane 15 represent *E. canadensis* (G6/7); lanes 17 and 18 are positive controls of *E. granulosus* s. s. and *E. canadensis* (G6/7); lane 19 represent negative control (uncut PCR product).

The samples that failed to amplify using the NAD1 PCR were subjected to the Nadnest PCR which targets a smaller portion of the *nad1* gene than NAD1 PCR. Results of Nadnest PCR amplification are shown in Figure 4.4. Restriction digest of Nadnest PCR with *Hph*I enzyme is shown in Figure 4.5.

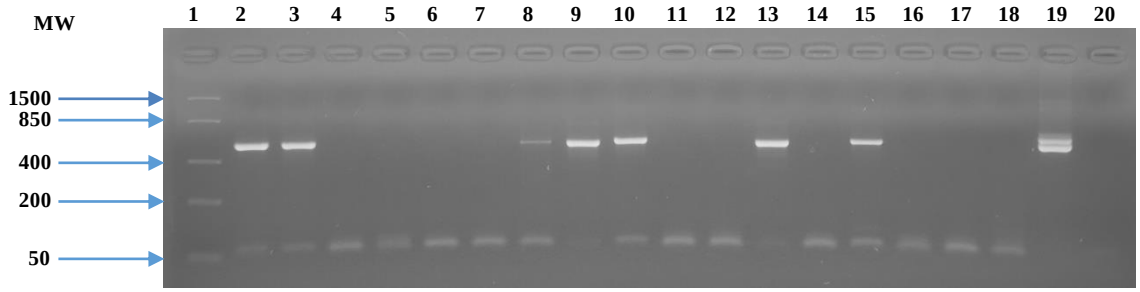


Figure 4.3: A gel showing results of Nadnest PCR.

PCR products were separated on 2% agarose gel and stained in ethidium bromide**.** Lane 1 represent molecular weight marker; lanes 2, 3, 8 – 10, 13 and 15 represent PCR positive samples; lanes 4 – 7, 11, 12, 14, 16 – 18 represent PCR negative samples; lanes 19 and 20 are positive and negative controls respectively.

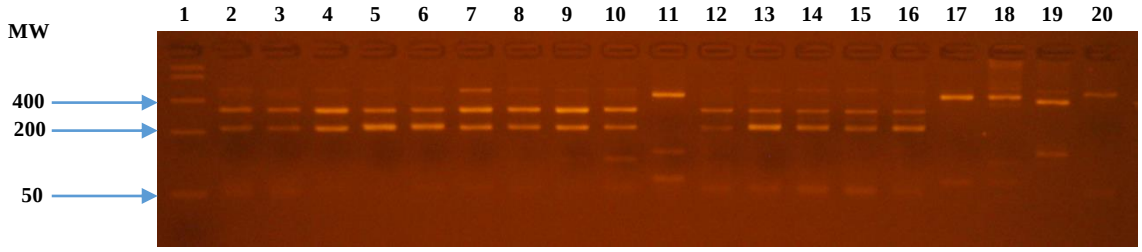


Figure 4.4: A gel showing results of RFLP of Nadnest PCR digested with *Hph*I.

Digests were separated on 3% agarose gel and stained in ethidium bromide. Lane 1 represent Molecular weight marker; lanes 2 – 10, 12 – 16 represent *Taenia* species; lane 11 represent *E. equinus*; lanes 17 – 19 are positive controls for *E. granulosus* s. s., *E. equinus,* and *E. canadensis* G6/7 respectively; lane 20 represent negative control (uncut PCR product).

4.6 *Echinococcus* species in livestock species

Majority of the cysts identified as *E. granulosus* s. s. 39.6% were from sheep, followed by cattle 36.8% and least in goats 23.6%. *E. ortleppi* was detected in 57.1% cattle, 28.6% goats and

14.3% in sheep. *E. canadensis* (G6/7) was common in goats 65.4%, followed by sheep 26.9% and least in cattle 7.7% (Table 4.5).

Table 4.5: Genotypes of cysts isolated in livestock

	Echinococcus species			
Livestock	*E. granulosus* s. s.	*E. ortleppi*	*E. canadensis* (G6/7)	Total
Cattle	131 (36.8%)	4 (57.1%)	2 (7.7%)	137
Goat	84 (23.6%)	2 (28.6%)	17 (65.4%)	103
Sheep	141 (39.6%)	1 (14.3%)	7 (26.9%)	149
Totals	**356**	**7**	**26**	**389**

4.7 Mixed *Echinococcus* species infection in livestock

There were three cases of mixed infections in cattle with one case of all three *Echinococcus* spp. (*E. granulosus* s. s., *E. ortleppi*, and *E. canadensis* (G6/7) two instances with double infection of *E. granulosus* s. s./ *E. ortleppi* and *E. ortleppi* /*E. canadensis* (G6/7). In goats, there were three cases of mixed infections, all with *E. granulosus* s. s. and *E. canadensis* (G6/7), and, in sheep, only one case of *E. granulosus* s. s. and *E. canadensis* (G6/7) mixed infection was observed (Table 4.6).

Table 4.6: Single or mixed *Echinococcus* species infections in livestock

	Echinococcus species							
Livestock	*E. g.*	*E. o.*	*E. c.*	*E. g./E. o.*	*E. g./E. c.*	*E. o./E. c.*	*E. g./E. o./E. c.*	Total
Cattle	51	1	0	1	0	1	1	55
Goat	50	2	13	0	3	0	0	68
Sheep	79	1	6	0	1	0	0	87
Total	180	4	19	1	4	1	1	210

E. g.* –** *E. granulosus* ***E. o. *–E. ortleppi* ***E. c****. – E. canadensis* (G6/7)

4.8 Prevalence of *Taenia* and *Echinococcus* species in dogs in Kajiado West

A total of 345 dog faecal samples were collected. The prevalence of *Echinococcus* infection was 0.6% (2/345) (Table 4.7).

Table 4.7 *Echinococcus* and *Taenia* species in dogs Kajiado-west sub-county

Ward Name	No. of samples	Taeniid positive samples	No. of taeniid eggs	PCR positive faecal samples	PCR positive taeniid eggs	*Echinococcus* spp. (eggs)	*Taenia* spp. (eggs)
Kiserian	115	1	20	0	0	0	0
Keekonyokie	115	4	60	0	0	0	0
Magadi	115	14	268	8*	32	2*	7*
						E. equinus (1)	1 *T. hydatigena* (3)
						E. felidis (1)	2 *T. ovis* (8)
							3 *T. multiceps* (6)
							1 *Taenia* spp.(1)
Totals	345	19	348	8	32*	2	7

*** One faecal sample had a coinfection of *E. equinus* and *T. hydatigena***

4.9 Genotyping of the isolated taeniid eggs from dog faeces

In total, 345 dog faecal samples were collected, 316 from homesteads and 29 around the slaughter houses. Taeniid eggs were isolated from 19 out of 345 faecal samples (5.5%). Of the 19 samples, 5.2%, 21.1% and 73.7% were from Kiserian, Keekonyokie and Magadi respectively. A total of 348 taeniid eggs were isolated from the samples and subjected to nested PCR, only 32 were PCR positive and originated from 8 faecal samples. PCR products of 6 taeniid eggs representing 3 faecal samples yielded faint bands and were therefore not typed. PCR products of 20 taeniid eggs were sequenced thus identified as *Echinococcus* species (2) and *Taenia* species (18). Only two faecal samples (0.6%) contained *Echinococcus* eggs. As shown in Table 4.7, *E. equinus* and *E. felidis* were the only *Echinococcus* spp. detected. The *E. equinus* isolate was 99% identical to sequence KY766905 (Kinkar *et al*., 2017), while the *E. felidis* isolate was 100% identical to sequence MG271925 (Mulinge *et al*., 2018). *T. multiceps* was the most common species and was detected in 3 faecal samples, *T. ovis* in 2, *T. hydatigena* and unknown *Taenia* species in 1 faecal sample each. Of the 18 taeniids identified as *Taenia*

species, 6 were *T. multiceps*, 8 *T. ovis*, 3 *T. hydatigena* and 1 unknown *Taenia* species. The *Taenia* species sequences were 99% identical to those deposited in the NCBI database (Table 4.7). The PCR positive samples marked using Global Positioning System (GPS) are shown in the Figure 4.5.

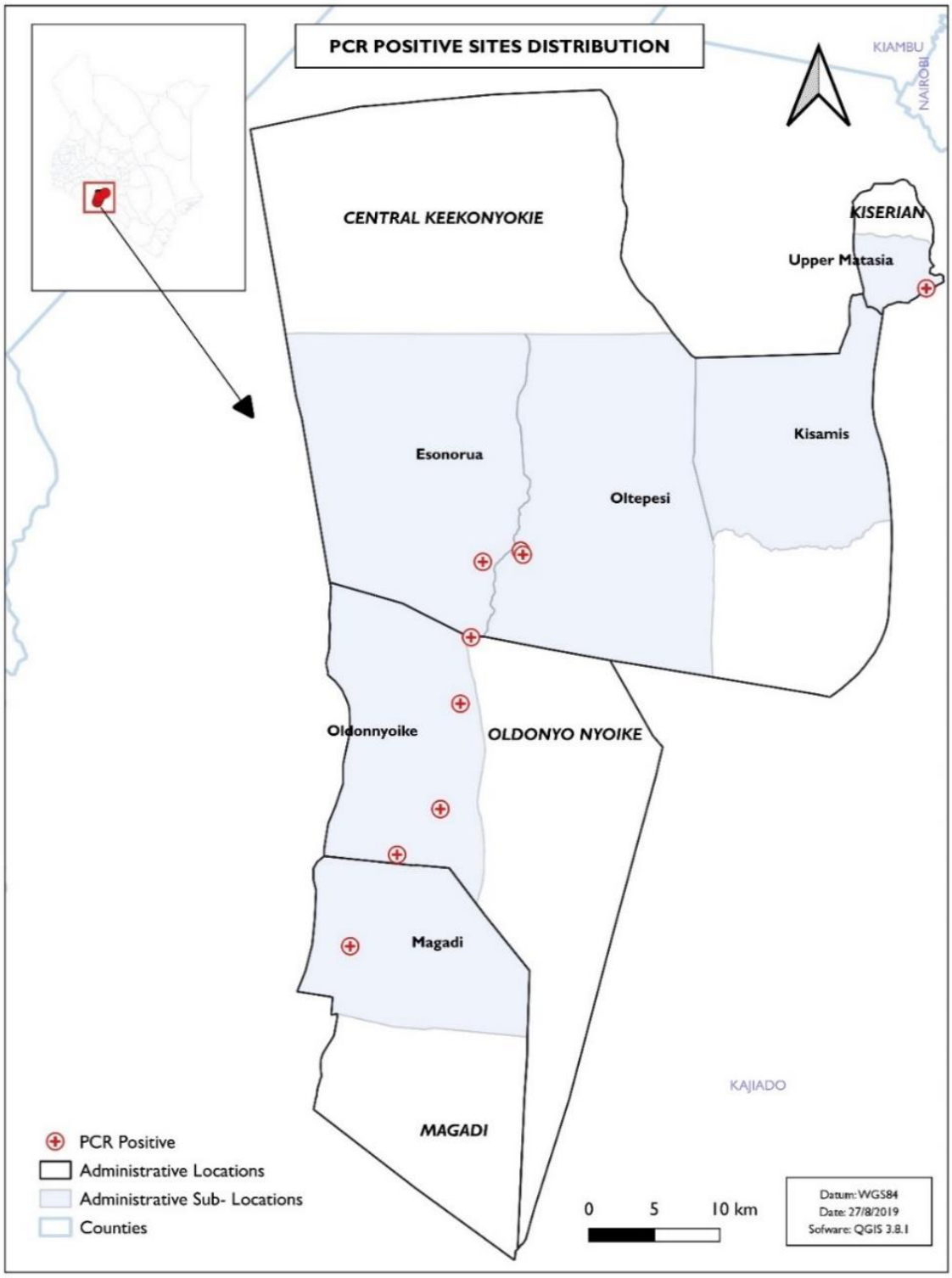


Figure 4.5: A map of Kajiado - West showing the location of PCR positive faecal samples

Table 4.7: Genotyping of taeniid eggs isolated from dog faecal samples

Sequence ID	Species	Accession number /Identity (%)	References
KMD 010_2	*T. multiceps*	FJ495086 (99%)	(Liu *et al*., 2011)
KMD 010_4	*T. multiceps*	FJ495086 (99%)	(Liu *et al*., 2011)
KMD 010_11	*T. multiceps*	FJ495086 (99%)	(Liu *et al*., 2011)
KMD 036_5	*T. multiceps*	FJ495086 (99%)	(Liu *et al*., 2011)
KMD 036_15	*T. multiceps*	FJ495086 (99%)	(Liu *et al*., 2011)
KMD 043_15	*T. hydatigena*	MN175587 (99%)	(Ohiolei *et al*., 2019)
KMD 043_18	*T. hydatigena*	MN175587 (99%)	(Ohiolei *et al*., 2019)
KMD 043_20	*T. hydatigena*	MN175587 (99%)	(Ohiolei *et al*., 2019)
KMD 058_8	*E. equinus*	KY766905 (99%)	(Kinkar *et al*., 2017)
KMD 058_13	*T. multiceps*	FJ495086 (99%)	(Liu *et al*., 2011)
KMD 060_4	*T. ovis*	AB731675 (99%)	(Nakao *et al*., 2013a)
KMD 060_8	*T. ovis*	AB731675 (99%)	(Nakao *et al*., 2013a)
KMD 060_17	*T. ovis*	AB731675 (99%)	(Nakao *et al*., 2013a)
KMD 060_19	*T. ovis*	AB731675 (99%)	(Nakao *et al*., 2013a)
KMD 060_20	*T. ovis*	AB731675 (99%)	(Nakao *et al*., 2013a)
KMD 063_8	*T. ovis*	AB731675 (99%)	(Nakao *et al*., 2013a)
KMD 063_17	*T. ovis*	AB731675 (99%)	(Nakao *et al*., 2013a)
KMD 063_20	*T. ovis*	AB731675 (99%)	(Nakao *et al*., 2013a)
KMD 103_12	*Taenia* spp.	AB905200 (99%)	(Terefe *et al*., 2014)
KMD 037_17	*E. felidis*	MG271925 (100%)	(Mulinge *et al*., 2018)

4.10 Demographical data of dog owners

A total of 316 questionnaires were administered using ODK (Open Data Kit) tool. In terms of the level of education, most of the respondents were primary school leavers (48%) and secondary school leavers (31%). Those with post-secondary education were 14% while those with no formal education were 7% (Figure 4.6).

