THE OCCURRENCE OF CYSTIC ECHINOCOCCOSIS AND MOLECULAR CHARACTERIZATION FROM LIVESTOCK IN ISIOLO, GARISSA AND WAJIR COUNTIES, KENYA

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DECLARATION

I declare that this thesis is my original work and has not been presented for a degree award in any university.

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DEDICATION

I dedicate this work to my mum whose prayers have gotten me this far and my father, a source of continuous encouragement. This work is also dedicated to my sisters: Camilla, Nureen, Gladwell and Angellah, we did it!

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ABBREVIATIONS

^{o}C	Degrees Centrigrade
CA-ELISA	Copro-Antigent Enzyme Linked Immunosorbent Assay
CDC	Center for Disease Control
CE	Cystic Echinococcosis
DALYs	Disability Adjusted Life Years
DNA	Deoxyribonucleic Acid
dNTPs	Deoxynucleotide triphosphates
ELISA	Enzyme Linked Immunosorbent Assay
Hph I	*Haemophilus parahaemolyticus* endonuclease I
IHAT	Indirect Hemagglutination Test
ILRI	International Livestock Research Institute
KEMRI	Kenya Medical Research Institute
min	Minute(s)
ml	Milliliter
NaoH	Sodium Hydroxide
nad-1	Nicotinamide Adenine Dinucleotide Dehydrogenase 1
OR	Odds Ratio
PAIR	Puncture, Aspiration, Injection and Re-aspiration
PCR	Polymerase Chain Reaction
RFLP	Restriction Fragment Length Polymorphism
s	Seconds
Spp	Species
µl	Micro-liter
w/v	Weight/Volume
Taq	*Thermus aquaticus*
WHO	World Health Organization

ABSTRACT

Cystic Echinococcosis (CE) is a zoonotic disease, spread worldwide and caused by the larvae of wild and domestic canids tapeworm, *Echinococcus granulosus*. Its prevalence in livestock has been reported in the wider African continent and in Kenya notably in the endemic regions of Maasailand and Turkana. Recent studies demonstrate its occurrence in non-endemic regions including Laikipia, Migori and Isiolo counties. CE's prevalence is high among rural pastoralist communities where socioeconomic and cultural conditions facilitate its transmission. Isiolo, Garissa and Wajir counties are regions with large numbers of pastoralist communities whose livelihood depends on livestock keeping.

The current study aimed to estimate the prevalence of CE in livestock in the north-eastern counties of Kenya including Isiolo, Garissa and Wajir and characterize the species of *E. granulosus* sensu lato present. Slaughter house surveys was used. Data collected included the total number of animals slaughtered, species, number infected and location of the cyst. The data was analyzed using Stata 15 with risk factors associated with the disease occurrence analyzed at both univariable and multivariable levels using chi-square and logistic regression. The overall prevalence rates for CE included 29.1% in camels (68/234), 14.4% in bovines (17/118), 9.9% (68/689) in caprine and 8.2% in ovine (27/329). A total of 295 cysts were recovered from the 180 infected animals including 153(51.86%) from camels, 80(27.12%) from caprine, 35(11.36%) from ovine and 27(9.15%) from bovines. Isiolo County had the highest number of recovered cysts at 61.4% (181/295), Garissa County at 20.3% (60/295) and Wajir County at 18.3% (54/295). The liver was the most frequently infected organ with 72.88%(215/295) and 26.44%(78/295) from the lungs, the heart and masseter muscle each

had one cyst. Cysts termed fertile were only recovered from the lungs of camels in Isiolo county.

There was a significant association between the county of animal origin and animal species with the occurrence of CE. Livestock in Isiolo county were more likely to be infected than those in Garissa and Wajir counties. Under similar environmental conditions, camels were more likely to be infected compared to the other livestock species.

PCR-RFLP combined with the sequencing of the nad-1 gene was carried out to characterize the genotypes of *Echinococcus granulosus* as the pathogen is associated with extensive genetic variations. Echinococcus species identified were *Echinococcus granulosus* G1 and *Echinococcus canadensis,* G6 which are both zoonotic. G6 was the most dominant species recovered in the livestock species. In the sheep, G6 was the only recovered species as most cysts analyzed from sheep were from Isiolo county where camels were seen to be actively infected with G6. Mixed infections with Taeniid species was also noted.

The findings here-in demonstrate the epidemiological situation in Isiolo, Garissa and Wajir counties which are emerging foci due to the huge slaughter volume with camels seen to play a key role in disease transmission. The G6 and G1 species were dominant in the study counties. Hence, similar studies that include molecular characterization of *Echinococcus granulosus* in livestock, humans and dogs should be carried out in the non-endemic areas to estimate the prevalence of the disease. In addition, dog population control coupled with community health education should be carried out.

CHAPTER ONE

1.0 INTRODUCTION

One prevalent zoonotic disease is Cystic echinococcosis (CE). It occurs due to the larvae of the tapeworm named *Echinococcus granulosus*. It is a public health concern as it is widespread especially in Africa. In its transmission, dogs are often the final hosts of the tapeworm *Echinococcus granulosus* whereas other domestic animals among them camels, goats, cattle and sheep serve as its intermediate hosts (Deplazes *et al*., 2017; Radfar & Iranyar 2004). Humans are accidental intermediate hosts commonly representing the culmination of the cycle. However, Magambo *et al*. (2006) in their study suggested that the tapeworm may also thrive in independent wildlife cycles.

The World Health Organization (WHO) affirms that CE has spread throughout every continent except for Antarctica. However, in developing countries, the distribution of *Echinococcus granulosus* is high. This situation is especially real in rural communities. It arises because, in these regions, dogs and other animals co-exist closely (Deplazes *et al*, 2017; Magambo *et al.*, 2006).

The asymptomatic incubation period of CE can last a long time (Jenkins *et al*. 2005). Often, one may realize its presence after an animal experiences growth of hydatid cysts which may then trigger clinical signs in the animal and often affect its primary organs which include the lungs and liver (Jenkins *et al.* 2005). In most instances, one may discover it in livestock during slaughter. Four forms of Echinococcosis exist. They include Cystic echinococcosis caused by *Echinococcus granulosus*; Alveolar echinococcosis which results from infection by the *Echinococcus multilocularis* infection; Polycystic echinococcosis which results from infection by the *Echinococcus vogeli*; and Unicystic echinococcosis which results from infection by the

Echinococcus oligarthra. However, the most relevant forms of the disease include cystic and alveolar echinococcosis (Deplazes *et al.,* 2017).

Extensive genetic variations exist within the genus of *E. granulosus*. At present, researchers have demonstrated ten distinct genotypes (G1-G10) within the *E. granulosus* through molecular analysis of nuclear genetic and mitochondrial markers (Latif *et al*., 2010). They associated them with epidemiological patterns and distinct host specificity. The genotypes, however, differ in various criteria that include host specificity, infectivity, and pathogenicity to humans, sensitivity to chemotherapeutic agents and biochemistry (Deplazes *et al.,* 2017; Romig *et al.,* 2011; Kia *et al.*, 2010). The G1, a strain prevalent in sheep, is most pervasive and often carried by cattle and sheep and is commonly found in semi-arid breeding areas. Moreover, most human infections often arise due to the G1 strain. However, reports indicate that few human infections emerge as a consequence of the camel strain. Presently in Africa, there are 5 distinct species of *Echinococcus granulosus* genotypes that have been illustrated in disease causation (Romig *et al.,* 2017; Huttner *et al*. 2009). This study not only investigated and identified the *Echinococcus* species present in Isiolo, Garissa and Wajir counties but also estimated the disease prevalence in each livestock species per County. This information is paramount for the formulation of effective intervention programs.

1.1 Study objectives

1.1.1 General objective

The general objective of the study was to estimate the prevalence of cystic echinococcosis and use molecular techniques to characterize the *Echinococcus* species present in livestock in Isiolo, Garissa and Wajir counties, Kenya.

1.1.2 Specific objectives

- To estimate the prevalence of cystic echinococcosis and factors associated with transmission among livestock in Isiolo, Garissa and Wajir counties, Kenya.
- To characterize *Echinococcus* species occurring in Isiolo, Garissa and Wajir counties, Kenya using molecular techniques.

1.2 Problem Statement

CE remains a highly neglected zoonotic disease. This situation persists even though CE is relevant to the promotion of public health especially amongst pastoral communities in developing countries (Budke *et al.*, 2006). According to the WHO reports, over one million individuals across the globe contract the disease on numerous occasions annually. If left untreated, CE's clinical symptoms are adverse or even life threatening. For instance, the 2015 WHO Foodborne Disease Burden Epidemiology Reference Group (FERG) reported that CE accounts for the death of over 19,300 individuals worldwide each year.

The disease often culminates in livestock production losses but this is dependent on the infected species involved. These often involve liver condemnation and may include a massive decrease in carcass weight, reduction in hide value and decreased milk production and fertility (Kere *et al.*, 2019; Abdulhameed *et al.*, 2018). WHO (2015), estimates that the livestock industry losses nearly $3 billion annually in treating cases and losses of livestock due to the disease.

1.3 Justification

Despite the enormous economic losses, often, there is minimal political will for CE's effective control, since it does not seriously affect the urban populations' health. CE's prevalence is high among pastoralists with minimal access to health facilities. Moreover, control programs, once instituted are difficult to sustain due to financial and logistical inputs (Deplazes *et al.*, 2017; Magambo *et al.*, 2006).

Data on CE is also very scarce since it is a disease focused mainly in remote areas. Minimal published information exists regarding the manifestation of CE in both domestic animals and human beings in regions outside the Turkana and Maasai land in Kenya. The latter indicates the need for research to focus on the distribution of CE and implementation of effective control programs. Garissa, Isiolo and Wajir counties are regions with a large number of pastoral communities. The human population together with their livestock often serves as CE's hosts. Additionally, the prevalence of untended dogs, the informal and home slaughtering of livestock and other socioeconomic and cultural conditions that facilitate CE transmission characterize Isiolo, Garissa and Wajir counties.

As such, the design of an active control program signifies the need for solid epidemiological information about the disease especially regarding aspects such as its infection level in different livestock species and the risk factors associated with its spread and distribution in a given region. Moreover, since *E. granulosus* has multiple genetically defined species that differ from each other epidemiologically and morphologically, species identification is rendered essential for effective prevention and control of cystic echinococcosis.

Given the scarcity of molecular data and other statistics on the pervasiveness of *E*. *granulosus* in Garissa, Isiolo and Wajir Counties, this study relied on molecular techniques to address the knowledge gap. Interventions to aid eradication of the disease have also been proposed.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Aetiology and Epidemiology of Echinococcosis

Echinococcosis is a cyclozoonotic disease of worldwide importance. Its causation is the metacestodes of the *Echinococcus* genus (Deplazes *et al.,* 2017). Four of the cestode species have been associated with the disease namely: *Echinococcus granulosus* that results in cystic echinococcosis, *Echinococcus multilocularis* causing alveolar echinococcosis, *Echinococcus vogeli* and *Echinococcus oligarthra* causing polycystic and unicystic echinococcosis respectively. Of these, the two most important forms of the disease are alveolar and cystic echinococcosis (Deplazes *et al.,* 2017; Romig *et al*., 2011).

Cystic echinococcosis refers to the larval cystic stage of the small taeniid-type cestode, *Echinococcus granulosus* (Jenkins *et al*., 2005). It occurs as an infection in intermediate hosts which mostly consist of herbivorous and omnivorous animals and man as an accidental host. The culmination of the disease is the occurrence of a hydatid cyst or cysts of varying sizes in the lungs, liver or other internal organs in these animals (Jenkins *et al*., 2005).

Dogs, wolves and foxes are most frequently the infected animals that form the definitive hosts. Others that are intermediate hosts include goats, camel, cattle and sheep. Deplazes *et al*., (2017) reported that the disease's significance is higher among the rural populace of developing countries where people rear domestic animals close to dogs.

The vast global distribution of these parasites occurs in relation to the animal husbandry. Majority of the lifecycles depend on livestock and domestic dogs as hosts while few regions have established secondary cycles in wildlife (Kagendo *et al.,* 2014; Jenkins & Morris, 2003).