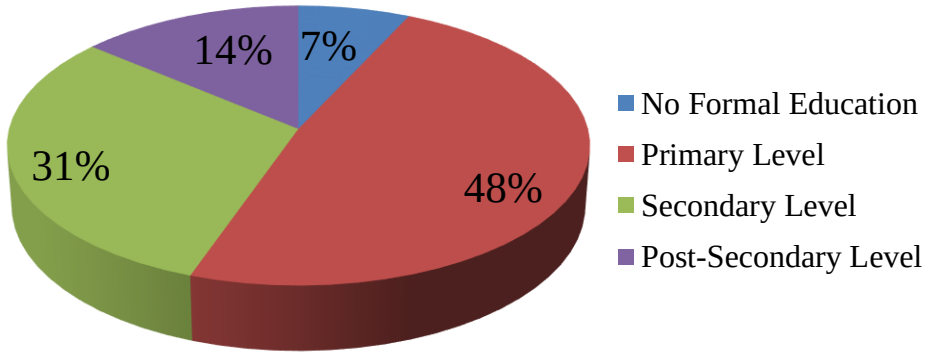


Figure 4.6: Level of education of the respondents

4.11 Description of demographic results of key risk factors

Among those interviewed 76.1% practiced home slaughter and only 38.3% of these respondents admitted that their dog(s) had access to raw offal, however 26.3% of them did not know. Only 16.8% of respondents admitted to deworming of dogs. Of the taeniid positive dogs 88.9% came from household with no history of deworming dogs. Only 6.3% of the interviewees had knowledge on CE and its transmission. Furthermore, all taeniid positive dogs originated from households where the heads did not know the cause of CE and the transmission cycle. Majority of the faecal samples were collected from male dogs 64.9% with female being the minority (35.1%). Taeniid eggs were more frequent in male dogs (55.6%) than female dogs (44.4%). Dog aged below 1 year (1.3%) and above 5 years (3.5%) were few with majority of them belonging to age groups 1 – 2 years (32.0%) and 2 – 3 years (43.7%). All (100%) of the taeniid

positive dogs were aged between 2 – 3 years. Free roaming of dogs was common with 97.2% of those interviewed reporting that their dogs roam regularly.

Table 4.9: Description of demographic results of key risk factors

Risk factor	n = 316
Sex	
Male	205 (64.9%)
Female	111 (35.1%)
Deworming of dogs	
Yes	53 (16.8%)
No	263 (83.2%)
Home slaughter	
Yes	73 (23.1%)
No	243 (76.9%)
Dog access to raw offal	
Yes	121 (38.3%)
No	112 (35.4%)
Do not know	83 (26.3%)
Age of dogs (years)	
≤1	4 (1.3%)
1 – 2	101 (32.0%)
2 – 3	138 (43.7%)
3 – 4	24 (7.6%)
4 – 5	38 (12.0%)
≥5	11 (3.5%)
Knowledge of CE	
Yes	20 (6.3%)
No	296 (93.7%)
Free roaming of dogs	
Yes	307 (97.2%)
No	9 (2.8%)

CHAPTER FIVE

DISCUSSION

5.1 Discussion

This study reports the prevalence of cystic echinococcosis (CE) in cattle, goats and sheep and *Echinococcus* spp. in dogs in Kajiado-West sub- County, Kenya. The general prevalence reported in livestock was 14.8% (220/1486). Goats had the highest prevalence at 15.2% (72/473) followed by sheep 14.9% (93/625) and 14.2% (55/388) for cattle. This is within the range known of Kajiado county from older accounts, such as in the works of Macpherson (1985) (8.9%, 8.1%, and 7.1% in cattle, sheep, and goat, respectively) and (Addy *et al*., 2012) (25.8 % in cattle, 16.5 % in sheep, and 10.8% in goats). It confirms the persistence of CE in Kajiado County. High livestock stocking intensity, conducive environmental conditions, and movement of livestock (Kebede *et al.,* 2011) may have influenced the infection pressure and the persistence of the CE in livestock in Kajiado County. Goats having a higher prevalence (15.2%) than cattle (14.2%) and sheep (14.9) is unusual given that they are browsers and sheep are grazers an implication that sheep would be exposed more to *E. granulosus* eggs. The unusual occurrence could be associated with the importance of goats as intermediate hosts of *E. canadensis (G6/7) genotype* which existed in a higher level. (Addy *et al*.,2012)

The liver was the most affected organ just as was shown before from previous study (Addy *et al*., 2012). The predilection site of *E. granulosus* s. l. is not fully understood and some studies (Macpherson, 1985; Njoroge *et al*., 2002; Kebede *et al*., 2011) indicated the lungs to be the most affected. In this study majority of the cysts from livestock were non-fertile, and show a similar result from a recent survey which reported 80% of cysts from sheep in Turkana being calcified (Zeyhle unpublished data). This observation is not clearly understood, because regular deworming of ruminants is less likely to have a significant effect on the calcification of cysts. Previous studies have shown that long-term treatment with high doses of anthelmintic (e.g. Albendazole) drugs is required to arrest cyst development (Gemmell *et al*., 1981; Schantz *et al*., 1982). Sheep in which most fertile cysts were isolated in the present study would be more important in the transmission and maintenance of CE in Kajiado County. The cysts fertility

rates reported indicate the need for control measures such as health education, regular deworming of dogs, dog population control, good slaughter hygiene, and proper disposal of slaughter offal to avert transmission.

Majority of the cysts in this study were *E. granulosus* s. s. which confirms its predominance observed in other survey in Kajiado south (Addy *et al*., 2012) and in Kenya at large (Wachira *et al*., 1993; Dinkel *et al*., 2004; Casulli *et al*., 2010; Mutwiri *et al*., 2013; Mbaya *et al*., 2014). The high fertility rates of *E. granulosus* s. s. cysts in sheep indicate that they are important intermediate host of this taxon in this area. Sheep are the most common home-slaughtered livestock species in Kajiado county and that may enhance transmission of *E. granulosus* s. s. (Macpherson, 1985). However, both cattle and goats may also play a role in transmission of *E. granulosus* s. s. based on the fertility rate observed in this study. Although goats are considered important intermediate host of *E. canadensis* (G6/7) in areas where camels are not kept, none of the cysts belonging to this taxon were fertile in this study (Soriano *et al*., 2010; Addy *et al*., 2012). Isolation of *E. ortleppi* from all three livestock species reveals a wider host range of this species an aspect that is less understood. Generally, *E. ortleppi* is a rare species even in cattle who are the principal intermediate hosts, possibly due to the fact that cattle are rarely slaughtered at home, and therefore dogs have less access to slaughter offal from cattle (Addy *et al*., 2012). However, in a recent development, due to poor disposal of condemned viscera in poorly managed slaughter facilities in urban centres, dogs have readily access to slaughter offal and this might be a reason for the increased cases of *E. ortleppi* in the present study (Mulinge *et al*., 2018). Elsewhere in Brazil home slaughter of cattle is believed to be a factor that facilitates the recent rise of *E. ortleppi* prevalence in Brazil (Romig *et al*., 2017).

The prevalence of *Echinococcus* infection in dogs in this study was lower (0.6%) than reported previously elsewhere; 27.3% in Kajiado County (Eugester, 1978), and 4.0% in Maasai Mara (Narok County) (Mulinge *et al*., 2018). Post-mortem examination of dogs around abattoirs in Kiserian, Rongai and Dagoretti revealed that 72% of dogs were *Echinococcus* positive (Wachira *et al*., 1994). In Turkana county, two necropsy-based studies reported 39.4% (Macpherson *et al*., 1985) and 33% (Buishi *et al*., 2006) *Echinococcus* infections in dogs. The low prevalence of *Echinococcus* infection in dogs in this study could be due to the sampling

method and detection method used. This study applied ground faecal sampling and microscopic detection of taeniid eggs followed by nested PCR for identification of *Echinococcus* spp. infection in dogs while previous studies in Kenya used necropsy and arecoline bromide purgation. Microscopic detection of eggs tends to under report infection in dogs because of prepatent infections and the periodic shedding of taeniid eggs. (Lillis, 1967). Furthermore, the presence of taeniid eggs does not guarantee PCR results as shown in this study, where of the 19 taeniid positive faecal samples, PCR positive eggs were only obtained from 8 faecal samples. Taeniid eggs are vulnerable to desiccation and degradation of genetic material when exposed to harsh environmental conditions (Wachira *et al*., 1991; Hüttner *et al*., 2009). A recent study (Mulinge *et al*., 2018), applied the same methodology as used in this study, however, these authors reported a higher *Echinococcus* infection in dogs. This difference could be due to the small sample size used in the present study and the differences in CE prevalence and infection intensities in intermediates hosts.

This study reports for the first time the detection of *E. equinus* in dogs in Kenya. The detection of *E. equinus* and *E. felidis* as the only two species in dogs in this study, was unusual as both taxa were not reported in livestock examined in this study. *E. granulosus* s. s. being the most dominant species in livestock (Wachira *et al*., 1993; Dinkel *et al*., 2004; Addy *et al*., 2012; Odongo *et al*., 2018) and in dogs (Mulinge *et al*., 2018) in Maasai Mara, a fact that was confirmed in livestock in this study. *Echinococcus equinus* is a rare species in intermediate hosts in Kenya, owing to the fact that donkeys are not considered as source of meat for human consumption in many communities in Kenya and therefore rarely slaughtered at home. The only known case of *E. equinus* in Kenya, was detected recently in a donkey from Maasai Mara (Mulinge *et al*., unpublished data). It is postulated that the source of this *E. equinus* infection in a dog could be as a result of dogs' access to donkey carcasses infected with this species. The detection of *E. felidis* in dogs follows the findings of a recent study in Maasai Mara that reported this species eggs in two dogs (Mulinge *et al*., 2018). The source of *E. felidis* in a dog in the present study, could be due to dogs scavenging wild intermediate hosts originating from the neighbouring Nairobi National park. There are no previous reports of *E. felidis* in livestock in Maasailand (Wachira *et al*., 1993; Dinkel *et al*., 2004; Addy *et al*., 2012; Odongo *et al*., 2018). The presence of *E. felidis* eggs in a dog in this study could also be due to heterospecific

coprophagy (dog feeding on lion or hyena faeces), where the eggs pass out through the gastro intestinal tract unaffected.

In this study, four *Taenia* species were detected in dogs; *T. hydatigena*, *T. multiceps*, *T. ovis* and an unknown *Taenia* spp. The detection of the first three species indicate the domestic cycle transmission of *Taenia* species due to the fact that dogs have readily access to raw slaughter offal or fallen animals. The unknown *Taenia* species was identical to a species isolated from hyena in Ethiopia (Terefe *et al*., 2014). *Taenia hydatigena,* whose larval stage *Cysticercus tenuicollis* cause cysticercosis in small ruminants and is common in Kenya. A recent study reported *T. hydatigena* as the most common *Taenia* spp. in dogs in Turkana and Narok Counties (Mulinge *et al*., 2020). *Taenia hydatigena* was also the most common species reported in two previous studies in Turkana (Jenkins *et al*., 1991; Buishi *et al*., 2006). *Taenia multiceps* was the most common species in dogs in this study, a similar observation was reported in Isiolo (Mulinge *et al*., 2020). The metacestode stage of *Taenia multiceps*, *Coenurus cerebralis,* cause coenurosis in small ruminants and rarely coenurosis in humans (Scala *et al*., 2007).