2.2 Host range of Echinococcosis

Echinococcus granulosus parasites perpetuate in life-cycles that involve carnivores harboring the adult cestode in the intestine, as definitive hosts while omnivores and herbivores are intermediate hosts where the metacestodes develop. Metacestodes may also develop in humans, as their accidental hosts. Humans, however, have no role in the parasite's development cycle (Mandal & Mandal, 2012). In the definitive hosts, the infection is asymptomatic unlike in the intermediate hosts where hydatid cysts develop in various organs and are of significance economically and medically (Battelli, 2009). Similarly, clinical signs in the intermediate hosts are not pathognomonic and are therefore rarely diagnosed before slaughter. Sometimes, depending on cysts localization, signs seen may include; bronchopneumonia, heart failure, hepatic disorders resulting in jaundice and ascites, growth retardation and weakness. Intermediate hosts are infected upon accidental feeding on contaminated pastures or ingestion of contaminated drinking water with the infective eggs shed by the definitive hosts (Deplazes *et al*., 2017).

In humans, cystic echinococcosis begins as a slow-growing mass commonly localized in the liver or lung whereas the spleen, heart, kidney and brain are other organs rarely affected. The site location of the cyst is relevant because the case can be asymptomatic even with a large cyst or symptomatic even with tiny cysts (Mandal & Mandal, 2012). Also, symptoms arise as a consequence of the cysts' area of localization. Presence of cysts in the lungs leads to symptoms such as chest pain, coughs and shortness of breath. A cystic liver, however, may lead to the development of symptoms such as hepatomegaly, abdominal pain and swelling with an unusual abdominal tenderness (CDC, 2012). Anaphylactic shock may result from rupture of the cyst. Torgerson *et al*. (2010) reported that despite alveolar echinococcosis being less frequent in humans, it is more lethal, more pathogenic and hard to treat compared to cystic echinococcosis.

2.3 Morphology of *Echinococcus granulosus*

2.3.1 Adult worm

Primarily, the adult *Echinococcus* is but a few millimeters long (rarely exceeding 10mm) and is made up of segments (Figure 2.1). Also, it has no gut and therefore, all its metabolic interchange occurs across its syncytial external covering, called the tegument (John & Petri, 2013). Anteriorly, an adult *Echinococcus* has a specific connection organ known as the scolex. This organ is made up of four muscular suckers and two rows of hooks on the rostellum which aid in its attachment to the gut wall. The body, also called strobili, is segmented and consists of proglottids (reproductive units) in the terminal end that varies in number. Often, they range from two to six (Figure 2.1). Also, it is crucial to note that the adult worm is bisexual. It has reproductive ducts whose opening is at a common lateral genital pore. Moreover, its uterus dilates after fertilization. Thereafter, the uterus occupies a majority of the terminal segment upon the culmination of the development of the eggs (John & Petri, 2013).

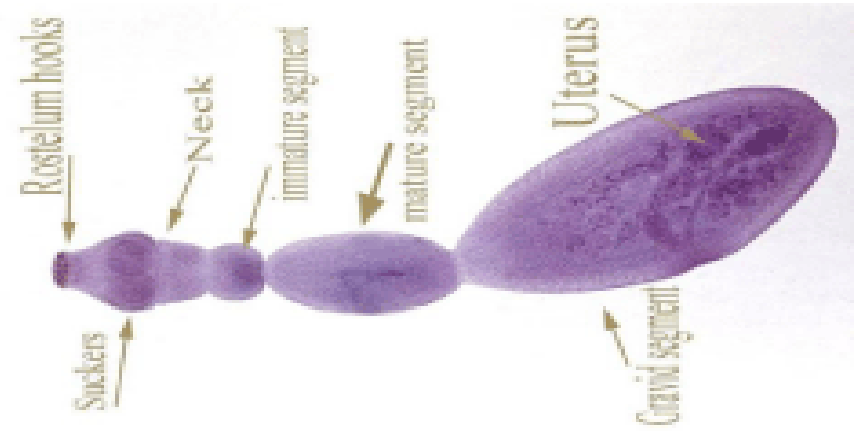

Figure 2.1: Shows an adult *Echinococcus granulosus* worm with segments. Source; https://www.researchgate.net/figure/Morphology-of-Adult-Worm-of-E-granulosus_fig2_318878214

2.3.2 Eggs

It is difficult to morphologically distinguish the eggs of *Echinococcus* species from the eggs of other tapeworms of the Taenia species. *Echinococcus granulosus* eggs are ovoid and about 30-40 micrometers in diameter. The eggs consist of a hexacanth embryo (oncosphere =first larval stage). A number of envelopes usually surround this embryo with the most prevalent being the highly resistant keratinized embryophore that results in the dark striated appearance of the egg. As soon as the eggs are released from the host, the outer capsule quickly disappears. The eggs, therefore, are sensitive to temperature extremes but can withstand freezing temperatures (John & Petri, 2013).

2.3.3 Metacestode, Hydatid Cyst

It is the second larval stage and primarily comprises of a bladder. This bladder bears an external acellular laminated layer and an inner nucleated germinal layer that usually allows for the development of brood capsules by asexual brooding (John & Petri, 2013). Protoscolices on their part stem out of the internal wall of the brood capsules. It is also crucial to note that the structure and development of the metacestodes profoundly differ amongst the four *Echinococcus* species.

Hydatid cysts are clear, colorless fluid-filled cysts of varying sizes. Often, the cysts contain highly antigenic material and the latter usually lead to anaphylactic reactions upon cyst rupture. Small ‘brood capsules’ are formed in large numbers within the cyst. The brood capsules contain protoscolices. Brood capsules normally float freely in the hydatid fluid (‘hydatid sand’) (John & Petri, 2013). The pericyst (periparasitic host tissue) surrounds the cyst. Usually, the endocyst of metacestode origin is encompassed by the pericyst (Figure 2.2). The endocyst contains an outer cellular wall. This wall is made up of concentric hyaline layers which lay on top of each other (John & Petri, 2013). The entire wall acts as a filter for micro-organisms and inflammatory host

cells while allowing the parasite access to nutritional factors. The inner layer on its part covers the inside of the cyst. It is usually made up of a single layer of viable pluripotent cells (Derbel *et al*., 2012). These cells often proliferate forming brood capsules filled with protoscolices (Figure 2.2).

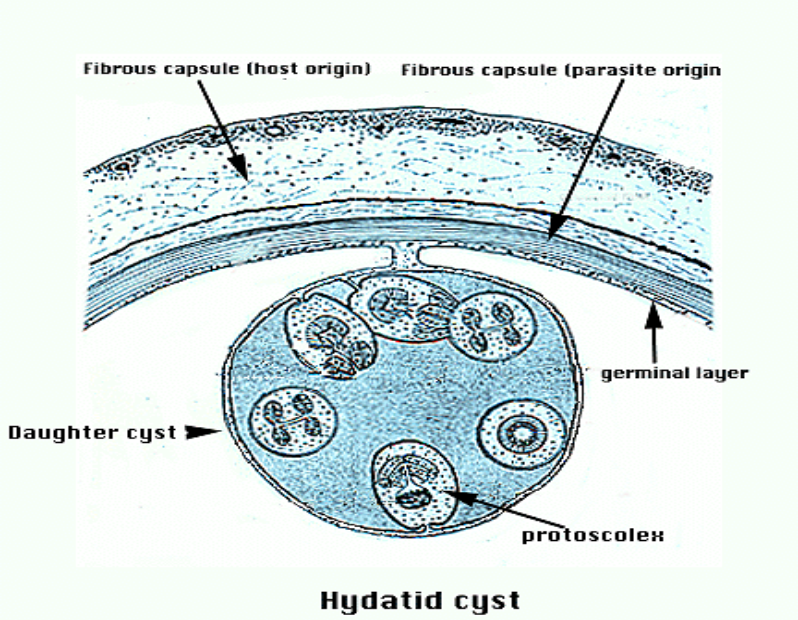

Figure 2.2 A diagram showing a cross section of hydatid cyst. Source; (Odero, 2015).

Hydatid cysts are of two main types; viable and non-viable. The non-viable cyst is made up of the pluripotent germinative layer (Thompson & McManus, 2001). However, it is often without a brood capsule and is mainly calcified. The viable cyst contains clear fluid and can be fertile which permits for its analysis through a parasitological or macroscopic diagnosis. However, it can also be 'infertile' in the event where it presents a clear fluid, but one fails to detect the presence of protoscolices. Overall, in both situations, the cysts expand slowly (Thompson & McManus, 2001).

Hydatid cysts, in the intermediate hosts, develop in different organs. The commonly affected organ is the liver with lungs being the second most affected organ (Derbel *et al*., 2012). However, any/every tissue can suitably harbor the metacestode.

2.4 Life-cycle of *Echinococcus granulosus*

The basic life cycle pattern of *E. granulosus* is considered to be either the sylvatic cycle (only wild animals) or the domestic cycle (just domestic animals). It is important to recognize that human factors have fostered the interaction between these cycles resulting in the creation of a peri-domestic cycle pattern (involves both domestic and wild animals), (Nakao *et al*., 2010).

For a life cycle to be complete, *Echinococcus* species like all cestodes, require two different vertebrate host species. Definitive hosts, exclusively of the cat and dog families, harbor the adult cestodes in the small intestine. The adult tapeworm is about 3-10 mm in length, does not invade tissue and feeds on intestinal contents (Nakao *et al*., 2010). This therefore makes the infection asymptomatic even with several thousand worms present. In the small intestine, the adult tapeworm produces gravid segments that release infective eggs passed in feces into the environment. *Echinococcus* eggs which pass with fecal matter into the environment do not directly infect the definitive hosts. The eggs need an intermediate larval (metacestode) stage to manifest in their various host species. Many herbivores and omnivores, both domestic and wild and including human beings are vulnerable to becoming infected with the *Echinococcus* species' metacestode. Infection often occurs when they accidentally ingest eggs that contaminate pasture, water or human food.

Upon ingestion, the eggs hatch in the small intestines of the intermediate host (CDC, 2012) (Figure 2.3). Thereafter, they release an oncosphere that actively penetrates into the walls of the

host's intestines and travel through the hepatic portal system to the liver. The metacestode then forms a hydatid cyst in the organ and grows for months or years. Eventually, through non-sexual reproduction, brood capsules which contain protoscolices form within the metacestode.

The definitive host feeds on the protoscolices-containing metacestode and completes the parasite's development through feeding on infected organs and offals of intermediate hosts (CDC, 2012). Once in the small intestine, the protoscolices evaginate and affix to the mucosa. This allows them to grow into an adult tapeworm in about two months.

Humans are epidemiologically unsuitable hosts that develop the disease although the resulting metacestode is unable to produce protoscolices. Typically, humans are the dead-end hosts (Mandal & Mandal, 2012). (Figure 2.3).

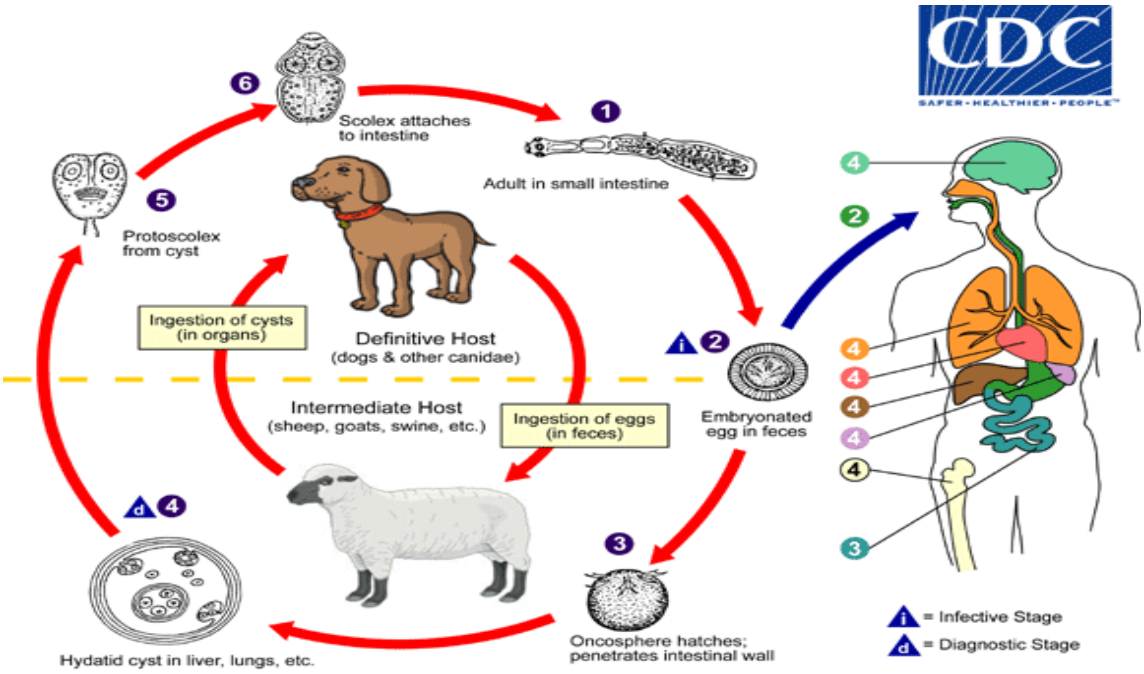

Figure 2.3 The life cycle of *Echinococcus granulosus* (CDC, 2012). Source: https://www.cdc.gov/parasites/echinococcosis/biology.html

2.5 Transmission of *Echinococcus granulosus*

Ferreira and Irabedra, (2007) established that illiteracy, lack of access to portable water and inadequate sanitation increase the risk of transmission of Echinococcosis. Transmission to humans can either be direct when they come into close contact with an infected dog whose fur contains *Echinococcus* eggs or indirectly when they ingest contaminated food or water. The eggs can also be carried mechanically in the wind and subsequently inhaled by man resulting in cyst development in the lungs (Morar & Feldman, 2003). Many link the prevalence in the rates of cystic echinococcosis in humans to the upsurge of the disease amongst livestock. The infection pressure to humans is also primarily determined by the genotype of the parasite and the hygiene conditions (Mandel & Mandel, 2012).

The intermediate hosts (herbivores and omnivores) acquire infection through ingestion of contaminated pastures while grazing. The definitive hosts acquire infection through feeding on contaminated offals and viscera common in pastoral communities that practice backyard slaughtering of animals. In endemic areas, as found out by Ferreira and Irabedra (2007) and Craig *et al*., (2007), hunting dogs are commonly provided with raw infected viscera. The frequency of CE throughout Africa, the Middle East, the Mediterranean, and Asia is linked to the livestock production system and affected by climate. Areas that receive a right amount of rainfall base their agriculture activities on the production of plants and rear animals in production systems that foster the utilization of well-maintained slaughtering facilities. In pastoral communities, the regions are usually arid and semi-arid with an economy largely dependent on extensive livestock production, often involving nomadism and transhumance. Such areas experience the most severe CE transmissions (Deplazes *et al*., 2017; Magambo *et al*., 2006).

Depending on the prevailing environmental temperatures, the eggs can last in the environment for several days. Many eggs, however, die naturally due to desiccation and temperature extremes.

2.6 Subspecies of *Echinococcus granulosus*

Previous studies have reported extensive genetic and phenotypic variation among isolates of *E. granulosus* drawn from various intermediate host species. Phenotypically, differences include; shape and size of rostellar hooks, number of segments and the number of testes. These studies also pointed out distinct genotypes (G1-G10) within *E. granulosus* through molecular investigations based on nuclear genetic and mitochondrial markers. The species include *E. granulosus sensu stricto* G1-3, sheep/buffalo strains; *E. equinus* G4, horse strain; *E. ortleppi* G5, cattle strain; *E. canadensis* G6-7, camel-pig strain G8/10 cervid strains and *E. felidis* the lion strain. The genotypes also differ in various criteria including; pathogenicity, host specificity, infectivity to man, biochemistry and responsiveness to chemotherapeutic agents (Kia *et al*., 2010). Several techniques have been used to establish variation within *E. granulosus*. To characterize strain grouping within *E. granulosus*, PCR based methods have been used (Deplazes *et al*., 2017). This led to the realization of the prominence of the sheep strain prevalent in semiarid breeding areas as transmitted by sheep and cattle.

Since the species differ epidemiologically and morphologically from each other, strain identification is considered paramount to aid in controlling and preventing the upsurge of cystic echinococcosis (Thompson & McManus, 2001). Several molecular studies undertaken in the African continent have shown the occurrence of distinct species of *Echinococcus granulosus* genotypes that have been identified in disease causation and include: *Echinococcus granulosus sensu stricto* (G1/3), *Echinococcus equinus* (G4), *Echinococcus ortleppi* (G5), *Echinococcus*

canadensis (G6-8, G10) and *Echinococcus felidis* (lion strain, a sister taxon of *E. granulosus sensu stricto*) (Romig *et al*., 2017). Wassermann *et al*., (2016), propose that genotype *G Omo,* closely related to *E. granulosus* s.s, be designated as independent species. However, the *Echinococcus granulosus sensu stricto* remains the most dominant strain in Africa.

The *E. granulosus sensu stricto* G1, has a near worldwide distribution in areas that practice extensive sheep farming. Such regions include, northern and eastern Africa, southern South America, Australia and Asia. Its occurrence, however, is dominant in areas which record the high prevalence of CE in humans (e.g. Kenya, Morocco, western China and Tunisia). The main intermediate hosts are sheep but various livestock species and wild herbivores are also infected. Moreover, the genetic analysis of isolates also points to the fact that G1 remains predominantly high amongst humans (Mandel & Mandel, 2012; Thompson & McManus, 2001).

E. equinus makes use of equines predominantly as intermediate hosts and occurs in Europe, South Africa, and the Middle East. At present, no human cases have been recorded. Also, epidemiological evidence points out the fact that it may be non-pathogenic to the human species (Mandel & Mandel, 2012).

E. ortleppi is predominantly associated with transmission by cattle. In western and southern Kenya, it has been described in pigs and cattle respectively (Odero, 2015: Addy et al., 2012). It has recently been described as transmitted by goats (Mbaya *et al*., 2014). It is prevalent in Europe, several African regions and South America. It is mildly pathogenic to humans compared to the G1 strain (Mandel & Mandel, 2012).

The camel species, cervid species and pig species are poorly differentiated from one another even though they are markedly different from the sheep species. The camel species chiefly

affects camels and goats. Reports have been made in Africa, the Middle East, South America and southern Asia (Romig *et al*., 2017). However, countries such as Kenya, Iran, and Mauritania continue to witness sporadic cases of human infection (Mutwiri *et al*., 2013; Dinkel *et al*., 2001).

2.7 Prevalence of Cystic Echinococcosis

Echinococcus granulosus is widely distributed globally in all continents except Antarctica. However, the parasite's prevalence is high in regions of Europe, Africa, Asia and South America (Figure 2.4). In endemic areas, the parasite's prevalence differs significantly ranging between sporadic and high. Very few countries including Iceland and Ireland are considered as free of *E. granulosus*. Cystic echinococcosis is unevenly distributed geographically (Deplazes *et al*., 2017). For example, the United States, western and central Europe, few livestock cases are reported while most human cases are documented. In most regions worldwide, however, CE is considered an emerging zoonosis. For instance, in Eastern Europe, cases of the disease have been observed to be on the rise (Deplazes *et al*., 2017).

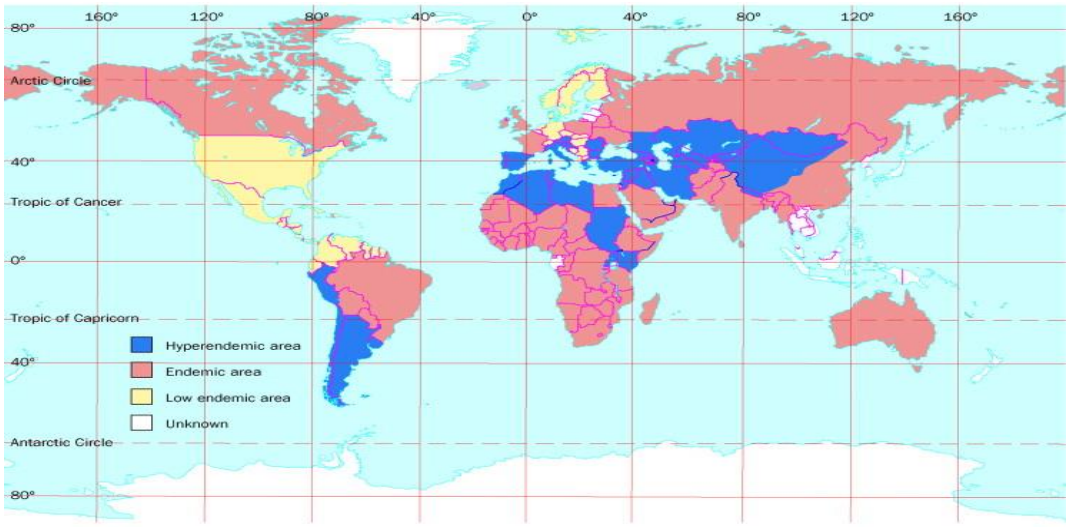

Figure 2.4 A map showing the distribution of cystic echinococcosis worldwide.

In Sub-Saharan Africa, CE is endemic. Deplazes *et al.,* (2017) and Magambo *et al.,* (2006) reported that CE is common in people especially amongst nomadic pastoralists in West African and North African countries. In East Africa, variation in the distribution of CE in livestock also exists. For instance, in Tanzania, a prevalence of 16.6% and 22.2% was reported in sheep and goats respectively by Miran *et al.*, (2017). Oba *et al.*, (2016) reported a 12.2% CE prevalence in slaughtered animals in Uganda.

Kenya remains a high CE endemic country worldwide with prevalence being high in Maasailand where pastoralists are dominant. In Turkana country, prevalence of the disease was high in the 1980s but has steadily been declining due to the intervention programs by AMREF to the 3.8% recorded by Solomon *et al.* (2017). Its prevalence in sheep, goats, cattle and camels has been reported in both endemic (Nungari *et al.*, 2019; Odongo *et al.*, 2018; Addy *et al.*, 2012) and non-endemic regions (Kere e*t al.,* 2019; Gachengo *et al*., 2017; Mbaya *et al.,* 2014). The epidemiology of CE in humans in Kenya is poorly understood despite the high incidence rates that continue to be recorded (Mutwiri *et al*., 2013). Prior reports had recorded high prevalence in middle aged humans with a higher prevalence in females (Odero, 2015). However, Solomon *et al*. (2017) observed a shift in prevalence to older people in addition to a prevalence reduction in females. The patterns of occurrence in the human population in other regions of Kenya are not well recorded.

Among domestic animals, camels demonstrate the most abundance and highest infection intensity with cysts. The predilection sites reported in livestock include the liver, lung, kidney and spleen while in humans apart from the lungs and liver other organs include the bladder, heart, spine and eye orbit (Eshrat *et al*., 2019). Four Echinococcus species have been reported and they include: *E. granulosus* s.s. that has been shown to occur in humans, livestock (sheep,

goat, cattle and camels) and wild herbivores in various conservancies within the country (Gachengo *et al*., 2017: Addy *et al*., 2012). *E. canadensis* has been reported in goats, camels and humans (Nungari *et al.*, 2019: Mbaya *et al*., 2014: Mutwiri *et al*., 2013: Addy *et al*., 2012). Concerning *E.ortleppi* it has been shown to occur in cattle, goats, sheep and irregularly in pigs in Western Kenya (Nungari *et al*., 2019: Mbaya *et al.*, 2014: Odero, 2015: Addy *et al.*, 2012) while *E. felidis* has been reported in wild carnivores from conservation areas through the examination of fecal material (Kagendo *et al*., 2014).