Coenurosis in small ruminants is usually characterized by severe clinical manifestation and eventual death of affected animals making this cestode of major economic significance to pastoralists (Avcioglu *et al*., 2011). Although the prevalence and economic burden of coenurosis in small ruminants in Kenya is unknown, the frequency of this cestode in dogs highlights a high infection pressure to small ruminants. In Maasailand, pastoralists have confirmed coenurosis as a major cause of morbidity and mortality in small ruminants (Zeyhle personal communication). This study reported *Taenia ovis* for the first time in dogs in Kajiado county. Previously this species was reported in dogs in Turkana and Isiolo counties (Mulinge *et al*., 2020). The prevalence of *Cysticercus ovis* in small ruminants (mainly sheep) in Kenya is unknown, partially due to the nature of meat inspection where the muscle tissues are rarely examined. As observed in a previous study, this species also occurs sporadically in dogs (Mulinge *et al*., unpublished data), compared to both *T. hydatigena* and *T. multiceps*. The presence of *T. hydatigena*, *T. multiceps* and *T. ovis* in domestic dogs confirms the existence of ongoing transmission of cestodes from livestock to dogs even in absence of the major

Echinococcus species (*E. granulosus* s. s., *E. canadensis* (G6/7) and *E. ortleppi* in dogs in this study and should be studied further.

The current study reports demographic characteristics of dogs in relation to *Echinococcus* infection. Due to the low prevalence of *Echinococcus* infection in dogs (2/345), it was not possible to carry out risks factors analysis, and therefore only demographic characteristics are described herein. As mentioned above the low prevalence of *Echinococcus* infection in dogs is attributed to the methodology used in this study. A previous study applying PCR to evaluate risk factors associated with *Echinococcus* infection in dogs found no association (Ziadinov *et al*., 2008). However, the use of Copro-ELISA in a previous study in Turkana showed a higher prevalence of *Echinococcus* infection in dogs (26%) and reported six risk factors to be significant (Buishi *et al*., 2006).

Feeding dogs with raw offal has been reported as a risk factor for *Echinococcus* infection in dogs in Kenya, Libya and Wales (Buishi *et al*., 2005a; Buishi *et al*., 2005b; Buishi *et al*., 2006). In this study 76.9% of respondents slaughtered sheep or goats at home and of these 35.4% said that their dog(s) accessed raw offal. The two *Echinococcus* positive dogs came from households where home slaughter was practiced, although one of the household reported not feeding dogs with raw offal. Only 1 of the 7 dogs that were infected with *Taenia* species (*T. hydatigena, T. multiceps, T. ovis* and the unknown *Taenia* spp.) originated from a household that practiced home slaughter. This particular dog was co-infected with *E. equinus*, the other dogs (6) are likely to have been infected through scavenging, as they were reported to be free roaming. In the present study 97.2% of interviewees admitted that their dogs were never restrained and therefore roamed freely. Dogs that are not restrained or free roaming have been shown to be at increased risk of *Echinococcus* infection compared to those restrained (Buishi *et al*., 2005b; Buishi *et al*., 2006; Mastin *et al*., 2011).

Deworming of dogs was not commonly practiced in this study with only 16.8% of those interviewed reported treating their dogs with anthelmintic drug. This was supported by the fact that 88.9% of dogs infected with taeniids were not dewormed. Previous studies in Wales, Libya and Chile have shown a positive correlation between *Echinococcus* infection in dogs and failure

to deworm (Buishi *et al*., 2005a; Buishi *et al*., 2005b; Acosta-Jamett *et al*., 2010). Lack of knowledge on CE and mode of transmission were reported to be risk factors of *Echinococcus* infection in dogs (Moro *et al*., 2005; Mastin *et al*., 2015). From this study 93.7% of the respondent did not know the cause of CE and its transmission. All taeniid positive dogs came from households who did not know CE and how it is transmitted and therefore they were likely to engage in practices that enhance transmission of CE and related *Taenia* species.

Taeniid infection was more common in male dogs (55.6%) than in their female counterparts (44.4%), however both *Echinococcus* positive dogs were female. Female dogs have been reported to be at high risk of *Echinococcus* infection elsewhere in Peru and Uruguay (Parada *et al*., 1995; Moro *et al*., 2005). This observation is contrary to other previous studies in Turkana, Kenya, where *Echinococcus* infections were more common in male dogs than in females (Macpherson *et al*., 1985; Buishi *et al*., 2006). In this study all the taeniid positive dogs were aged 2 – 3 years, other studies report contrasting findings relating dog's age and *Echinococcus* infection. Dogs aged 2 years and those below 5 years were more likely to be infected with *Echinococcus* (Buishi *et al*., 2006; Acosta-Jamett *et al*., 2010). The young dogs have a low immunity than older dogs who may acquire parasite derived immunity overtime.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

1 The current study confirms the persistence of cystic echinococcosis in Kajiado County, with *E. granulosus* s. s. being the dominant species. The high fertility rate of cysts in sheep and its regular home slaughter make it the most important intermediate host in the transmission of CE in Kajiado County of Kenya.

2 *Echinococcus ortleppi* (G5) and *E. canadensis* (G6/7) may be important CE agents in Kajiado County. This is the first study to report *E. equinus* in dogs in Kenya. The detection of all the five species of *E. granulosus* s. l. in a single study is also reported for the first time in Kenya.

3 The presence of *T. hydatigena*, *T. multiceps* and *T. ovis* in domestic dogs confirms the existence of ongoing transmission of taeniid cestodes from livestock to dogs even in absence of the major *Echinococcus* species (*E. granulosus* s. s., *E. canadensis* (G6/7) and *E. ortleppi* in dogs in this study. The presence of *E. felidis* and unknown *Taenia* spp. in dogs indicate a possible involvement of dogs in sylvatic cycles.

6.2 Recommendation

1. Studies involving humans are recommended in the area because of high prevalence of CE in livestock and close relationship between human and dogs
2. An integrated control program focusing on interrupting transmission from dogs to livestock and humans is recommended. These measures should include public health education, proper management of slaughter houses, dog population control and regular treatment, maintaining high standards of hygiene and proper disposal of dog faeces
3. Prevalence and economic importance of cysticercosis, coenurosis in the study area needs to be undertaken
4. More sensitive methods for copro-detection of *Echinococcus* and other taeniids such as copro-ELISA and real-time PCR are recommended

5. The collection of faecal samples from the rectum of individual dogs is recommended to have direct association of results and demographic factors such as sex, age and location

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APPENDICES

Appendix I: Livestock Data Entry Form

DATE…….

LIVESTOCK SPECIES……

TOTAL SLAUGHTER NUMBERS……

CARCASS CODE No:	**PRESENCE OF CYSTS**	**ECHINOCOCCOSIS**		**OTHER DISEASES**
		Positive	**Negative**	
001				
002				
003				
004				
005				
006				
007				
008				
009				
010				
011				
012				
013				
014				
015				
016				
017				

018				
019				
020				

Appendix II: Questionnaire

CODE OF THE ENUMERATOR ……

LOCATION ID…….

ANIMAL ID…….

HOUSE HOLD QUESTIONNAIRE NO: ……

DATE…….

SECTION A: QUESTIONS ABOUT THE DOG OWNER

1. Dog Owner…….
2. Occupation?

   Teacher ☐ Business ☐

   Farmer Company ☐

   Housewife ☐ Others …….

   Driver ☐
3. Gender Male ☐ Female ☐
4. Age group

   18 – 25 ☐ 34 – 39 ☐

   26 – 33 ☐ 40 and above ☐
5. Education level

   No formal education ☐

   Primary level ☐

   Secondary level ☐

   Post-secondary level ☐
6. How many dogs do you have in your home? ☐
7. Do you deworm the dog(s)? Yes ☐ No ☐
8. If yes, how often?

   Weekly ☐ Monthly ☐

Quarterly ☐ After more than a year ☐

After 6 months ☐ Never ☐

Annually ☐ Any other (specify)……

9. Name of the drug used.....

10. Do you slaughter sheep, goats or cattle at home? Yes ☐ No ☐

11. If yes, do you ensure meat inspection by a veterinary officer? Yes ☐ ☐ No

12. How do you dispose of the visceral organs?

Feed the dog ☐ Eaten by people ☐

Throw away in the bush ☐ Others (specify)…….

Burry ☐

13. What is your main Source of water?

Closed source ☐

Open source ☐

14. How does your dog(s) mainly get fed?

Cooked food

Raw butchery/slaughter house waste ☐

Scavenging ☐

Others ☐

15. Do you know of a disease(s) you can acquire from a dog? Yes ☐ No ☐

16. If yes which one(s) are they?

Malaria ☐

Rabies ☐

Brucellosis ☐

Others ☐

17. Do you know CE (Hydatid disease)? Yes ☐ No ☐

18. Who does the disease affect? People ☐ Domestic animals ☐ wild animals ☐

dont know ☐ Others (specify)……..

19.Which organs does the disease mainly affect? Lungs ☐ liver ☐ skin ☐ Don't know ☐ Others(specify)……..

20.What is the source of transmission of CE to man? Domestic animals ☐ wild animals ☐ Dogs ☐ Don't know ☐ Others(specify)

SECTION B: QUESTIONS ABOUT THE DOG

1. Name…… Animal ID……...
2. Age……
3. Sex? Male ☐ Female ☐
4. Breed ……
5. Dog use
   - Shepherd dog ☐
   - Guard dog ☐
   - Others (Specify) ☐
   - Pet dog ☐
   - Hunting dog ☐

6. Dog restraining

Is your dog(s) restrained all the time? ☐

Is your dog(s) allowed to roam some or all the time? ☐

7. Place where dog keep and sleep

Inside the house ☐ Outside the house ☐

8. Access to viscera Yes ☐ No ☐ Don't know ☐

Appendix III: Consent form for dog owners

STUDY TITLE: ECHINOCOCCOSIS IN LIVESTOCK AND *ECHINOCOCCUS* INFECTION IN DOGS IN KAJIADO WEST SUB COUNTY, KENYA.

1. **Introduction**

This study will involve determination of the prevalence of echinococcosis in cattle, sheep, goats and *Echinococcus* infection in dogs. It is a widespread neglected zoonotic disease caused by dog tapeworm *Echinococcus granulosus.* The study will also determine the strains/species of Echinococcosis in cattle, sheep, goats and *Echinococcus* infection in dogs and the factors associated with Echinococcus infection. There is no commercial interest in this study at the study regions. The aim is to have data on Echinococcosis for purpose of control measures. The participation to the study is voluntary and you have the right to withdraw from the study. Please read the explanation of the study and feel free to ask any question for clarification.

2. **Purpose of the study.**

The purpose of this study is to determine the prevalence of Echinococcosis in cattle, sheep and goats and Echinococcus infection in dogs and the factors associated with the infection

3. **Procedures**

The dog owners will be requested to consent for their involvement in questionnaire interview, and provide permission to allow dog faecal sample collection in identified homesteads.

4. **Confidentiality**

The records of this study will be kept privately and Lockable cabinets will be used. Any publication or presentation that will arise from this study will not include any information that will make it possible to identify your dog as a subject. However, this information will be available to the dog owners involved in the study.

5. **Benefits**

Though no payments, participating in this study, and answering our questions will help you understand the disease better, this can enhance community participation in prevention.

6. Risks

During this study, there are no risks at all as we will just collect your dog's faecal sample.

7. Compensation

There will be no compensation because no losses or risks involved in this study.

8. Sample storage and transportation

Dog faecal samples will be collected in clean labelled containers and preserved in 80% ethanol and carefully stored in boxes for transportation to KEMRI, CMR parasitology lab for analysis.

Contacts:

The main investigator in this study is Lucy Wanjiru Nungari, ID Number 14483124. In case of any problem you can contact me through 0723208967 or lugitau75@gmail.com. Also the following can be contacted.

Supervisor	**Institution**	**Contact**
Dr. Cecilia Mbae	Kenya Medical Research Institute	+254-722485819
Prof. Joseph K. Gikunju	Jomo Kenyatta University of Agriculture and Technology	+254-722808671

In case you need to enquire about your security and right to participate in this study, the involved institute will be;

Scientific Ethical Review Unit, KEMRI.

P.O Box 54840, Telephone 2722541 (Weekday, daytime).

Jomo Kenyatta University of Agriculture and Technology (JKUAT) JKUAT

P.O BOX 62,000-00200, Nairobi Kenya

Telephone +254 (067) 52124,

Declaration

Dog owner……... ……....

Address: ……

I……. have full capacity to consent to Lucy Wanjiru Nungari to involve my dog in the study. I have been informed about the study in details. Having read the information explained to me and understood it, I give consent for my dog to participate in the study. I also understand that I can withdraw my dog from this study.

Dog owner's signature or left thumb print

Date……

Person obtaining consent Date……

Name……

Signature

Witness (in case of illiterate participants) Date…….

Name…….

Signature

Appendix IV: Kiswahili translations for questionnaire

VIAMBATISHO

KIAMBATISHO I: DODOSO

KANUNI ZA ENUMERETA .....

LOKISHENI.....

NAMBARI YA MBWA .....

NAMBARI YA DODOSO: .....

TAREHE……...