The main definitive host for CE in Kenya is the domestic dog (Odero, 2015) with wild carnivores such as the lion, hyena and jackal as the definitive hosts in the sylvatic cycle (Kagendo *et al.*, 2014). The intermediate hosts are herbivores, omnivores ungulates and humans. Risk factors for CE transmission include lack of awareness about the disease, unsanitary conditions in abattoirs, poor offal disposal, close contact with dogs and lack of appropriate dog deworming practices (Eshrat *et al.*, 2019).

2.8 Diagnosis of *Echinococcus granulosus*

2.8.1 Diagnosis in Definitive Hosts

It is often difficult to diagnose an infection with *Echinococcus* species in definitive hosts. This situation arises because it is impossible to morphologically distinguish between the eggs of all Taenia and *Echinococcus* species. The two major methods of diagnosis used in dogs are necropsy of the small intestine and purgation with arecoline hydrobromide or arecoline acetarsol (Lahmar *et al*., 2007). The gold standard, at necropsy, is the sedimentation and counting technique (Conraths & Deplazes, 2015). Purgation is costly, laborious and with low sensitivity - approximately 43% following one arecoline dose administration (Lahmar *et al.*, 2007). This resulted in the development of Coproantigen detection by enzyme-linked immunosorbent assay

(CA-ELISA) as an alternative. CA-ELISAs are quick tests. They are important for large epidemiological screenings (Deplazes *et al*., 2017). Moreover, the coproantigens are stable. As such, these tests are also applicable to field fecal samples.

2.8.2 Diagnosis of Cystic Echinococcosis in Intermediate Hosts

This concept is largely based on the findings at necropsy at meat inspection in abattoirs. This is because frequently, the clinical symptoms may be overlooked. The examination of herbivores and omnivores for *E. granulosus* cysts is paramount for baseline surveys and surveillance of control programs (Guisantes, 2014). The predilection sites are often the liver and lungs. The age-dependent prevalence becomes the most important information needed in such cases for establishing the epidemiological status of *E. granulosus* (Guisantes, 2014).

Alternatively, one may use an ultrasound examination in the diagnosis of cystic structures especially amongst small animals like sheep and goats and also horses. Sonography best detects hepatic but not lung cysts. They have poor sensitivity. In Kenya, studies by Zhang *et al*., (2012) demonstrated that ultrasounds sensitivity is approximately 54% for the detection of CE in goats and sheep. It is however highly specific at 94%.

Immunodiagnosis has had challenges of specificity and sensitivity. ELISAs, IHAT and Double Diffusion tests have had minimal success (Gatti *et al*., 2007). Cross-reactivity with other taeniids is often the implicated cause of poor specificity. Various serological tests appropriate for detection of cystic echinococcosis has been availed but they have low sensitivity and high specificity (Zhang *et al*., 2012). However, it is possible to identify *E. granulosus* exposure at the level of the herd. This situation would necessitate the use of mean values drawn from serum antibody activity through the use of hydatid cyst fluid antigens in ELISA (Zhang *et al*., 2012). It

is useful in programs tailored towards screening and surveillance. In 4 weeks' post-infection, it is possible to detect reactive antibodies to the hydatid cyst fluid in the serum of experimentally infected sheep.

There are a number of DNA techniques described that enable for distinction between the various species of *Echinococcus* and between the *E. granulosus* strains. These mechanisms require for the utilization of metacestode material from intermediate hosts, e.g., PCR restriction fragment length polymorphism and multiplex PCR through the use of protoscolices or cystic structures.

2.8.3 Diagnosis in Humans

For humans, highly specific tests should be used. Multiple tests (ultrasound and serology) are recommended and accurate result interpretation is paramount. Ultrasound use is widely employed for the confirmation of an abdominal echinococcosis diagnosis. These tests also indicate whether the lesions are viable (Macpherson *et al*., 2003). Their greatest drawback is that they fail to detect pulmonary echinococcosis.

Advanced imaging techniques including computed tomography and nuclear magnetic resonance imaging help confirm CE diagnosis in man (Apaydin, 2019). They have high sensitivity and are able to detect the size, location and number of cysts including cyst wall degeneration.

Serology is an alternative line tool for CE diagnosis in humans (Pawlowski *et al*., 2001). Assays available for antibody detection include ELISA, immunoelectrophoresis, and immunoblots (Ortona *et al*., 2000). These tests have low sensitivity hence specific antibodies may fail to be detected in most patients. The viable metacestode masked by the hyaline layer may be sequestered from the immune response of the host (Vuitton *et al*., 2006). It may also have already evolved other means of evading the immune response.

The PCR is a molecular-based technique for establishing *Echinococcus granulosus* genotypes. It is carried out on DNA originating from the protoscolices, larval tissues or eggs refrigerated, frozen or preserved in 70% ethanol. PCR can either solely demonstrate cestodes' DNA in the sample or differentiate the genotypes present. PCR distinguishes G1/G2/G3 from G5 and G6/G7 (Deplazes *et al*., 2017)).

2.9 Treatment of Echinococcosis

2.9.1 Treatment in the Definitive Hosts

Echinococcus granulosus in dogs is treated with praziquantel administered either orally or parenterally. In addition, Jiang *et al.*, (2017), recommended the administration of praziquantel as an in-situ slow release formulation by injecting dogs subcutaneously. Here, the formulation not only eliminates both juvenile and adult echinococci but it also enables dogs to resist new echinococcus infection for about 6 months.

2.9.2 Treatment in the Intermediate Hosts

In livestock, three treatment modalities that include chemotherapy, ultrasound-guided aspiration and surgery are recommended (Gavidia *et al*., 2010; Blanton *et al*., 1998). Chemotherapy with benzimidazoles such as mebendazole and albendazole is best suited for circumstances where the cysts are inoperable or too many. Moreover, chemotherapy is used in combination with surgery to avoid the anaphylactic reactions that may occur when contents of the cysts spill over. Gavidia *et al*., (2010) in a trial carried out in naturally infected sheep established that high doses of Oxfendazole, a combination of Oxfendazole and Praziquantel or a combination of Albendazole and Praziquantel are successful schemes in control of CE in the specie. This is because Albendazole inhibits cyst growth while Praziquantel paralyzes the worm.

Surgery is the most effective treatment for cystic echinococcosis combined with chemotherapy using albendazole before and after surgery in humans. However, cyst puncture, chemotherapy and PAIR (percutaneous aspiration, injection of chemicals and re-aspiration) are also effective treatments in cases where cysts are in multiple organs or locations (Kern *et al*.; 2017). Park *et al*., (2009) hints at many studies which suggest that PAIR coupled with chemotherapy proves efficacious compared to surgery regarding morbidity, mortality and disease recurrence. In regards to chemotherapy, however, albendazole is recommended twice daily for 1-5 months or mebendazole for 3-6 months (Kern *et al*., 2017).

2.9.1 Prevention and control of Echinococcosis

Prevention and control strategies that usually target the major risk factors associated with the disease and its transmission cycle may include: Health education programs that primarily focus on cystic echinococcosis coupled with enhanced water and personal hygiene. The reduction of stray dog populations, regular and appropriate deworming of dogs and proper disposal of carcasses and offal from slaughterhouses to avoid dog access will help curb the disease spread. In addition, it is recommended that ovine be vaccinated with the EG95 vaccine which enhances the effectiveness of control measures (Larrieu *et al*., 2019). Home slaughter of livestock without proper meat inspection should also be discouraged. However, following home slaughter, boiling of cyst-containing lungs and livers can be done for half an hour. This kills infectious larvae (Li et al., 2014).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area

The study was undertaken in three counties in Kenya including Isiolo, Garissa and Wajir. Wajir county is located in the north eastern region of Kenya. Wajir town is its capital and largest town. It expands over an area of 55,840.6 km^2 with a populace of 661,941 with an anticipated populace of about 800,000 individuals in 2017. It lies between latitudes 1.7471 North and 40.0573^o East. The rise of Wajir above sea level is 258m. The county borders the following counties; to the north and north east is Mandera, to the east is the republic of Somalia, Isiolo and Marsabit to the west, Garissa to the south and south west. Also, it borders the republic of Ethiopia to the northwest. In the previously conducted livestock census for the Northern Kenya region of 2009, Wajir county was estimated to have more than 15.3 million animals. Wajir county is characterized by ample sunshine all through the year, with a large land area lacking vegetation. The mean annual temperature is 28^{o}C, the amounts of rainfall range between 250mm and 700mm yearly in various regions of the county. The poverty level is at a sky rocketing 84%. The region is dominated by pastoralists and the main economic activity is livestock keeping and trading.

Garissa county is also situated in the north eastern area. Garissa town is its capital. Garissa county neighbors the republic of Somalia to the East, Lamu county to the South, Isiolo county to the north-west, Tana River county is to its West and to the North is Wajir county. It lies between latitudes 0.1112° North and 40.3142° East, with an elevation of 1,138 meters above sea level. The populace is about 923,060 people with a land mass stretching to about 45,720.2 km^2. The

poverty rate is at 49%. The Garissa climate is termed “desert” with an average temperature of 29.3^{o}C.

Isiolo county has an area of 25, 336 km^{2} with a populace of 143,294 individuals. It lies between latitudes 1.0475° North and 38.5879° East. The county borders seven other counties: Wajir to the north-east, to the East are Samburu and Garissa counties, Tana River to the south-east, Meru and Kitui to the south-west, Marsabit in the north-west region. Most regions are dry and receive less than 150 mm of rainfall annually while temperatures range between 12^{o}C and 28^{o}C. On average, Isiolo county’s elevation above sea level is 200-300 meters however in other areas the altitude can be up to 1000 meters (KNBS, 2009).

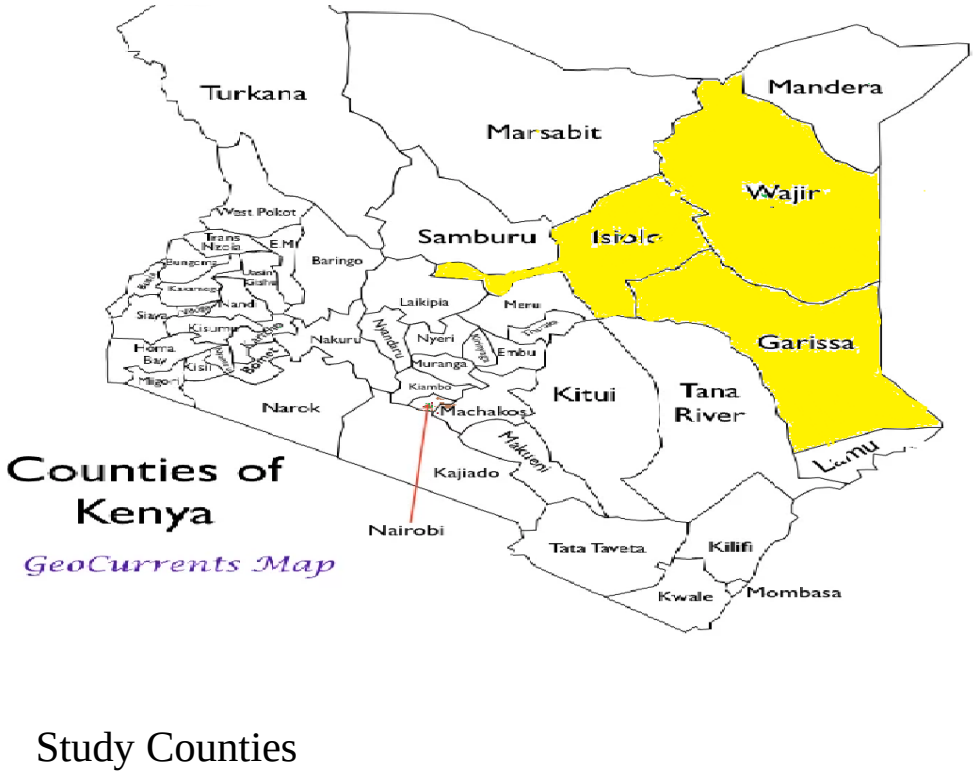

Figure 3.1: Map of Kenya showing the selected counties to be investigated.