SEHEMU A: MASWALI KUHUSU MMILIKI WA MBWA

1. Mmiliki wa mbwa ....
2. Kazi

1.Mwalimu 2. Mkulima 3. Mkenyumbani 4. Dereva 5. Biashara 6. Kampuni7. Ingine

3. Jinsia

Mwanaume ☐ Mwanamke ☐

4. Umri

18-25 ☐

26-33 ☐

33-39 ☐

☐

Juu ya 40

5. Kiwango cha Elimu

   Hakuna elimu rasmi ☐

   Masomo ya msingi ☐

   Kiwango cha sekondari ☐

   Kiwango cha baada ya sekondari ☐

6. Je, una mbwa wangapi katika boma lako? ☐
7. Je, wewe huwapa dawa za minyoo mbwa wako? Ndiyo ☐ La ☐
8. Kama ndiyo, ni mara ngapi?

   Kila wiki ☐

   Kila mwezi ☐

   Robo mwaka ☐

   Baada ya miezi sita ☐

   Kila mwaka ☐

   Baada ya zaidi ya mwaka ☐

   Nyingine yoyote (taja) ..... ☐

   Kamwe

9. Jina la dawa inayotumika ....
10. Je, wewe huchinja kondoo, mbuzi au ng'ombe nyumbani? Ndiyo ☐ La ☐
11. Kama ndiyo, nyama hukaguliwa na maafisa wa mifugo? Ndiyo ☐ La ☐

12. Viungo vya ndani huelekezwa wapi?

Kulisha mbwa ☐

Kutupa katika kichaka ☐

Kuzika ☐

Kuliwa na watu ☐

Zingine (taja) .....

13. Je,wewe huchota maji wapi?

Kisima ☐

Mto ☐

Kwingineko(taja).....

14. Ni kwa jinsi gani mbwa wako hasa kupata kulishwa?

Chakula hupikwa ☐

Taka za kichinjio / nyumbani ☐

Kuokoteza ☐

Zinginezo (taja)…….

15. Je, unajua ugonjwa unaoweza kuupata kutokana na mbwa? Ndiyo ☐ La ☐

16. Kama ndiyo, ni ugojwa gani?

Malaria ☐

Rabies ☐

Brucellosis ☐

Zingine ☐

17. Je, unajua CE (Cystic Echinococcosis)? Ndio ☐ La ☐
18. Ugonjwa huu huathiri nani? Watu ☐ Mifugo ☐ Wanyama pori ☐

    Sijui ☐ Wengine (taja) .....

19. Sehemu gani hasa ugonjwa huu huathiri? Mapafu ☐ Maini ☐ Ngozi ☐

    Sjui ☐ zingine (taja) .....

20. Chanzo cha maambukizi ya CE kwa mwanadamu ni nini? Mifugo ☐ Wanyama pori ☐

    Mbwa ☐ Sijui ☐ Wengine (taja).....

SEHEMU B: MASWALI KUHUSU MBWA

1. Jina ..... Kitambulisho.....
2. Umri .....
3. Jinsia? Wakiume ☐ Wakike ☐
4. Aina .....
5. Mbwa hutumika aje?

    Mbwa wa kuchunga ☐

    Mbwa mlinzi ☐

    Mbwa wa nyumba ☐

    Mbwa mwindaji ☐

Wengine (Taja).....

6. Uzuilizi wa Mbwa

Mbwa wako huwa amezuiliwa wakati wote? Ndiyo ☐ La ☐

Mbwa wako huruhusiwa kuzurura baadhi au wakati wote? Ndiyo ☐ La ☐

7. Sehemu ambapo mbwa hukaa na kulala

Ndani ya nyumba ☐

Nje ya nyumba ☐

8. Mbwa hupata viungo vya ndani Ndio ☐ Hapana ☐ Sijui ☐

Appendix V: Kiswahili translation for dog owner's consent

NYONGEZA: IDHINI YA WAMILIKI MBWA

TITLE STUDY: ECHINOCOCCOSIS IN LIVESTOCK AND ECHINOCOCCUS INFECTION IN DOGS IN KAJIADO WEST SUB COUNTY, KENYA.

1. **Kuanzishwa**

Utafiti huu utahusisha uamuzi wa kiwango cha maambukizi ya Echinococcosis katika ng'ombe, kondoo, mbuzi na maambukizi ya *Echinococcus* katika mbwa. Ni ugojwa ulioenea sana na unaathiri watu na wanyama na unsababishwa na mnyoo ya mbwa, *Echinococcus granulosus*. Utafiti huu pia utaamua aina ya Echinococcosis katika ng'ombe, kondoo, mbuzi na ya maambukizi ya *Echinococcus* katika mbwa na mambo yanayohusiana na maambukizi ya *Echinococcus*. Hakuna maslahi ya kibiashara katika utafiti huu katika mikoa ya utafiti. Lengo ni kuwa na data juu ya Echinococcosis kwa lengo la hatua za kudhibiti. Ushiriki na utafiti huu ni wa hiari na una haki ya kuondoka kutoka utafiti. Tafadhali soma maelezo ya utafiti na ujisikie huru kuuliza swali lolote kwa ufafanuzi.

2. **Madhumuni ya utafiti.**

Lengo la somo hili ni kuamua kiwango cha maambukizi ya Echinococcosis katika ng'ombe, kondoo na mbuzi na maambukizi ya *Echinococcus* katika mbwa na mambo yanayohusiana na maambukizi hayo.

3. **Utaratibu**

Wamiliki wa mbwa wataombwa kwa idhini kuhusika katika dodoso la mahojiano, na kutoa ruhusa kwa ajili ya kuruhusu ukusanyaji wa kinyesi cha mbwa wao kama sampuli katika boma lililotambuliwa.

4. **Usiri**

Rekodi ya utafiti huu itawekwa faragha na makabati za kufugwa zitatumika. uchapishaji wowote au mada itakayotokea kutokana na utafiti huu haitahusisha kamwe habari za mbwa na haitawezekana kamwe kutambua mbwa wako kama somo. Hata hivyo, habari hii itakuwa inapatikana kwa wamiliki wa mbwa watakaoshiriki katika utafiti.

5. **Faida**

Ingawa hakuna malipo, kushiriki katika utafiti huu, na kujibu maswali yetu itakusaidia kuelewa ugonjwa bora, hii inaweza kuongeza ushiriki wa jamii katika kuzuia ule ugojwa.

6. **Hatari**

Wakati wa utafiti huu, hakuna hatari yeyote maana sisi tutakukusanya tu kinyesi cha mbwa wako kama sampuli.

7. **Fidia**

Hakutakuwa na fidia kwa sababu hakuna hasara au hatari ya kushiriki katika utafiti huu.

8. **Uhifadhi wa sampuli na usafirishaji**

Sampuli za kinyesi cha mbwa zitakuwa zikikusanywa katika vyombo safi na kuhifadhiwa katika 80% ethanol na kwa makini kuhifadhiwa katika masanduku kwa ajili ya usafiri kuelekea KEMRI, CMR vimelea maabara kwa ajili ya uchambuzi.

Mawasiliano:

Mpelelezi mkuu katika utafiti huu ni Lucy Wanjiru Nungari, Idadi ya kitabuliso 14483124. Katika matatizo yoyote unaweza kuwasiliana na mimi kupitia 0723208967 au lugitau75@gmail.com. Pia unaweza kuwasiliana na;

Msimamizi	Taasisi	Mawasiliano
Dr. Cecilia Mbae	Kenya Medical Research Institute	+254-722485819
Prof. Joseph K. Gikunju	Jomo Kenyatta University of Agriculture and Technology	+254-722808671

Kama unahitaji kuuliza kuhusu usalama wako na haki ya kushiriki katika utafiti huu, taasisi wanaohusika watakuwa;

Scientific Ethical Review Unit, KEMRI.

SLP 54840, Simu 2722541 (siku za wiki, mchana).

Jomo Kenyatta University of Agriculture and Technology (JKUAT)

SLP 62,000-00200, Nairobi Kenya

Namba +254 (067) 52124,

TAMKO

Mmiliki wa Mbwa ....

Mtaa: .....

Mimi ..... nina uwezo kamili kuridhia Lucy Wanjiru Nungari kuhusisha mbwa wangu katika utafiti. Nimefahamishwa kuhusu utafiti katika maelezo. Baada ya kusoma

maelezo alinieleza na kulielewa, mimi nimetoa idhini kwa mbwa wangu kushiriki katika utafiti. Mimi pia nimeelewa kwamba naweza ondoa mbwa wangu kutoka utafiti huu.

Sahihi ya Mshiriki au alama ya kidole cha kushoto

Jina la mtafiti.....Tarehe.....

Sahihi

Jina la shahidi.....Tarehe.....

Sahihi

Appendix VI: Maasai translations questionnaire

EMATUA A: NGILIKUANAT NAIPIITA ELOPENY OLDIE

1. Olepeny oldie …….
2. Emanyiso?

   Olmalimui ☐ Olaramatani loo akuluku ok ☐ Ereshata ebiotisho oo seseni ☐ Onkulie.
3. Ko lee ☐ elipon'g ☐
4. Erisiore

   18-25 ☐

   26-33 ☐

   34-39 ☐

   Nalang 40 ☐
5. Enebaiki engisoma

   Metii engisoma ☐

   Ereshata enkiterunoto ☐

   Ereshata empolos ☐

   Ereshata enkitoo ☐
6. Kaja ildiein liata te njan'gino
7. Inchos oshi oldie asho ildiein olnjani lenjoga Ee ☐ Aa ☐
8. Tenesepa, katiaa reshata

   Te wiki ☐

   To lapa ☐

   lapaitini ☐

   Ketuluseiye lapain ile ☐

   Tolari ☐

   Ketuluseiye olari obo nelang ekululie (ntadede) ☐

   Etu aikata ☐
9. Enkaina olchani……

10. Iyen'giyieng'e ngerra,nkineji arachu inkishuu tiang' Ee ☐ Aa ☐

11. Tanaa,kedede,kepisa ajo keing'uraa olkitari loo swam ingiri Ee ☐ Aa ☐

12. Kai intoraki robat oo Mbopong'i

Intotiyie oldia ☐

Intoraa te ndim ☐

Inukaa ☐

Kenya ilntong'ana ☐

Onkulie (ntadede)…….

13. Kaji entonata enkare?

Eweji nepising'a ☐

Eweji newang' ☐

14. Kaji oshiinko te nitoti oldie (ildiein) lino?

Edaa naiyera ☐

Nkiri najon /legato o naitore te gichinjo (olale eyieng'ieki nkishu) ☐

Nkiri kongata (mpopong'i) ☐

Onkulie…….

15. Ijiolo (emoyian moiyaritin) nidim anotoyo oldia? Ee ☐ Aa ☐

16. Tanaa nejia,kaa nabo (kakua)

Ka ninje? ☐

Erikajong'ani ☐

Ilpepedo ☐

Enekole ☐

Onkulie ☐

17. Iyiolo ena muyan CE (Hydatid disease) Ee ashu Aa

18. Kang'ae eibung emoyian indung'ana

Swam ☐

Nguesi endim ☐

Maiyola ☐

Ongulie (ndadede) ☐

19. Kakua robat oshi eibun'g emoyian?

Inkipiu ☐

Emonyua ☐

Olnchoni ☐

Maiyolo ☐

Onkulie(ntadede) ☐

EMATUA B: NKILIKUANAT NAIPIRITA OLDIE

1. Enkarna……embukunoto eng'ues
2. Erisiori……
3. Pukunoto? Olee ☐ Elipong' ☐
4. Ankabila oldie…….
5. Pukunot oldie

   Oldie airitani ☐

   Oldie oriponi ☐

   Oldie liaji ☐

   Onkulie (ntadede) ☐
6. Aresh oldie

   Indirishaki oldia (ildieni) linono anaake? ☐

   Ipalaa oshi oldia (ildiein) pee ololoito pooki kata? ☐
7. Kaji etonie neirag ildia

   Atua enkaji ☐ Boo enkaji ☐

Appendix VII: Maasai translations for consent form

1. **Enkiterunoto**

   Koro ena kisema keipirta enetiu empong'et tiatua inkishu,nkerra ,nkinyeji wo roirirua loo ildiein.emoiyian ena natamanal yie tolusho nayauwa ildien tengoitoi oo ilpuduk (*Echinococcus granulosus*) kore ena kisoma keitondolu errishata /weiya empong'et tiatua inkishu,nkerra,ngineji wo roirirua loo ildiein wesipata.naipirta empong'et .meeta esipata sapuk ena kisonia tiatua elosho.kore esipata na ashum ilkigerot eipinta na ashum ilkigerot eipinta na ashum ilkigerot eipinta engibooruto empong'it .kore ena kisoma na enepesho ,kake tenimidim ,niata esipata nipalie soma ebolunoto ena kisoma naa idim aikilikuano nemidim peiye kiutare

2. **Esipata enkisoma**

   Kore esipata ena kisoma, naa neikoni peiboori ena keeya empongit tiatua inkishu, nkerra ngineji lo leiricua loo lindiein engulie pukunot naaroba ie, nena baa.

3. **Naishiakinore**

   Keishiakinore loopeny (ildiein)netii atua nkikilikuanat neiruk sii pedumuni inkik ooileliein ntiata ilalea lenye

4. **Nkisudorot**

   Kore kona bolunot,neishiakinore nemeitodoluni ,neshumi,tiatua sadukuni oikeni.kore kona kigerot ena kisoma ,meishiakinore neitau arashu aibalunye ajo kere ena keisho ilitung'ana lolmarei pee eiboyo moiyarilin.

5. **Isidan**

   Hoona nemeta elaat, kore peiye isoma ena kisoma,nishuk ingilikuanat ,iyolou nenadede naiparta ena moiyan ,kore eina keisho ilitung'ana lolmarei pee aiboyo moiyarilin.

6. **Intorrok**

Meeta ena kisoma entorroni tenemee inkik ake oldia lino kiyie anaa enkitanyanyu koto

7. **Elaata**

   Metii elaata amu metii entoroni naipirta ena kisoma.

8. **Enchoma or kitanyanyukot we Napata**.

   Keishiakinore nepiki nkitanyanyukot ooinkik oo oldiein atua aweei sidai neshumi tiatua 80% ethanol too mpuyai nasira nepiki atua sadukuni nryae KEMRI, CMR,parasitology lab too nking'urat nadede.

Nambain:

Ore Olajuroni tena ngisoma na Lucy Wanjiru Nungari, namba engitambulisho na 14483214, Enetii enyamali intumia namba ee esimu 0723208967 ashu lugitau75@gmail.com.

Endim atosho guna simu:

Supervisor	**Institution**	**Contact**
Dr. Cecilia K.Mbae	Kenya Medical Research Institute	+254-722485819
Prof. Joseph K. Gikunju	Jomo Kenyatta University of Agriculture and Technology	+254-722808671

Enioli niyolou enalagua alangu ena ngisoma ninger empala te;

Scientific Ethical Review Unit, KEMRI.

P.O Box 54840, Telephone 2722541 (ngoloni, dama).

Jomo Kenyatta University of Agriculture and Technology (JKUAT) JKUAT

P.O BOX 62,000-00200, Nairobi Kenya

Telephone +254 (067) 52124,

Aibarie

Olpeny oldia…..…….

Namba empala: ……

Ore nanu.……. ategelua Lucy Wanjiru Nungari veitumia oldie lai tena ngisoma.Atolikio enangisoma naimbonga pi.Ore aidepa aisoma ena ngisoma naimbonga pi, atonyoraiye oldie lai veitumia tenangisoma. Naidipa ayiolou kaiden aitau oldie lai tena ngisoma

Olkimajino olopeny oldie

Appendix VIII: Certificate of translation: English to Maasai language

PROTOCOL TITLE: ECHINOCOCCOSIS IN LIVESTOCK AND *ECHINOCOCCUS* INFECTION IN DOGS IN KAJIADO WEST SUBCOUNTY, KENYA.

PI: LUCY WANJIRU NUNGARI

To whom it may concern

I BENSON S. TITIAN do hereby testify that I translated the English version of the questionnaire and informed consent forms from version 1.2 dated 06/01/2017 into Maasai language for the above named study. I certify that this is an accurate and true translation to the best of my ability.

Signed _____ Dated 10/01/2017

Address 564 GPO NRB

Email: bsiwonka@gmail.com

Tel: +254-721539993

Appendix IX: Certificate of back translation: Maasai into English language

CERTIFICATE OF BACK TRANSLATION

PROTOCOL TITLE: ECHINOCOCCOSIS IN LIVESTOCK AND *ECHINOCOCCUS* INFECTION IN DOGS IN KAJIADO WEST SUBCOUNTY, KENYA.

PI: LUCY WANJIRU NUNGARI

To whom it may concern

I Jennifer Sanaipei do hereby testify that I back translated the Maasai version of the questionnaire and informed consent forms from version 1.2 dated 06/01/2017 into English language for the above named study. I certify that this is an accurate and true translation to the best of my ability.

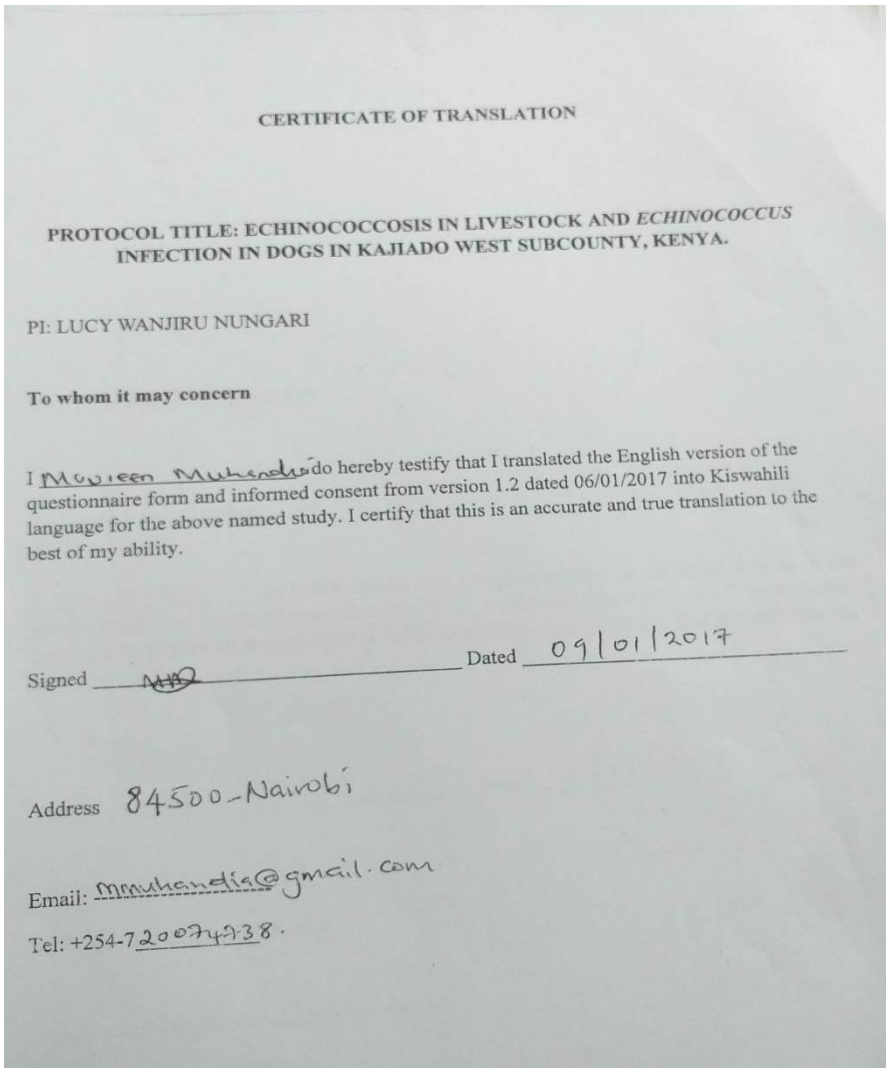
Signed ~~AAK~~. Dated 12/01/2017.

Address 54840 NRB.

Email: jsanaipei@gmail.com

Tel: +254-7 0720-749891

Appendix X: Certificate of translation: English into Kiswahili language



CERTIFICATE OF TRANSLATION

PROTOCOL TITLE: ECHINOCOCCOSIS IN LIVESTOCK AND *ECHINOCOCCUS* INFECTION IN DOGS IN KAJIADO WEST SUBCOUNTY, KENYA.

PI: LUCY WANJIRU NUNGARI

To whom it may concern

I Moureen Muhendo do hereby testify that I translated the English version of the questionnaire form and informed consent from version 1.2 dated 06/01/2017 into Kiswahili language for the above named study. I certify that this is an accurate and true translation to the best of my ability.

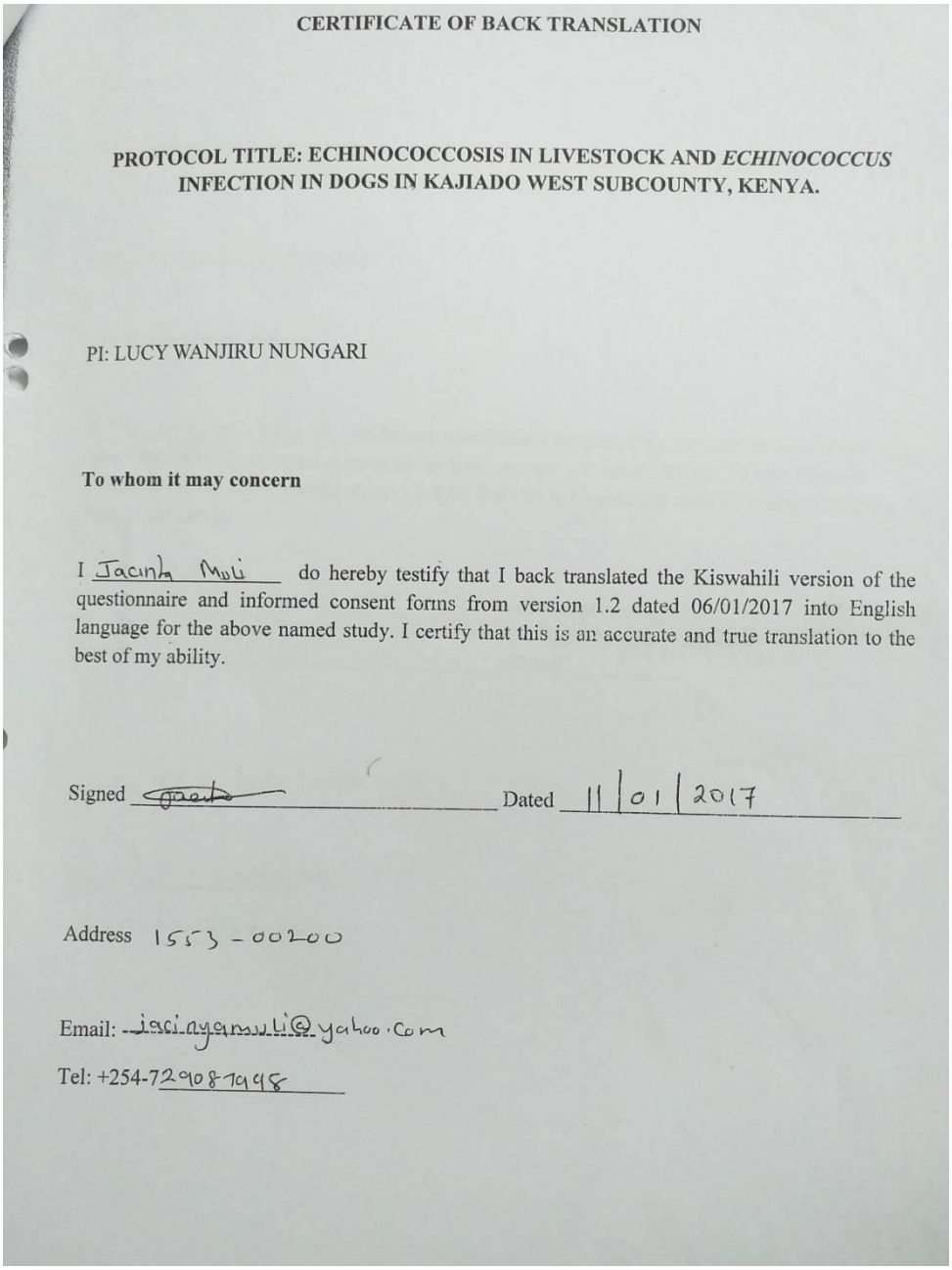
Signed MM Dated 09/01/2017

Address 84500-Nairobi

Email: mmuhendia@gmail.com

Tel: +254-720074938.

Appendix XI: Certificate of back translation: Kiswahili into English language



CERTIFICATE OF BACK TRANSLATION

PROTOCOL TITLE: ECHINOCOCCOSIS IN LIVESTOCK AND *ECHINOCOCCUS* INFECTION IN DOGS IN KAJIADO WEST SUBCOUNTY, KENYA.

PI: LUCY WANJIRU NUNGARI

To whom it may concern

I Jacinta Muli do hereby testify that I back translated the Kiswahili version of the questionnaire and informed consent forms from version 1.2 dated 06/01/2017 into English language for the above named study. I certify that this is an accurate and true translation to the best of my ability.