3.2 Study design

The study comprised abattoir (slaughter house) surveys for hydatid cyst identification in the infected carcasses or organs and was undertaken in Isiolo, Garissa and Wajir counties. This study was carried out between October 2018 and November 2018 during which visitations to the abattoirs were made.

3.3 Sampling and sample size determination

Sample sizes for various livestock species were determined as per the formula in Dohoo et al., (2009). Studies have been done on cystic echinococcosis in Kenya with prevalence established in various regions (Kere *et al*., 2019; Nungari *et al*., 2019; Gachengo *et al*., 2017; Mbaya *et al*., 2014; Addy *et al*., 2012; Njoroge *et al*., 2002; Gathura & Kamiya, 1990). The expected prevalence was set at 30%, 15% and 13% for cattle, goats and sheep respectively according to Gathura & Kamiya (1990) in a study carried out in Turkana.

Cattle sample size:

$$n = Z^2_{\alpha} pq/L^2$$

Where:

n- Requisite sample size.

p- Estimated prevalence.

q = (1-p)

L = precision of the estimate (0.05)

Z = 1.96 (normal deviate)

Sample size = $1.96^2 * 0.3 * 0.7 / 0.05^2 = 322.69 \sim 324$ cattle.

Sheep sample size:

Expected prevalence used 13%

$n = 1.96^2 * 0.13 * 0.87 / 0.05^2$

173.79 ~ 176 sheep.

Goats sample size

Expected prevalence used 15%

$n = 1.96^2 * 0.15 * 0.85 / 0.05^2 = 195.9 \sim 196$ goats.

Camel sample size:

Determining the sample size for camels, the prevalence estimate from the study by Njoroge *et al*., (2002) in Turkana was used.

Expected prevalence used was 61.4% as per Njoroge *et al*., (2002).

$n = 1.96^2 * 0.614 * 0.386 / 0.05^2 = 364.19 \sim 364$ camels.

3.4 Data collection

Data on the animal species and presence of hydatid cysts was collected during the routine meat inspection at the abattoirs and infected organ/ carcass were also recorded. This was through palpation and later organ incision. The main abattoir per study region was visited for a week during the study period and information including total number of slaughtered animals, species, number infected with hydatid cysts and location of the cysts was recorded.

3.5 Identification of hydatid cysts

Hydatid cysts were identified macroscopically/visually in slaughtered animals. They are generally ovoid in shape with a varying diameter of about 2.5mm-10cm. The cysts were identified in organs inside the body, especially the lungs and livers. The hydatid cysts were

excised carefully using knives and after collection, carefully labeled and then stored in 75% ethanol in plastic containers while awaiting transportation to the laboratory for additional processing and diagnosis.

At the laboratory, the contents of all cysts were evaluated microscopically at 40x magnification for protoscolices presence. Fertile cysts were those that contained protoscolices while non-fertile cysts included the cysts that lacked protoscolices and the calcified cysts.

3.6 DNA extraction

The extraction of DNA was undertaken on the samples. Under a dissecting microscope, protoscolices or cyst wall tissue material were picked using a pipette and transferred into 0.2µl PCR tubes that contained 10µl of 0.02M NaOH. Lysis was at 99^{o}C for 10 minutes in the Applied Biosystems Gene Amp PCR System 9700 Thermal Cycler. These lysates were used as template for the primary PCR reaction.

In some instances, and especially with the calcified cysts where the afore mentioned method failed to give adequate DNA, DNA extraction was by the use of the Qiagen DNeasy Blood and Tissue Kit Protocol whereby approximately, 0.5g of the germinal layer was minced into fine pieces and 180µl Buffer ATL followed by 20µl proteinase K were added. Mixing was through vortexing and incubation was at 56 ^{o}C for 3 hours with occasional vortexing during incubation. Post incubation, 200µl Buffer AL was added and another incubation of 10 mins followed. 200µl of 96% ethanol was then added and mixed and the mixture was pipetted into a DNeasy mini spin column placed in a 2ml collecting tube and centrifuged at maximum speed for 1 min. The wash buffers were then respectively added with centrifugation being done at maximum speed for 1 min in between their additions. DNA elution was by adding 50µl Buffer AE into the membrane

of the spin column. This was contrary to the indicated 200µl Buffer AE but served to increase DNA yield. The DNA served as template for the PCR reaction.

3.7 Polymerase Chain Reaction of nad-1

A nested PCR was undertaken to amplify the NADH dehydrogenase subunit 1(nad-1). In both the primary and secondary reactions, the reaction mixture was a total volume of 25µl and was made up of 18.625µl distilled water, 2.5µl of 10X PCR buffer, 0.5µl of the 10mM dNTPs, 0.625µl of each 10µM primer, 0.625units of DreamTaq Green DNA Polymerase (Thermofisher) and 2µl of DNA template. The conditions for amplification included prior denaturation at 95^{o}C for 5 min, subsequently, denaturation at 94^{o}C lasting 30s. The forward and reverse primers were annealed at 55^{o}C for 30s; initial elongation was at 72^{o}C for 1min followed by elongation 72^{o}C lasting 5min post cycling. This was done using the Applied Biosystems Gene Amp PCR System Thermocycler and repeated for 40 cycles.

Table 3.1 Primer pairs for the PCR assay

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

Source: Huttner et al. (2008). **F**: Forward primer; **R**: Reverse primer.

Both reactions included a negative control to elucidate contaminations if present and a positive control sample of *Echinococcus granulosus* G1, *E. ortleppi,* G5 and *E. canadensis,* G6. The results of the amplification were then resolved on 2 % (w/v) agarose gel and visualized on UV transilluminator after staining with ethidium bromide.

Restriction Fragment Length Polymorphism of nad-1

The nad-1 amplicons were digested as earlier reported by Huttner *et al*., (2009). This employed the use of the restriction enzyme, HphI. The total reaction mixture was 20µl and was made up of 7.5µl distilled water, 2.0µl 10X buffer, 0.5µl HphI and 10µl PCR product. The reaction mixture was incubated at 37^{o}C overnight and then separated on 3% agarose gel. From the samples, the *Echinococcus* species were detected by contrasting their banding patterns to those of already established patterns. The reference pattern of bands used was; *E. granulosus* G1(485, 320, 204, 64 base pairs), *E. ortleppi* G5 (867, 107, 102 base pairs), *E. canadensis* G6 (442, 425, 107, 102 base pairs) and *E. equinus* (485, 380, 102, 64 base pairs) according to Huttner *et al*., (2009).

Sequencing of DNA

For samples whose banding patterns were not clear-cut and thus could not be categorized by the aforementioned method, DNA sequencing was carried out on the nad-1 gene. The secondary PCR product was first purified in accordance with the QIAquick PCR purification kit manual whereby, to 1 volume of the PCR reaction mixture, 5 volumes of Buffer PB were added and the mixture then placed in a QIAquick column placed in a 2ml collection tube. Centrifugation was at maximum speed for 1 min. The sample was then washed using 0.75ml Buffer PE and centrifuged at maximum speed for 1 min. The spin column was transferred into a clean collection tube and DNA elution done using 30µl Buffer EB and centrifuged for 1 min. The purified samples were carefully labelled and sent to Inqaba Biotec laboratory in South Africa for analysis.

3.8 Data handling and analysis

Data were first entered into MS Excel (Microsoft Inc., Sacramento, California, USA) and then imported to Stata 15 (StataCorp LLC, College station, Texas, USA) for analysis. The data were first checked for accuracy, coded and analysed using descriptive statistics. Proportions were determined for categorical variables.

Univariable analysis using simple logistic regression was performed to determine unconditional associations with the presence of CE infection. All the predictors in the dataset were considered and these included. Univariable associations with $p \leq 0.25$ were eligible for multivariable logistic regression analysis.

Candidate variables were also checked for collinearity by means of variance-covariance matrix of the estimators (VCE). Multicollinearity was considered to be present if $VCE > 5$ for any pair (Dohoo *et al*., 2009).

Multivariable logistic regression was performed to determine factors associated with the presence of CE infection, while controlling for possible confounding among model variables. The final model was built using backward stepwise elimination leaving those variables which had a p-value ≤ 0.05 and exploring the presence of interaction.

CHAPTER FOUR

4.0 RESULTS

A total of 1,368 carcasses were inspected during the study period (October – November 2018) for the occurrence of cysts in both the pleural and peritoneal cavities in Isiolo, Wajir and Garissa counties. This included; 687 goats, 329 sheep, 234 camels and 118 cattle (Table 4.1). In Isiolo county, there were 255 goats, 59 sheep, 62 camels and 93 cattle inspected. In Garissa county the number inspected included 310 goats, 222 sheep, 125 camels and 21 cattle. Wajir county had the lowest number of inspected animals including 122 goats, 48 sheep, 47 camels and 4 cattle (Table 4.2).

4.1 Proportions and distribution of Echinococcus cysts

The total number of animals with hydatid cysts was 180, including 68 caprine, 27 ovine, 68 camels and 17 bovines (Table 4.1). This translated to a proportion of 29.1% in camels (68/234), 14.4% in bovines (17/118), 9.9% in caprine (68/687) and 8.2% in ovine (27/329) (Table 4.1).

Table 4.1: Proportions of carcasses with Cystic Echinococcosis in Livestock in Isiolo, Garissa and Wajir counties in October-November 2018

Livestock Species	Number Inspected	Number Infected	Proportion Infected (%)
Caprine	687	68	9.9
Camel	234	68	29.1
Ovine	329	27	8.2
Bovine	118	17	14.4

In Isiolo county the proportions were 58% in camels (36/62), 15.3% in caprine (39/255), 16.9% in ovine (10/59) and 17.2% in bovines (16/93) (Table 4.2). Garissa county recorded a species proportion of 10.4% in camels (13/125), 8.4% in caprine (26/310), 3.6% in ovine (8/222) and 4.7% in bovines (1/21) (Table 4.2). In Wajir county, the prevalence rates were as follows 40.4% in camels (19/47), 2.5% in caprine (3/122), 18.8% in ovine (9/48) and 0% in bovines (0/4) (Table 4.2).

Table 4.2: Proportion of Cystic Echinococcosis in Livestock per county in October-November 2018

County	Livestock Species	Number Inspected	Number Infected	Proportion Infected (%)
Isiolo	Caprine	255	39	15.3
	Camel	62	36	58.0
	Ovine	59	10	16.9
	Bovine	93	16	17.2
Garissa	Caprine	310	26	8.4
	Camel	125	13	10.4
	Ovine	222	8	3.6
	Bovine	21	1	4.7
Wajir	Caprine	122	3	2.5
	Camel	47	19	40.4
	Ovine	48	9	18.8
	Bovine	4	0	0

4.2: Number of Cysts, Abundance and Mean Infection Intensity

The total number of cysts collected was 295. Isiolo county had the highest number of recovered cysts at 61.4% (181/295), Garissa county at 20.3% (60/295) and Wajir county at 18.3% (54/295) (Figure 4.1).