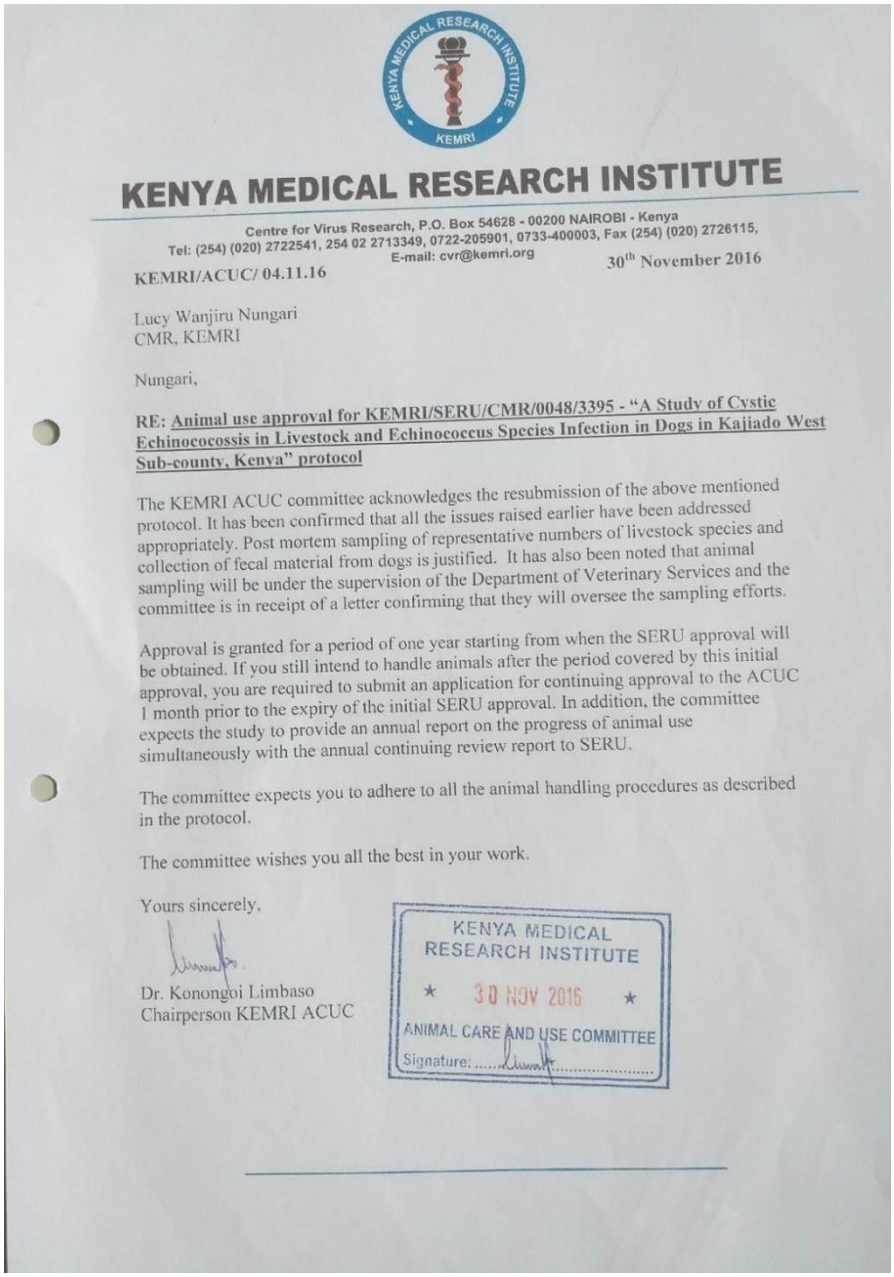
Signed _____ Dated 11/01/2017

Address 1553 - 00200

Email: jacinayamuli@yahoo.com

Tel: +254-729087998

Appendix XII: KEMRI Animal Care and Use Committee approval letter



KENYA MEDICAL RESEARCH INSTITUTE

Centre for Virus Research, P.O. Box 54628 - 00200 NAIROBI - Kenya
Tel: (254) (020) 2722541, 254 02 2713349, 0722-205901, 0733-400003, Fax (254) (020) 2726115,
E-mail: cvr@kemri.org

KEMRI/ACUC/ 04.11.16

30th November 2016

Lucy Wanjiru Nungari
CMR, KEMRI

Nungari,

RE: Animal use approval for KEMRI/SERU/CMR/0048/3395 - "A Study of Cystic Echinococossis in Livestock and Echinococcus Species Infection in Dogs in Kajiado West Sub-county, Kenya" protocol

The KEMRI ACUC committee acknowledges the resubmission of the above mentioned protocol. It has been confirmed that all the issues raised earlier have been addressed appropriately. Post mortem sampling of representative numbers of livestock species and collection of fecal material from dogs is justified. It has also been noted that animal sampling will be under the supervision of the Department of Veterinary Services and the committee is in receipt of a letter confirming that they will oversee the sampling efforts.

Approval is granted for a period of one year starting from when the SERU approval will be obtained. If you still intend to handle animals after the period covered by this initial approval, you are required to submit an application for continuing approval to the ACUC 1 month prior to the expiry of the initial SERU approval. In addition, the committee expects the study to provide an annual report on the progress of animal use simultaneously with the annual continuing review report to SERU.

The committee expects you to adhere to all the animal handling procedures as described in the protocol.

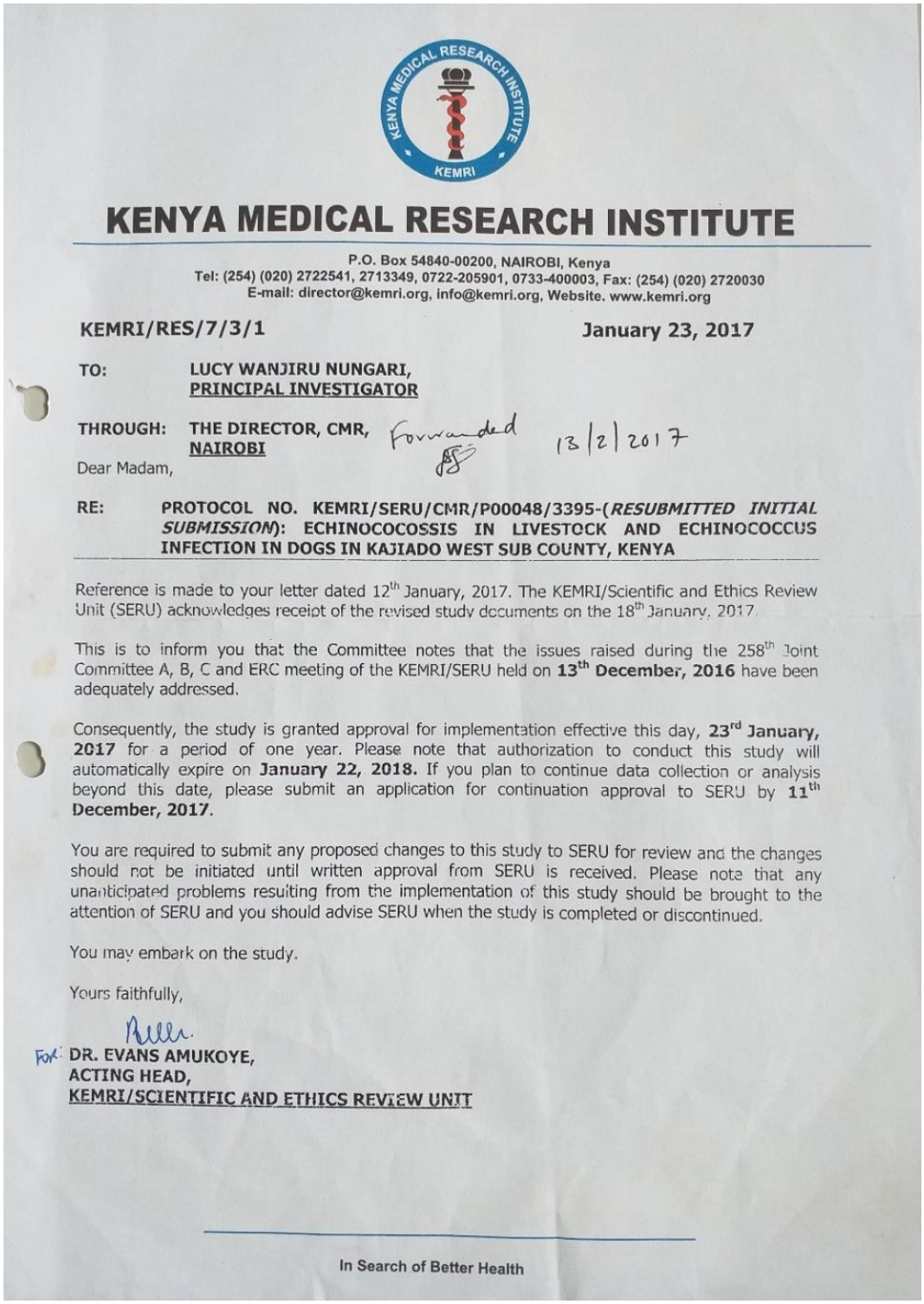
The committee wishes you all the best in your work.

Yours sincerely,

Dr. Konongoi Limbaso
Chairperson KEMRI ACUC

KENYA MEDICAL RESEARCH INSTITUTE
★ 30 NOV 2016 ★
ANIMAL CARE AND USE COMMITTEE
Signature: .....

Appendix XIII: KEMRI-Scientific Ethical Review Unit approval letter



KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel: (254) (020) 2722541, 2713349, 0722-205901, 0733-400003, Fax: (254) (020) 2720030
E-mail: director@kemri.org, info@kemri.org, Website. www.kemri.org

KEMRI/RES/7/3/1 **January 23, 2017**

TO: **LUCY WANJIRU NUNGARI,**
PRINCIPAL INVESTIGATOR

THROUGH: **THE DIRECTOR, CMR,**
NAIROBI

Forwarded 13/2/2017

Dear Madam,

RE: **PROTOCOL NO. KEMRI/SERU/CMR/P00048/3395-(*RESUBMITTED INITIAL SUBMISSION*): ECHINOCOCOSSIS IN LIVESTOCK AND ECHINOCOCCUS INFECTION IN DOGS IN KAJIADO WEST SUB COUNTY, KENYA**

Reference is made to your letter dated 12th January, 2017. The KEMRI/Scientific and Ethics Review Unit (SERU) acknowledges receipt of the revised study documents on the 18th January, 2017.

This is to inform you that the Committee notes that the issues raised during the 258th Joint Committee A, B, C and ERC meeting of the KEMRI/SERU held on **13th December, 2016** have been adequately addressed.

Consequently, the study is granted approval for implementation effective this day, **23rd January, 2017** for a period of one year. Please note that authorization to conduct this study will automatically expire on **January 22, 2018.** If you plan to continue data collection or analysis beyond this date, please submit an application for continuation approval to SERU by **11th December, 2017**.

You are required to submit any proposed changes to this study to SERU for review and the changes should not be initiated until written approval from SERU is received. Please note that any unanticipated problems resulting from the implementation of this study should be brought to the attention of SERU and you should advise SERU when the study is completed or discontinued.

You may embark on the study.

Yours faithfully,

FOR: **DR. EVANS AMUKOYE,**
ACTING HEAD,
KEMRI/SCIENTIFIC AND ETHICS REVIEW UNIT

In Search of Better Health

Appendix XIV: Approval letter of research proposal and of supervisors from Director, Board of Post- graduate, JKUAT.

JOMO KENYATTA UNIVERSITY
OF
AGRICULTURE AND TECHNOLOGY
DIRECTOR, BOARD OF POSTGRADUATE STUDIES

P.O. BOX 62000
NAIROBI – 00200
KENYA
Email: director@bps.jkuat.ac.ke
TEL: 254-67-5870000/1-5

REF: JKU/2/11/TM304-2781/2015

19TH JUNE, 2019

NUNGARI LUCY WANJIRU
C/o SOBMS
JKUAT

Dear M. Wanjiru,

RE: APPROVAL OF RESEARCH PROPOSAL AND OF SUPERVISORS

Kindly note that your MSc. research proposal entitled: "ECHINOCOCCOSIS IN LIVESTOCK AND *ECHINOCOCCUS* INFECTION IN DOGS IN KAJIADO WEST SUB COUNTY, KENYA" has been approved. The following are your approved supervisors:-