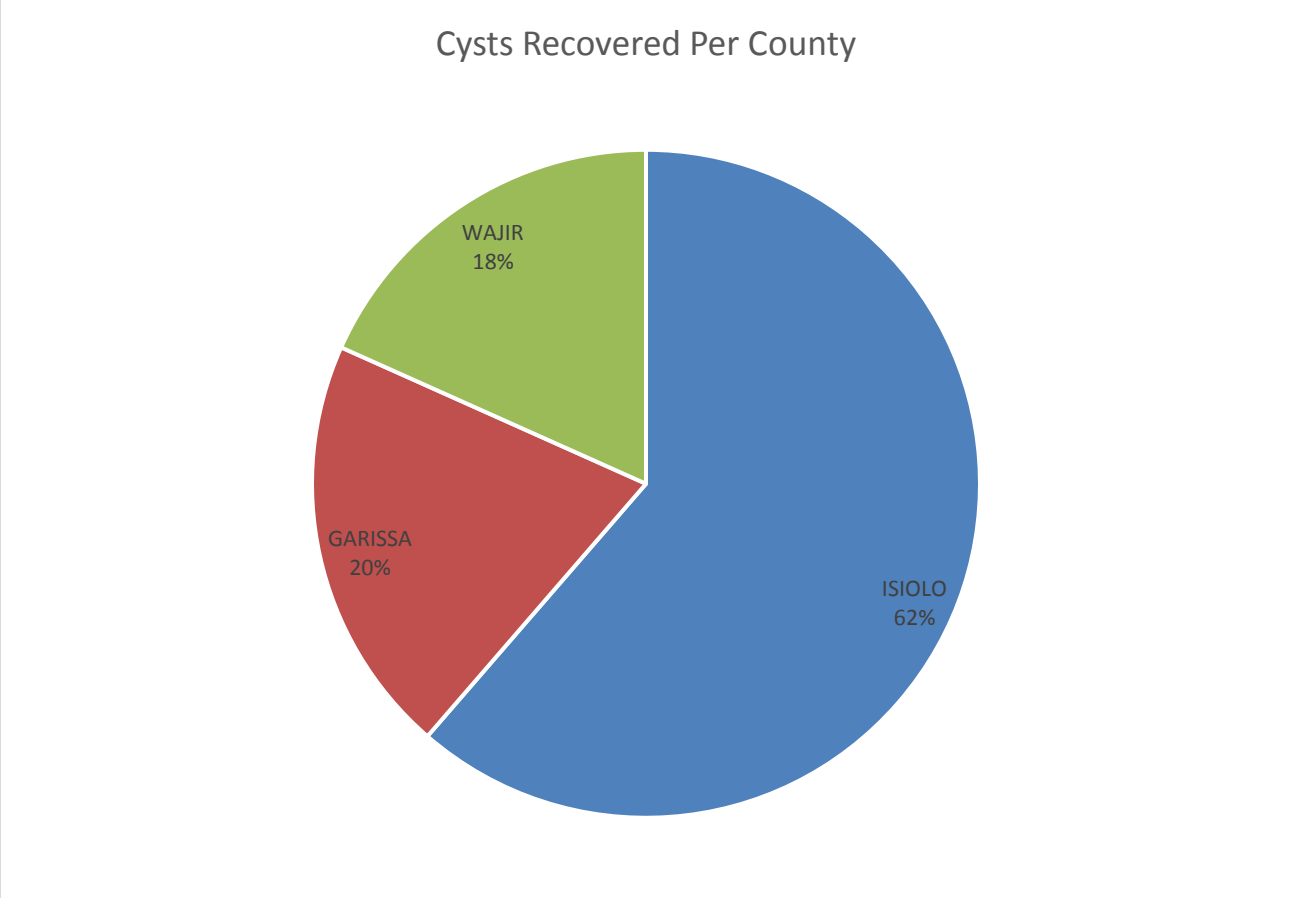

Figure 4.1: Number of Cysts per County in Isiolo, Garissa and Wajir in October-November 2018

Among the infected livestock species, camels had the highest number of recovered cysts at 51.7% (153/295) followed by goats 27.1% (80/295), sheep 11.7% (35/295) and cattle 9.1% (27/295) respectively (Figure 4.2).

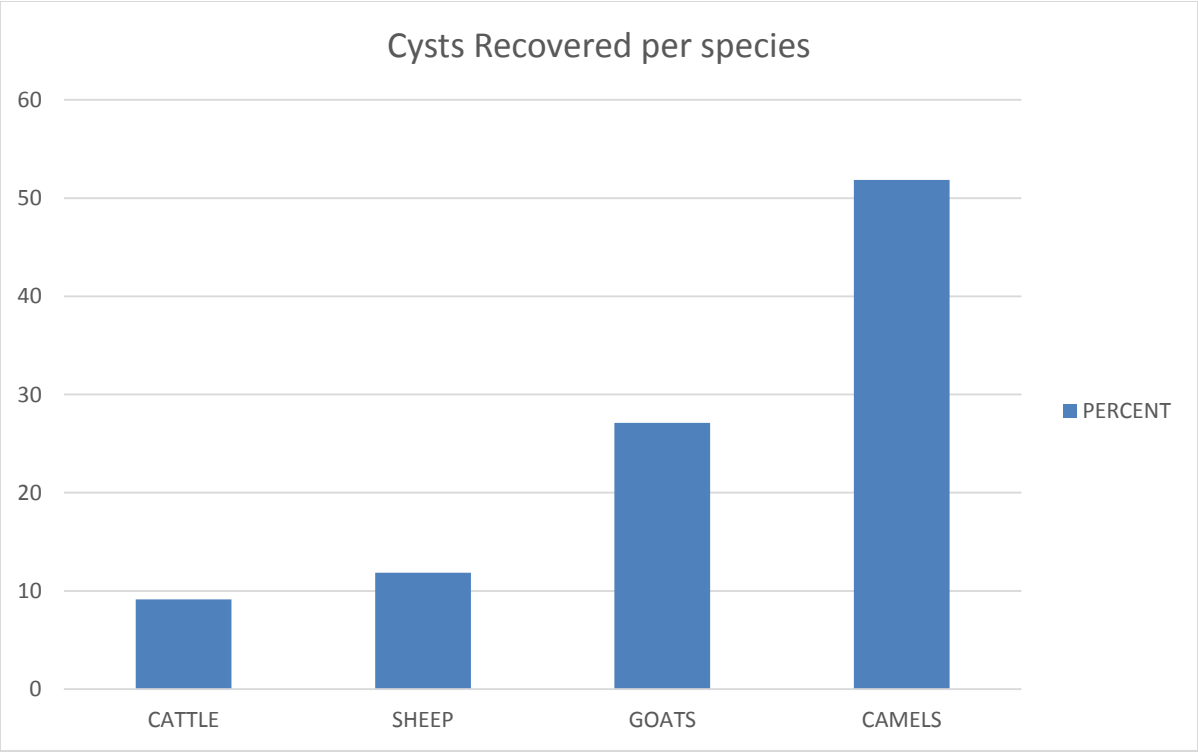

Figure 4.2: Cysts Recovered per Animal Species in Isiolo, Garissa and Wajir counties in October-November 2018

The number of cysts per animal largely differed among the sampled livestock ranging between 1 and 11 cysts (Table 4.3). Two camels had a total of 10 and 11 cysts respectively, recovered from their lungs. The highest number of cysts recovered from an individual bovine was 6 cysts in a case of mixed liver and lung infection. Among the caprine species the highest number of recovered cysts from an individual animal was 4 cysts while in the ovine it was 3 cysts.

Table 4.3: Number of Recovered Cysts per Species in Isiolo, Garissa and Wajir in October-November 2018

Infected Livestock Species	**Number Of Recovered Cysts**			
	1	**2-5**	**6-10**	**>10**
Caprine	58	10	0	0
Camel	33	30	3	2
Ovine	22	5	0	0
Bovine	13	3	1	0

The number of cysts per total number of inspected animals (abundance of cysts) was recorded as 0.65 (153/234) among camels, 0.12 (80/687) among caprines, 0.10 (35/329) among ovine and 0.23 (27/118) among bovine (Table 4.4). The number of cyst per infected animals (mean infection intensity) was 2.25 in camels (153/68), 1.18 in caprines (80/68), 1.30 in ovine (35/27) and 1.59 in bovines (27/17) (Table 4.4).

Table 4.4: Abundance of Cysts and Mean Infection Intensity in Isiolo, Garissa and Wajir counties in October-November 2018

Livestock Species	Number Inspected	Number Infected	Total Number of Cysts	Abundance of Cysts (cysts per inspected animals)	Mean Infection Intensity (cysts per infected animals)
Caprine	687	68	80	0.12	1.18
Camel	234	68	153	0.65	2.25
Ovine	329	27	35	0.10	1.30
Bovine	118	17	27	0.23	1.59

4.3: Echinococcus Cysts Predilection Site in the livestock species

The major predilection site of the *Echinococcus granulosus* cysts from this study was the liver and subsequently the lungs in all species (Table 4.5). Both single and multiple infections in organs were noted. In the infected caprine species, 8.8% (7/68) harboured lung cysts while 88% (61/68) harboured cysts in the liver. Among the infected camels; 26% (18/68) harboured cysts in the lung, 59% (40/68) in the liver, 1.5% (1/68) in the masseter muscle while 13.2% (9/68) harboured both lung and liver cysts. For the infected ovine, 3.6% (1/27) had cysts in the lung, 89% (24/27) had cysts in the liver, 3.6% (1/27) had a cyst in the heart while (3.6%) 1/27 had both liver and lung infections. In the infected bovine 5.8% (1/17) had a cyst in the lung, 82% (14/17) had cysts in the liver with 12% (2/17) having both liver and lung infections.

Table 4.5: Echinococcus Cysts Predilection Site in the livestock species in Isiolo, Garissa and Wajir in October-November 2018

Infected Livestock Species	**Organs infected**				
	Liver	**Lung**	**Liver & Lung**	**Heart**	**Masseter Muscle**
Caprine (n=68)	61	7	0	0	0
Camel (n=68)	40	18	9	0	1
Ovine (n=27)	24	1	1	1	0
Bovine (n=17)	14	1	2	0	0
Total (n=180)	**139**	**27**	**12**	**1**	**1**

The 295 cysts recovered from all the livestock species were distributed amongst individual organs as follows; liver at 72.9% (215/295) and the lung at 26.4% (78/295). The heart and the masseter muscle each had only one cyst, 0.34% (Figure 4.3).

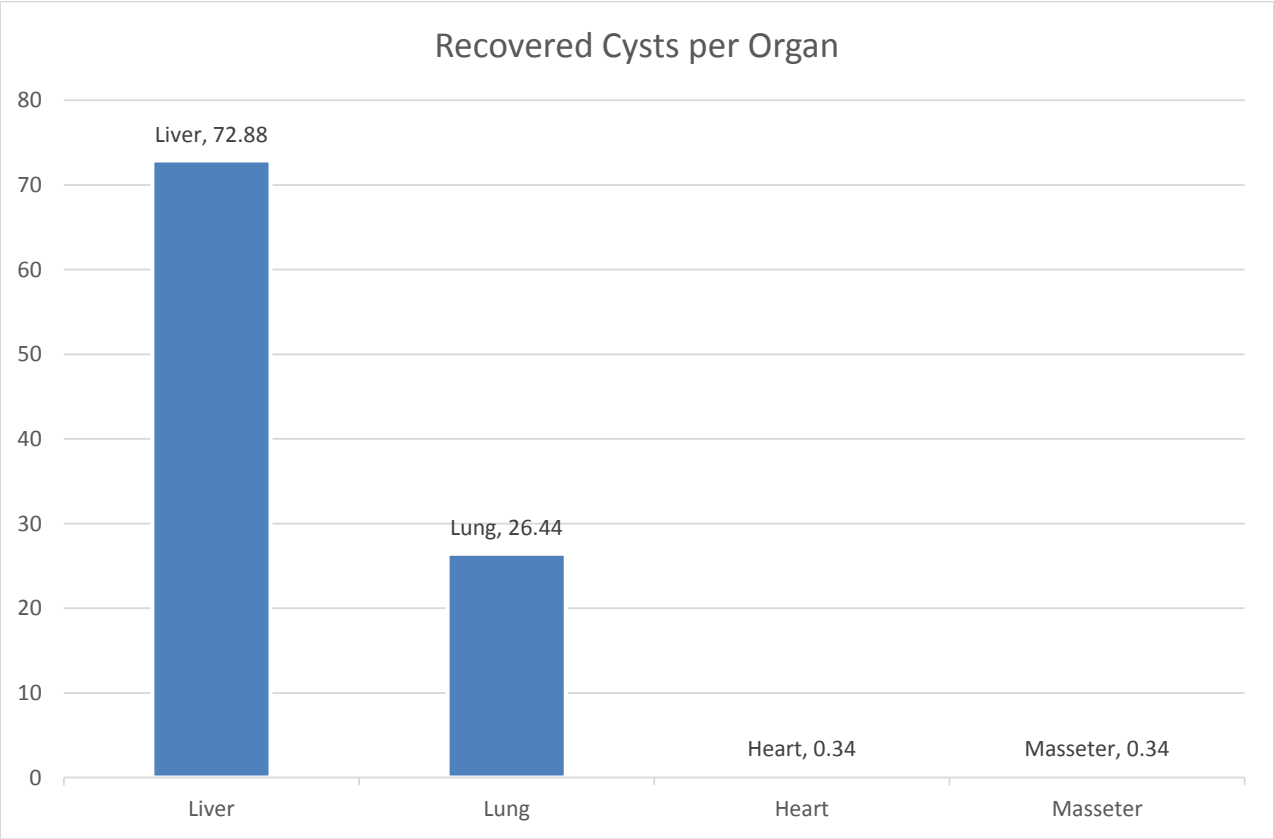

Figure 4.3: Cysts Recovered per Organ

4.4: Classification of Recovered Echinococcus Cysts in Isiolo, Garissa and Wajir counties

Echinococcus cysts were classified as fertile when the hydatid cysts present had clear, colorless fluid-filled sacs with identifiable protoscolices upon microscopy. The cysts were classified as infertile when they had fluid but lacked protoscolices or were generally calcified (Figures 4.4, 4.5, 4.6).

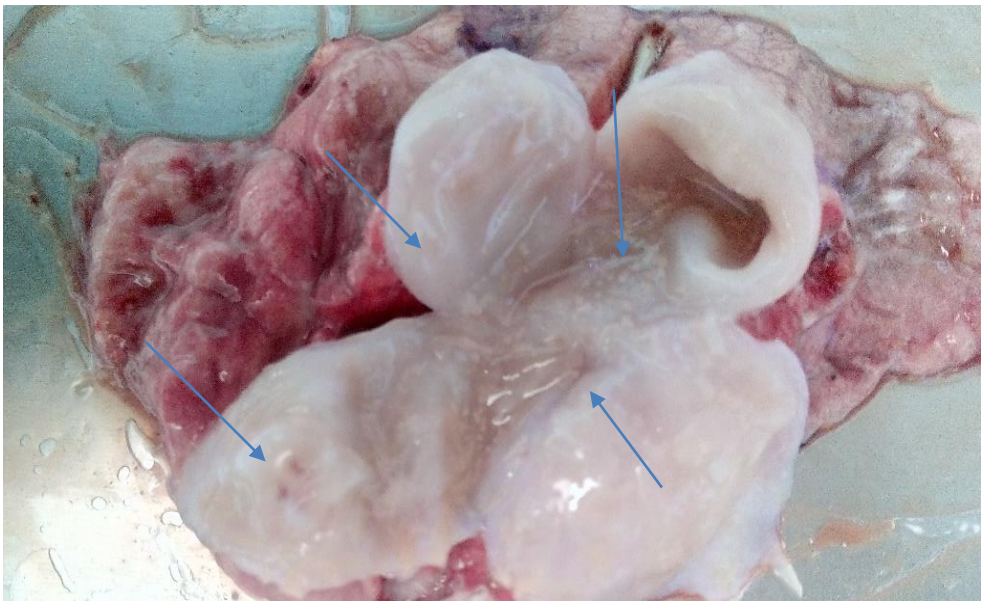

Figure 4.4: Image depicts a camel lung from Isiolo County. Arrows depicts cyst position. Photo courtesy of Eberhard Zeyhle.

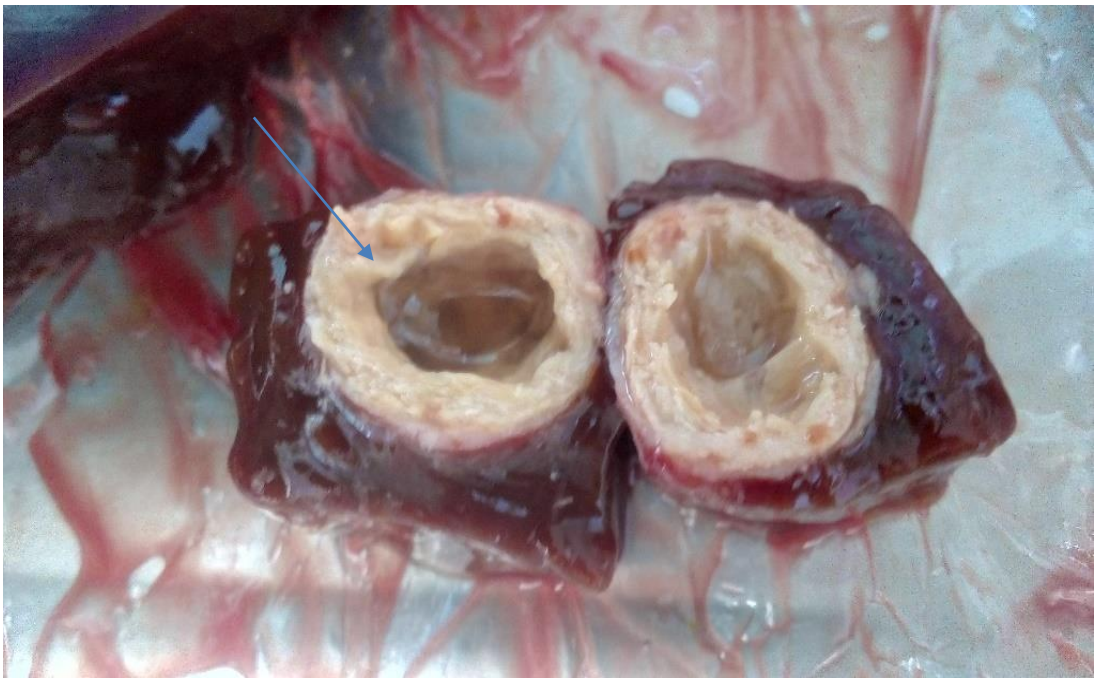

Figure 4.5: Image depicts a cattle liver from Isiolo County. Arrows depict the cysts. Photo courtesy of Eberhard Zeyhle.

Figure 4.6 Image of a calcified cyst retrieved from the liver of a camel in Isiolo County. Arrow depicts the calcified material. Photo courtesy of Eberhard Zeyhle.

There were two kinds of *Echinococcus granulosus* cysts that were identified during the study including fertile cysts with protoscolices and non-fertile cysts designating both the calcified and sterile cysts (Table 4.6). Cysts termed fertile were only demonstrated to occur in the lungs of camels in Isiolo County. Majority of the cysts from the small stock and bovines were calcified. All recovered cysts from sheep were calcified and thus non-fertile.

Table 4.6: Nature of recovered cysts in Isiolo, Garissa and Wajir in October-November 2018

Livestock	Nature	**Isiolo**					**Garissa**			**Wajir**		
Species	of Cyst	liver	lung	heart	muscle	**Total**	liver	lung	**Total**	liver	lung	**Total**
Caprine	Fertile	0	0	0	0	**0**	0	0	**0**	0	0	**0**
	Non-fertile	41	5	0	0	**46**	28	2	**30**	4	0	**4**
Camel	Fertile	0	14	0	0	**14**	0	0	**0**	0	0	**0**
	Non-fertile	29	51	0	1	**80**	20	1	**21**	38	0	**38**
Ovine	Fertile	0	0	0	0	**0**	0	0	**0**	0	0	**0**
	Non-fertile	14	0	1	0	**15**	8	0	**8**	10	2	**12**
Bovine	Fertile	0	0	0	0	**0**	0	0	**0**	0	0	**0**
	Non-fertile	21	5	0	0	**26**	1	0	**1**	0	0	**0**

4.5 Risk Factors for Detecting Cystic Echinococcosis Infection

By using cross tabulation and chi square test, there was a statistically significant association between animal county and occurrence of cystic echinococcosis, χ^2 (2) = 64.38, P<0.05. A statistically significant association was also noted between animal species and CE infection occurrence by use of cross-tabulation where χ^2 (3) = 119.67, P<0.05.

In the final multivariable logistic regression analysis, the occurrence of cystic echinococcosis was associated with: animal county and animal species. These variables statistically significantly predicted CE, (χ^2 (5) = 173.72, P<0.05). In the final model, while controlling for possible confounding, the odds of animals from Garissa county to have cystic echinococcosis were 0.05 times lower compared to animals from Wajir county with an odd of 0.17. There was a strong association between camels and the occurrence of echinococcus cysts, P=0.000. All other variables held constant, camels were twelve times more likely to get infected with CE compared to other livestock in the same region (Table 4.7).

Table 4.7: Final multivariable logistic regression analysis results for Echinococcosis in October-November 2018

Variable	Category	OR	0.95CI		P-value
			UCL	LCL	
County	Isiolo	Reference			<0.001*
	Garissa	0.05	0.11	0.02	<0.001
	Wajir	0.17	0.38	0.08	<0.001
Species	Cattle	Reference			<0.001*
	Sheep	0.89	2.69	0.29	0.834
	Goats	0.51	1.26	0.20	0.144
	Camel	11.84	27.89	5.03	0.000

*Overall p-value

4.6 Species of *Echinococcus granulosus*

A total of 295 cysts were retrieved from the inspected livestock species. Of these, 250 (84.75%) were typed by PCR and 132 (44.75%) cysts were PCR positive hence genotyped up to the species level (Figure 4.7 and Table 4.8).

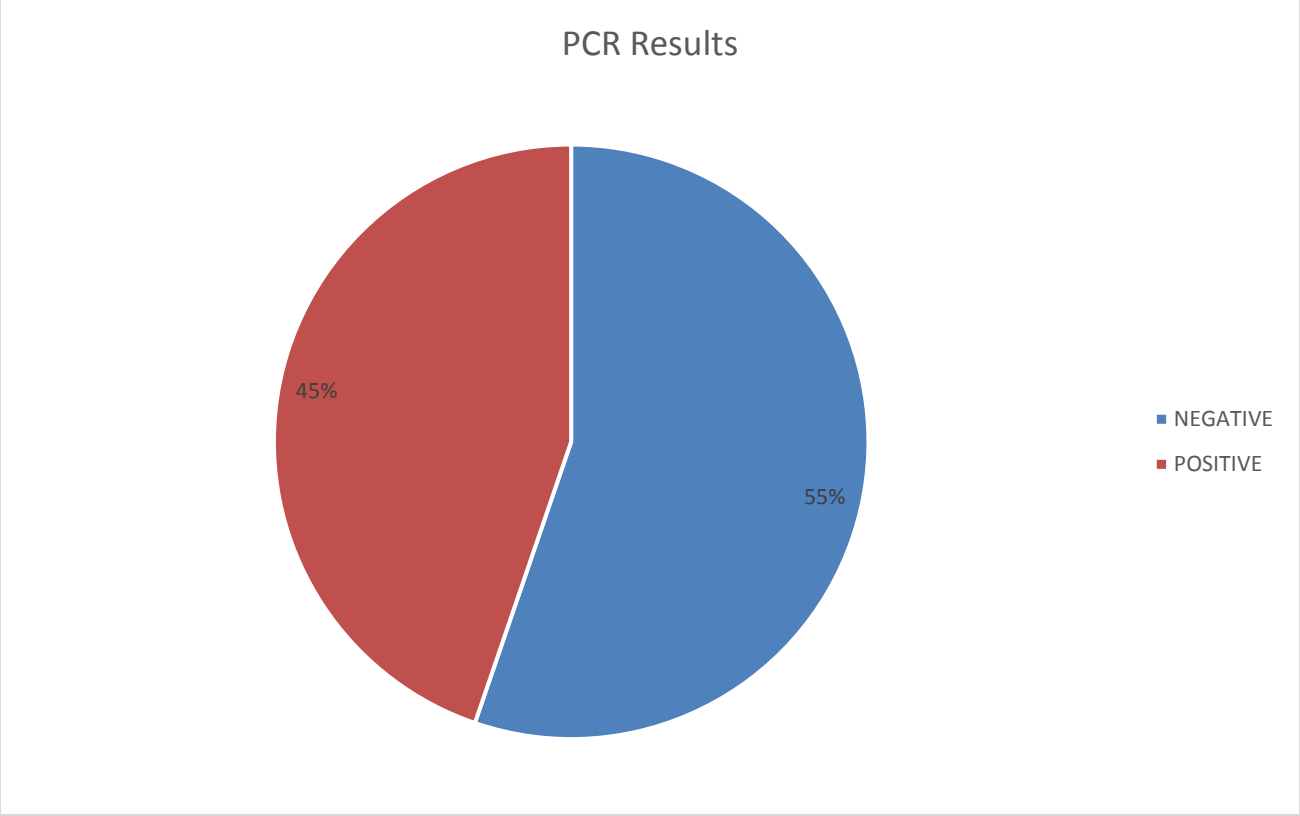

Figure 4.7: Polymerase Chain Reaction results for the Echinococcus species in October-November 2018

Majority of the cysts recovered from the liver failed to yield adequate genetic material for PCR. This was also the case in the lone cyst recovered from the heart. However, the cyst recovered from the masseter region was PCR positive.