1. Dr. Cecilia Mbae
2. Prof. Joseph K. Gikunju

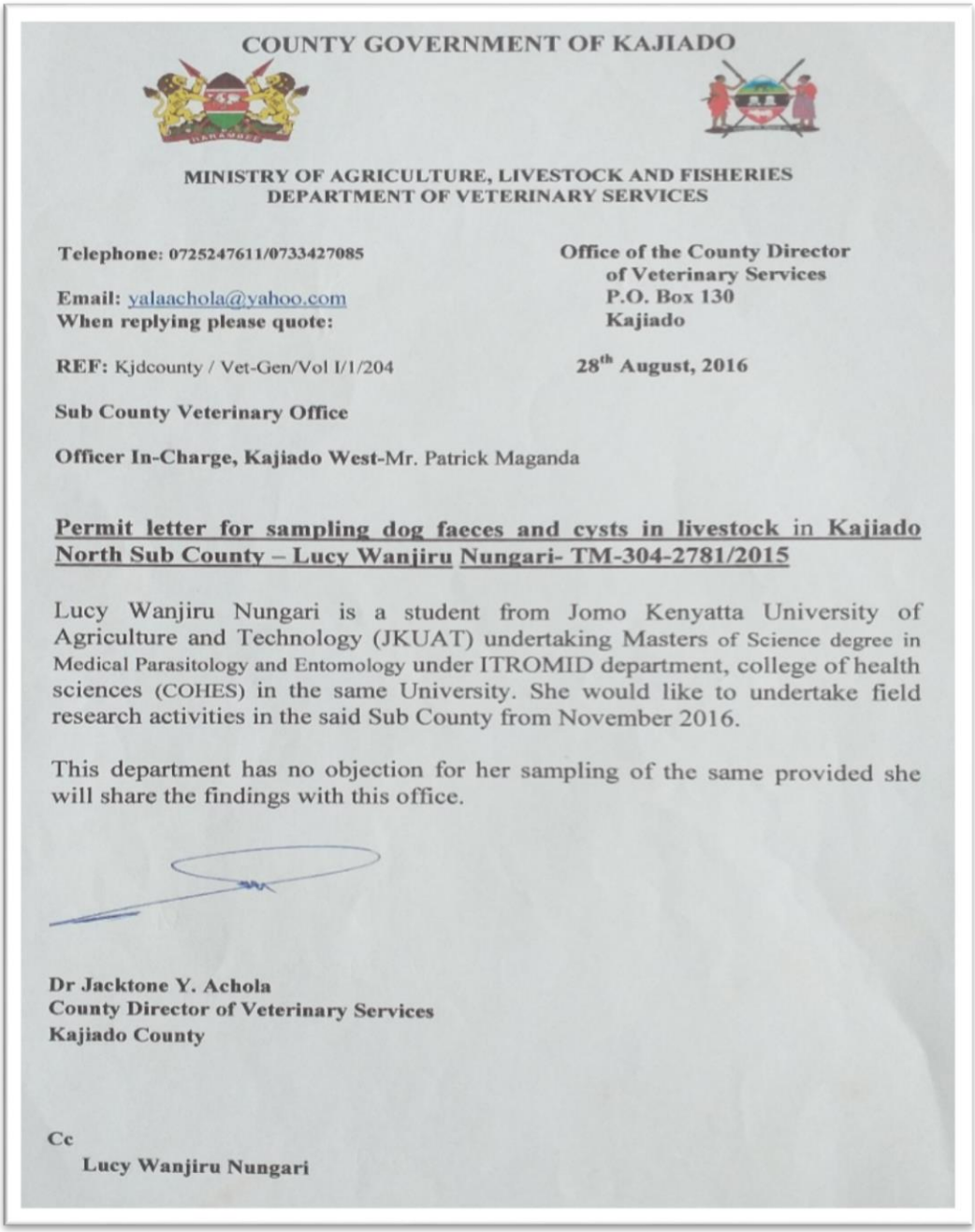
Yours sincerely,

PROF. MATHEW KINYANJUI
DIRECTOR, BOARD OF POSTGRADUATE STUDIES
Copy to: Dean, SOBMS

/cm

Setting trends in Higher Education, Research and Innovation

Appendix XV: Dog faecal sampling approval letter from Kajiado County Director of Veterinary Services.



COUNTY GOVERNMENT OF KAJIADO

MINISTRY OF AGRICULTURE, LIVESTOCK AND FISHERIES
DEPARTMENT OF VETERINARY SERVICES

Telephone: 0725247611/0733427085

Email: yalaachola@yahoo.com
When replying please quote:

Office of the County Director
of Veterinary Services
P.O. Box 130
Kajiado

REF: Kjdcounty / Vet-Gen/Vol I/1/204

28th August, 2016

Sub County Veterinary Office

Officer In-Charge, Kajiado West-Mr. Patrick Maganda

Permit letter for sampling dog faeces and cysts in livestock in Kajiado North Sub County – Lucy Wanjiru Nungari- TM-304-2781/2015

Lucy Wanjiru Nungari is a student from Jomo Kenyatta University of Agriculture and Technology (JKUAT) undertaking Masters of Science degree in Medical Parasitology and Entomology under ITROMID department, college of health sciences (COHES) in the same University. She would like to undertake field research activities in the said Sub County from November 2016.

This department has no objection for her sampling of the same provided she will share the findings with this office.

Dr Jacktone Y. Achola
County Director of Veterinary Services
Kajiado County

Cc
Lucy Wanjiru Nungari

Appendix XVI: Plagiarism Report

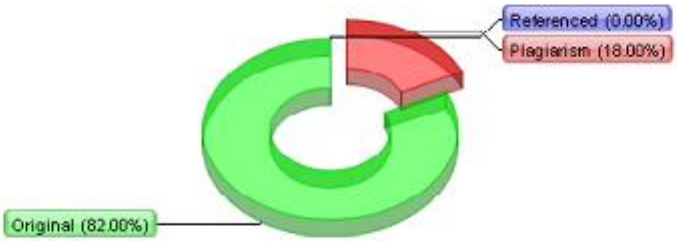
Plagiarism Detector v. 1092 - Originality Report:

Analyzed document: 13-Sep-19 16:19:32

"Lucy Wanjiru Nungariucy Thesis Draft 13.09.2019.docx"

Licensed to: **Jomo Kenyatta University of Agriculture and Technology**

Relation chart:



Distribution graph:

Comparison Preset: Word-to-Word. Detected language: English

Top sources of plagiarism:

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% 13	wrds: 696	https://www.hindawi.com/journals/bmri/2019/4798906/
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[Show other Sources:]

Processed resources details:

269 - Ok / 110 - Failed

[Show other Sources:]

Appendix XVII: Published Manuscript

Hindawi
BioMed Research International
Volume 2019, Article ID 4798906, 7 pages
https://doi.org/10.1155/2019/4798906

Research Article

Prevalence and Genotyping of *Echinococcus* Species from Livestock in Kajiado County, Kenya

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Cystic Echinococcosis (CE) is a widespread neglected zoonotic disease and is caused by the larval stage of the dog tapeworm *Echinococcus granulosus* sensu lato. CE is more frequent in livestock-rearing areas and where people live a nomadic or seminomadic lifestyle such as in Kajiado County, Kenya. There is limited data on CE disease situation in the county of Maasailand; the present study, therefore, reports on the prevalence of CE in cattle, sheep, and goats and their relative importance in CE transmission in Kajiado County. In total, 1,486 livestock (388 cattle, 625 sheep, and 473 goats) slaughtered in two abattoirs were examined for the presence of hydatid cysts in various organs. Cyst isolates were genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) of the NADH dehydrogenase subunit 1 gene (*nad1*). The overall prevalence of CE was 14.8% (220/1486), while prevalence per livestock species was 15.2% (72/473) in goats, 14.9% (93/625) in sheep, and 14.2% (55/388) in cattle. Out of the 421 cysts isolated, 389 cysts were successfully characterized to be either *E. granulosus* sensu stricto (s. s.), 356/389 (91.5%), *E. canadensis* (G6/7), 26/389 (6.7%), or *E. ortleppi*, 7/389 (1.8%). This record confirms predominance of *E. granulosus* s. s. in Maasailand and other parts of Kenya, while the importance of *E. ortleppi* and *E. canadensis* (G6/7) to the general CE burden in Maasailand might be higher than previously thought. More so, a higher infection pressure for humans by *E. granulosus* s. s. based on its abundance could be speculated. The study sheds significant light on CE situation in livestock in the nomadic/seminomadic society of the Maasai in Kajiado County and provides good bases to investigate human CE in the area.

1. Introduction

Cystic Echinococcosis is caused by the larval stage of the dog tapeworm *Echinococcus granulosus* sensu lato (s. l.) and is currently recognized by World Health Organization (WHO) as a neglected tropical disease [1]. CE is a common zoonotic disease of great public health significance globally due to its associated economic losses [2]. Approximately US$ 3 billion are lost annually on treatment of CE in humans and losses incurred due to the condemnation of infected organs in livestock [3]. Dogs and to a lesser extent other canids and felids are the primary definitive hosts of *Echinococcus* species, with herbivores acting as the intermediate hosts and the humans as aberrant intermediate hosts. The outcome of the infection in livestock and human is cyst development in the liver, lungs, or other organs [4]. *E. granulosus* s. l. consists of at least five species, namely, *E. granulosus* sensu stricto (s. s.), *E. equinus, E. ortleppi, E. canadensis* (G6–G10), and *E. felidis* [5, 6].

East Africa and Kenya, in particular, have long been known to be of the world's largest foci of CE in humans [7–9]. Previous data from Kenya have centered their focus on CE situations in Turkana and Maasailand [8]. Data from other endemic areas are available but only sparingly. Previous CE studies in Kenya, thus far, indicate the presence of all five *E. granulosus* s. l. and the recently discovered Gomo genotype [10–15]. To appreciate the CE situation in the whole of Kenya, epidemiological data from all endemic localities including Kajiado County is required. The only available data from this area is nearly three decades old and did not report *Echinococcus* spp. in livestock [16]. Furthermore, the recent study examined livestock originating mainly from Bissil area

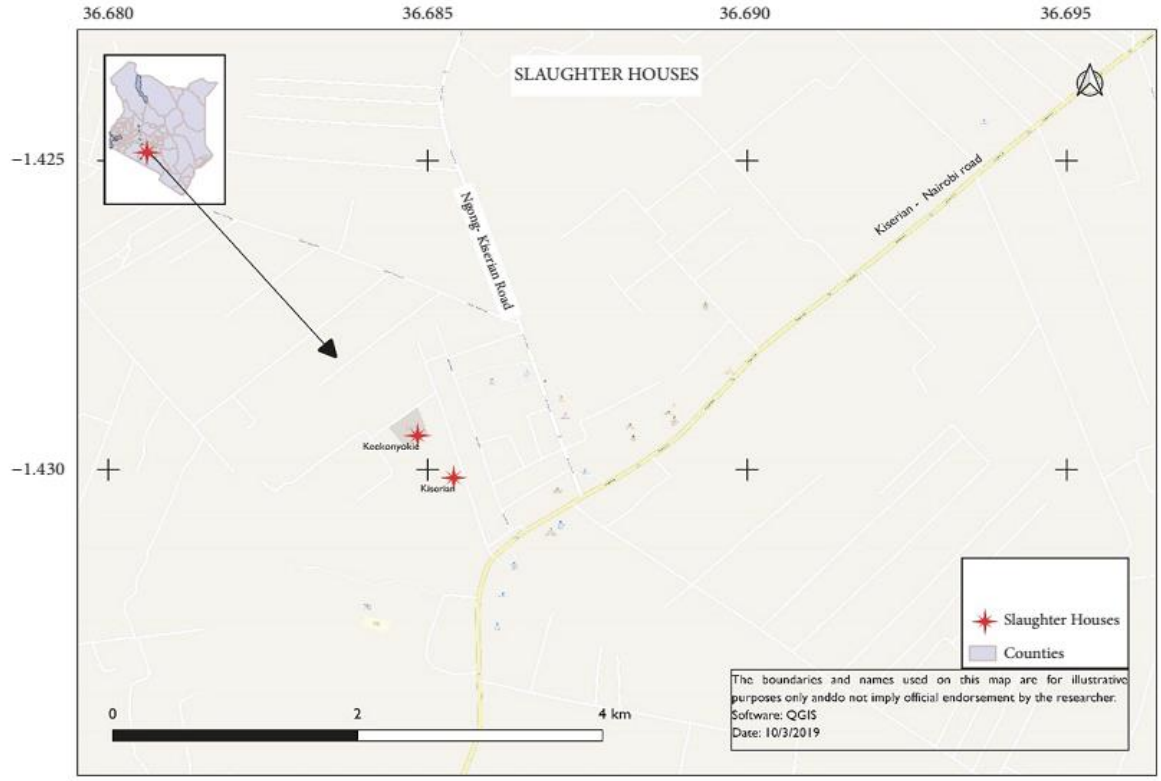

Figure 1: A map showing the location of the two abattoirs located in Kiserian and Keekonyokie.

(Kajiado South) [10]. Therefore, this study focused on the two main slaughterhouses receiving livestock from the wider scope of the Kajiado County. We report here the prevalence of CE in cattle, sheep, and goats and the *Echinococcus* spp. causing CE in Kajiado County. Findings from the study will improve our knowledge of CE in this county and establish the relative contribution of each livestock species in the distribution and transmission of CE.

2. Materials and Methods

2.1. Hydatid Cysts Collection from Cattle, Sheep, and Goats. Majority of the livestock examined for CE came from Kajiado County. The county is divided into five subcounties: Kajiado Central, Kajiado North, Kajiado East, Kajiado West, and Kajiado South. Sampling was done in two major abattoirs in Kajiado West Subcounty, namely, Kiserian and Keekonyokie, during slaughter days from December 2016 to February 2017 (Figure 1). A total of 1486 carcasses of livestock were inspected for the presence of hydatid cysts in all organs (lungs, liver, heart, spleen, as well as the kidneys) of the pleural and abdominal cavities. Visual inspection, palpation, and incision were done for all of the organs for the presence and cyst distribution. The lesions were carefully excised from all infected organs. Individual cysts were identified as those that had a continuous cyst wall while multiple cysts had a visibly separate cyst wall even for the calcified cysts. The isolated cysts were packed in clean polythene bags placed in cooler boxes and transported to the parasitology laboratory of the Kenya Medical Research Institute, for examination and further analysis. Cysts were dissected using a sterile scalpel blade and each cyst material was fixed and preserved in 70% Ethanol in individual tubes. The contents of the cysts were examined microscopically for the presence of protoscoleces (PS). Cysts were classified as fertile (with protoscoleces) sterile (fluid-filled without protoscoleces), degenerated (collapsed cyst walls with caseated protoscoleces and soft cheesy debris without calcification), and calcified (hard solid appearance of the ectocyst). All the cysts from the same organ were examined individually to confirm mixed infections.

2.2. DNA Extraction. DNA was obtained from cyst material and protoscoleces by lysing in 0.02 M NaOH at 99°C for 10 minutes. In a few instances where the above process failed to yield adequate DNA, genomic DNA was extracted using DNeasy Blood & Tissue Kit® (Qiagen, Hilden, Germany). The germinal layers or cyst walls were cut into small pieces and lysed in ATL lysis buffer (180 μl) and proteinase K (20 μl), and DNA was subsequently extracted using the manufacturer's protocol. Extracted DNA was eluted in 50 μL of elution buffer.

Table 1: Prevalence of cystic echinococcosis and cyst location in cattle, goats, and sheep in Kajiado County.

Livestock	Prevalence (%)	Liver	Lungs	Both organs
Cattle (n=388)	55 (14.18)	25	16	14
Goats (n=473)	72 (15.22)	45	21	6
Sheep (n=625)	93 (14.88)	67	18	8

Table 2: Cyst load in infected cattle, goats, and sheep in Kajiado County.

	Cysts n (%)				
Livestock	1	2	3	4	5 or More
Cattle (55)	26 (47.3)	14 (25.5)	2 (3.6)	8 (14.5)	5 (9.1)
Goat (72)	51 (70.8)	10 (13.9)	6 (8.3)	3 (4.2)	2 (2.8)
Sheep (93)	64 (68.8)	17 (18.3)	5 (5.4)	1 (1.1)	6 (6.5)

n = number of cysts.

Table 3: Condition of isolated cysts from cattle, goats, and sheep in Kajiado County.

		Condition of cysts					
Livestock		Fertile	Sterile	Degenerated	Calcified	Total	Fertility rate
Cattle	Liver	7	34	14	22	77	
	Lungs	30	26	6	4	66	
	Total	*37*	*60*	*20*	*26*	*143*	*25.9%*
Goat	Liver	14	0	10	51	75	
	Lungs	7	3	21	9	40	
	Total	*21*	*3*	*31*	*60*	*115*	*18.3%*
Sheep	Liver	25	4	8	71	108	
	Lungs	41	1	5	8	55	
	Total	*66*	*5*	*13*	*79*	*163*	*40.5%*

2.3. Polymerase Chain Reaction and Restriction Fragment Length Polymorphism (PCR-RFLP). Two nested PCR assays targeting part or the entire NADH dehydrogenase subunit 1 gene (*nad1*) were used for genotyping of cyst materials. The first nested PCR (entire *nad1* gene) was performed as described by Hüttner and Nakao [17]. The cyst materials negative using the first PCR assay were genotyped using a second nested PCR as described by Mulinge and Magambo [18], which amplifies part of the *nad1* gene (545-552 bp). In both PCR assays, the reaction mixture contained 2 μl of the DNA, 1 × DreamTaq Green Buffer (20 mM Tris-HCl (pH 8.0), 1 mM DTT, 0.1 mM EDTA, 100 mM KCl, 0.5 % (v/v) Nonidet P40, 0.5 % (v/v) Tween 20) (Thermo Scientific), 0.2 mM dNTPs, 0.25 μM of forward and reverse primers, 2 mM $MgCl_2$, and 0.625 units of DreamTaq Green DNA Polymerase (Thermo Scientific) in 25 μl final volume. The PCR cycling conditions were 5 min. for initial denaturation at 94°C, 40 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 60 s, and a final extension at 72°C for 5 min. Positive PCR products were genotyped by RFLP to the specific *Echinococcus* species. To this end, 10 μl of the nested PCR products was digested using 0.5 μl (5 U) of *Hph*I restriction enzyme, 1 × Buffer, and 7.5 μl of nuclease-free water and incubated at 37°C overnight [18, 19]. Positive controls for *E. granulosus* s. s., *E. ortleppi, E. canadensis* (G6/7), and *E. felidis were* resolved alongside the test samples for both methods.

2.4. Ethical Approval. Institutional approval was granted by the Institute of Tropical Medicine and Infectious Diseases (ITROMID) at the Jomo Kenyatta University of Agriculture and Technology (JKUAT). The study protocol received ethical clearance from the Department of Veterinary Services, Kajiado County, and by KEMRI's Scientific Ethics Review Unit (SERU) (P00048/3395) and Animal Care and Use Committee.

3. Results

3.1. Prevalence, Cysts Location, Load, and Conditions. A total of 1486 livestock at slaughter were screened during the survey. The general prevalence of CE in the current study area was 14.80% (220/1486): more common in goats 15.22% (72/473) than in sheep 14.88% (93/625) and in cattle 14.18% (55/388) (Table 1). In all the infected livestock, liver and lungs were the only organs harbouring cysts, and the liver was the most infected organ ($p = 0.013$). Across infected livestock, 421 cysts were isolated: 260 (61.76%) were from the liver and 161 (38.24%) from the lungs (Table 1). Though CE infection in goat was the highest, infections with more than one cyst were higher in cattle and sheep than in goat (Table 2). Majority of cysts of cattle origin were sterile (41.9%), while most of the calcified cysts were found in sheep (48.5%) and goats (52.2%) (Tables 3 and 4). On average, sheep had the greatest number

Table 4: Condition and frequencies of *Echinococcus* spp. isolated cysts from cattle, goats, and sheep in Kajiado County.

	Condition of cysts (n (%) and *Echinococcus* spp.			
Livestock	Fertile	Sterile	Degenerated	Calcified
Cattle	37 (25.9%) (37 *E. granulosus* s. s.)	60 (41.9%) (52 *E. granulosus* s. s., 3 *E. ortleppi*, 5 NC)	20 (14%) (19 *E. granulosus s. s.*, 1 *E. canadensis* (G6/7))	26 (18.2%) (23 *E. granulosus* s. s., 1 *E. ortleppi*, 1 *E. canadensis* (G6/7),1 NC)
Goat	21 (18.3%) (21 *E. granulosus* s. s.)	3 (2.6%) (3 *E. granulosus* s. s.)	31 (26.9%) (27 *E. granulosus* s. s., 4 *E. canadensis* (G6/7))	60 (52.2%) (33 *E. granulosus* s. s., 2 *E. ortleppi*, 13 *E. canadensis* (G6/7), 12 NC)
Sheep	66 (40.5%) (65 *E. granulosus* s. s.,1 *E. canadensis* (G6/7))	5 (3.1%) (5 *E. granulosus* s. s.)	13 (7.9%) (11 *E. granulosus* s. s.,1 *E. canadensis* (G6/7), 1 NC)	79 (48.5%) (60 *E. granulosus* s. s., 1 *E. ortleppi*, 5 *E. canadensis* (G6/7), 13 NC)
Total	*124*	*68*	*64*	*165*

n = number of cysts
NC = Not characterised

Table 5: Frequency of single or mixed infections in cattle, goats, and sheep in Kajiado County.

	Livestock n (%)		
Echinococcus spp.	Cattle	Goat	Sheep
E. granulosus s. s.	51 (92.7)	50 (73.5)	79 (90.8)
E. ortleppi	1 (1.8)	2 (2.9)	1 (1.1)
E. canadensis (G6/7)	0 (0)	13 (19.1)	6 (6.9)
E. granulosus s. s./*E. ortleppi*	1 (1.8)	0 (0)	0 (0)
E. granulosus s. s./*E. canadensis* (G6/7)	0 (0)	3 (4.4)	1 (1.1)
E. ortleppi/*E. canadensis* (G6/7)	1 (1.8)	0 (0)	0 (0)
E. granulosus s. s./*E. ortleppi*/*E. canadensis* (G6/7)	1 (1.8)	0 (0)	0 (0)
Total	*55*	*68*	*87*

n = number of cattle, goats, and sheep.

of fertile cysts at 40.5%, 25.9% in cattle and 18.3% in goats (Tables 3 and 4).

3.2. Genotyping. From a total of 421 cysts that were subjected to nested PCR-RFLP, 389 cysts were successfully genotyped and included 137/143 (95.8%) from cattle, 103/115 (89.57%) from goats, and 149/163 (91.4%) from sheep. A total of 32/421 cysts failed to amplify, thus, 6, 12, and 14 from cattle, goats, and sheep, respectively, and therefore were not characterized (Table 4). Three species of *Echinococcus* were identified and included *E. granulosus* s. s. 356 (91.5%), *E. canadensis* (G6/7) 26 (6.7%), and *E. ortleppi* 7 (1.8%). *E. canadensis* (G6/7) infection was higher in goats 17 (16.5%) than in cattle 2 (1.5%) and sheep 7 (4.7%). All the fertile cysts in cattle (25.9%), 18.3% in goats, and 40.5% in sheep except one belonged to *E. granulosus* s. s taxon. The other fertile cyst from sheep was *E. canadensis* (G6/7). All the remaining cysts identified as *E. canadensis* (G6/7) (25) and *E. ortleppi* (7) were not fertile (Table 4).

In addition to single infections, this study reports several cases of mixed infections. There were three cases of mixed infections in cattle and there was one case of all three *Echinococcus* spp. (*E. granulosus* s. s., *E. ortleppi*, and *E. canadensis* (G6/7) as well as two instances of *E. granulosus* s. s. and *E. ortleppi* and *E. ortleppi* and *E. canadensis* (G6/7). In goats, there were three cases of mixed infections, all with *E. granulosus* s. s. and *E. canadensis* (G6/7), and, in sheep, only one case of *E. granulosus* s. s. and *E. canadensis* (G6/7) mixed infection was observed (Table 5).

4. Discussion

This study reports the prevalence of cystic echinococcosis (CE) and *Echinococcus* spp. in cattle, goats, and sheep in Kajiado County, Kenya. The prevalence reported is within the range known of Maasailand from older accounts, such as in the works of Macpherson [16] (8.9%, 8.1%, and 7.1% in cattle, sheep, and goat, respectively) and Addy et al. [19] (25.8 % in cattle, 16.5 % in sheep, and 10.8% in goats). It confirms the persistence of CE in nomadic society. High livestock stocking intensity, conducive environmental conditions, and movement of livestock [20, 21] may have influenced the infection pressure and the persistence of the cestode in livestock intermediate hosts in Maasailand.

The liver was the most affected organ just as was known before from Maasailand [19]. The predilection site of *E. granulosus* s. l. is not fully understood and some studies [16, 20, 22] indicated the lungs to be the most affected. Cysts in the liver or the lungs could be either fertile containing protoscoleces/daughter cysts or nonfertile. The nonfertile cysts can further be divided into calcified, degenerated, or sterile. These nonfertile cysts are noninfectious and, therefore, have no epidemiological significance in CE transmission to definitive hosts. In this study majority of the cysts from livestock were nonfertile, and a recent survey reported 80% of cysts from sheep in Turkana being calcified (Zeyhle unpublished data). This observation is not clearly understood, because regular deworming of ruminants is less likely to have a significant effect on the calcification of cysts. Previous studies have shown that long-term treatment with high doses of anthelmintic drugs is required to arrest cyst development [23, 24]. Sheep in which most fertile cysts were isolated in the present study would be more important in the transmission and maintenance of CE in Maasailand. The cysts fertility rates reported indicate the need for control measures such as health education, regular deworming of dogs, dog population control, good slaughter hygiene, and proper disposal of slaughter offal to avert transmission.

Majority of the cysts in this study were *E. granulosus* s. s. which confirms its predominance observed almost a decade ago in Maasailand [10] and in Kenya at large [11, 13, 15, 25, 26]. The high fertility rates of *E. granulosus* s. s. cysts in sheep indicate that they are important intermediate host of this taxon in this area. Sheep are also the most common home-slaughtered livestock species in Maasailand and that may enhance transmission of *E. granulosus* s. s. However, both cattle and goats may also play a role in transmission of *E. granulosus* s. s. based on the fertility rate in this study. Although goats are considered important intermediate host of *E. canadensis* (G6/7) in absence of camels, none of the cysts belonging to this taxon were fertile in this study [10, 27]. Isolation of *E. ortleppi* from all three livestock species reveals a wider host range of the parasite, aspect that is less understood. Generally, *E. ortleppi* is a rare species even in cattle who are the principal intermediate hosts, possibly due

to the fact that cattle are rarely slaughtered at home, and therefore dogs have less access to slaughter offal from cattle [10]. However, in a recent development, due to poor disposal of condemned viscera in poorly managed slaughter facilities in urban centres, dogs have readily access to slaughter offal and this might be a reason for the increased cases of *E. ortleppi* in our study [18]. Elsewhere in Brazil home slaughter of cattle is believed to be a factor that facilitates the recent rise of *E. ortleppi* prevalence in Brazil [28].

5. Conclusion

Cystic echinococcosis continues to persist in Maasailand with *E. granulosus* s. s. being the dominant species. The high fertility rate of cysts in sheep and its regular home-slaughter make it the most important intermediate host in the transmission of CE in Kajiado County of Kenya. *Echinococcus ortleppi (G5)* and *E. canadensis* (G6/7) may be important CE agents in Maasailand more than previously thought.

Data Availability

The prevalence and genotyping data used to support the findings of this study are included in the article.

Disclosure

The proceedings from this study were presented in the Kenya Veterinary Association 53rd Annual Scientific Conference and World Veterinary Day Celebrations and the abstract was published in the Conference's Book of Abstract.

Conflicts of Interest

The authors declare no conflicts of interest.

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