Table 4.8: Polymerase Chain Reaction results per organ typed in October-November

PCR Result	Organ				
	Liver	Lung	Masseter	Heart	Total
Negative	153	9	0	1	163
Positive	62	69	1	0	132
Total	215	78	1	1	295

The nad- 1 gene 1073bp – 1078bp was successfully amplified by the selected primer pairs. To aid in species differentiation, the nad- 1 amplicons were subsequently digested with Hph I. Two *Echinococcus* species were found. A total of 84.9% (112/132) samples were identified as *Echinococcus canadensis* (G6/7) and 15.1% (20/132) of samples were found to be *Echinococcus granulosus sensu stricto* (G1/3) (Figures 4.8 and 4.9 and Table 4.9). The *E. granulosus* s. s. was mainly in livestock in Isiolo county while the *E. canadensis* dominated in Wajir and Garissa counties. All cysts recovered from the sheep were of *E. canadensis*. *E. granulosus* s. s was the dominant strain in goats and cattle. There were three cases of mixed infection observed. This included one bovine in Isiolo county having *E. granulosus* s. s in its lung and *E. canadensis* in the liver. Also still in Isiolo county, one camel presented a liver co-infected with both *E. granulosus* s. s and *E. canadensis*. In Wajir county, one camel demonstrated the simultaneous occurrence of *E. canadensis* and a *Taenia* species in its liver. Four samples from camels and one from the caprine species however could not be clearly ascertained as to what strain/species they

represented. A further two samples from camels depicted Taeniid species. One sample from the bovines was identified as *Taenia saginata*.

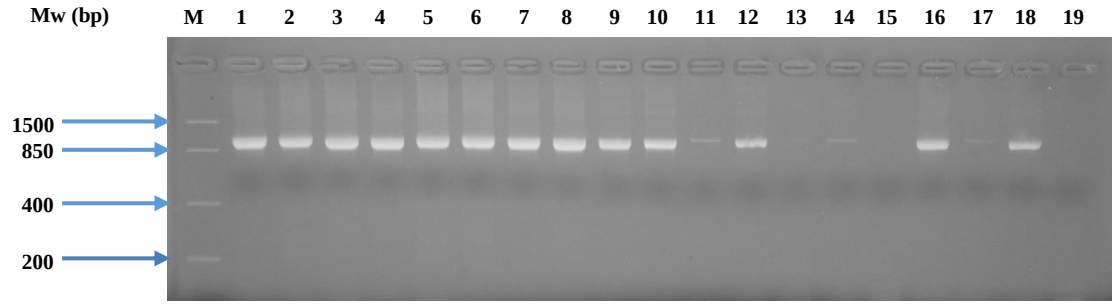

Fig 4.8: PCR of nad1 gene on *Echinococcus* cysts

Lane M: is the molecular weight marker (FastRuler middle range), lanes 1 – 12, 14, 16 and 17: represent *Echinococcus* cysts positive by PCR, lanes 13 and 15 represent PCR negative cysts, lane 18: represents a PCR positive control and lane 19: represents a PCR negative control.

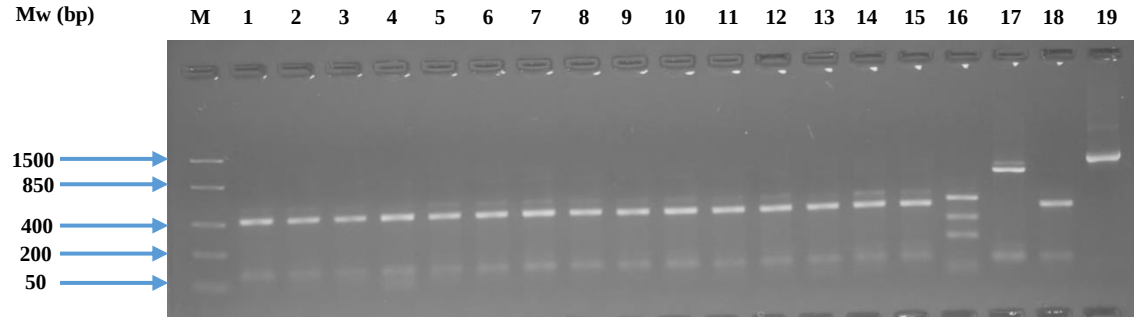

Fig 4.9: PCR-RFLP following restriction digestion with *Hph*I

Lane M: is the molecular weight marker (FastRuler middle range), lanes 1 – 15: represent *E. canadensis* (G6/7), lane 16: represents *E. granulosus* s. s. positive control; lane 17: represents the positive control for *E. ortleppi*; lane 18: represents the positive control for *E. canadensis* (G6/7); lane 19: represents uncut PCR product (negative control).

Table 4.9: Genotyped results showing *Echinococcus* species in October-November 2018

Genotyped samples	Isiolo		Garissa		Wajir	
	E. granulosus s. s.	*E. canadensis* (G6/7)	*E. granulosus* s. s.	*E. canadensis* (G6/7)	*E. granulosus* s .s.	*E. canadensis* (G6/G7)
Caprine n= 14	7	6	0	1	0	0
Camel n=99	3	81	1	4	1	9
Ovine n=6	0	4	0	1	0	1
Bovine n=13	8	5	0	0	0	0
Total 132	**18**	**96**	**1**	**6**	**1**	**10**

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

Abattoir surveys remain important when conducting prevalence studies of Cystic Echinococcosis in the intermediate host since the disease in livestock presents without obvious clinical symptoms. The results of this study reported for the first time the presence of Echinococcus species in Garissa and Wajir counties which were outside the much-studied regions of Maasailand and Turkana. These results also presented the current situation in Isiolo county and agree with the findings of Mbaya *et al*., (2014). The proportions of CE from this study included: 29.1% in camels, 9.9% in goats, 8.2% in sheep and 14.4% in cattle. These rates significantly differ from that of Mbaya *et al*., (2014) in their survey of Isiolo and Meru counties where the prevalence rates included; 6.9% in camels, 0.4% in goats, 4.6% in sheep and 1.9% in bovine species. However, this study is comparable to that of Addy *et al*., (2012) and Nungari *et al*., (2019) in their survey in Maasai land regions where infection rates were 10.8% goats, 16.5% in sheep, 25.8% in cattle and 15.2% in goats ;14.9% in sheep, 14.2% in cattle respectively.

There were marked variations in CE infection rates per county. These differences in CE prevalence could have been due to numerous factors including; religion and culture, awareness level on the disease and attitude of people towards dogs (Ibrahim, 2010). Moreover, egg survival once shed is also largely dependent on the vegetation cover and climatic conditions. Low vegetation cover combined with high environmental temperatures decrease the survivability of the eggs (Ibrahim, 2010). Isiolo county generally had the highest prevalence rates for CE in livestock. In Isiolo county, dogs could be seen around the slaughter house and dogs are also domesticated while further north, in Garissa and Wajir counties, interaction with dogs is limited

by virtue of religion so few dogs are kept. This serves to curb disease spread as dogs are the final or definitive hosts of *E. granulosus* and pass gravid segments or the adult worm through faeces to the environment.

The proportions of CE were high in cattle compared to sheep and goats. The high occurrence in cattle could be because cattle are grazers compared to goats that are browsers and also possibly influenced by the slaughter volume where few cattle are slaughtered. The proportions were higher in goats compared to sheep yet goats are browsers and sheep are grazers. This means that within the same environment, sheep are more likely to be exposed to CE than goats. The reason for the above observation was unclear.

Echinococcus granulosus cysts occur in various internal organs including lungs, liver, kidney and heart (Addy *et al*., 2012;Varcasia *et al*., 2007). In the present study, most cysts were isolated from the liver (72.88%) after which came the lungs (26.44%). This could be because oncospheres actively migrate from the intestines through the portal vein. The liver and lungs are consequently the first vast capillary regions that oncospheres encouter in migration. The oncospheres are thus first put through hepatic and pulmonary filtering systems prior to the involvement of other organs (Kumsa & Mohammedzein, 2012). The findings in this study concur with those of Addy *et al*., (2012), Odongo *et al*., (2018) and Nungari *et al*., (2019). Our results however differed with other studies that found the lung as the most parasitized organ (Njoroge *et al.*, 2002; Ibrahim *et al.*, 2011; Kere *et al.*, 2019). There was multiple organ infection of mostly the liver and lungs with one cyst recovered from the heart of an ovine species and another from the masseter muscle of camels. The multiple organ infection signifies the huge losses economically. These losses are accrued due to the condemnation of the infected organs at

slaughter. This also details the need for meat inspectors to be keen in conducting a thorough inspection of all internal organs and the carcass in general.

Fertile *Echinococcus granulosus* cysts were only recovered from the lungs of camels in Isiolo county. This may have come about since lungs have a softer consistency with loose connective tissue structure as compared to the higher reticuloendothelial cells and abounding connective tissue in the liver, hence onchospheres get trapped easily and mature into cysts (Kumsa & Mohammedzein, 2012). The lungs are thus seen as a greater source of dog infection than all other organs whereas camels are an important contributor to the spread of cystic echinococcosis among the livestock species. All cysts recovered from the other livestock species were non-fertile and mostly calcified and 'stone-like'. All cysts recovered from the ovine were calcified. This means that sheep in Isiolo, Garissa and Wajir counties may not play a significant role in CE transmission. Our findings differ from the findings of Gachengo *et al*., (2017) in his study in Laikipia, a CE non-endemic area, where all cysts from sheep were highly fertile. The high rates of calcification in sheep could have been due to the absence of *Echinococcus granulosus* G1/3.

The species of *E. granulosus* presented in this study are only *E. granulosus* s. s. and *E. canadensis* (G6/7). The various species of *E. granulosus* sensu lato (s. l.) are classified according to Nakao *et al*., (2013) that advocates for the grouping of genotypes that are closely related (G1/3 and G6-8, 10) as species. The isolation of G1 and G6 from livestock supports prior reports of the presence of these species in Kenya (Nungari *et al*., 2019: Mbaya *et al*., 2014: Addy *et al*., 2012). *E. canadensis* G6/7 was the most dominant taxa of Echinococcus recovered. This simulates the findings of Ibrahim *et al*., (2011) in his study in Sudan and differs from the findings of Addy *et al*., (2012) from the Maasailand survey where *E. granulosus* G1 species was found to be dominant. Sheep are known to be well adapted as hosts of *E. granulosus*, G1 strain. However, in

this study, all cysts from sheep were of the G6/7 species. This could have been due to the fact that majority of the cysts analysed from sheep were recovered in Isiolo county where camels were seen to be actively infected with the G6/7 species. Varcasia *et al*., (2007) reported that goats are well suited as hosts for the maintenance of the life cycle of the G6/7 species. This is in agreement with our study whereby goats infected with G6/7 species were almost equal in number to those infected with the G1 species however, the cysts were calcified.

Mixed infections with different species of *E. granulosus s. l* presumably occur when an animal accidentally ingests eggs of differing genotypes. Mixed infections were rare as most animals presented with only one strain of *E. granulosus* even with multiple cyst infections. In Isiolo county, one cattle and one camel presented with the G1 species in the lung and the G6 species in the liver while in Wajir county, one camel had simultaneous infection with both *E. canadensis* and a Taenia species in the liver. This amplification of the nad-1 of Taenia species means that the primer pairs used are also suitable for screening a range of Taenia spp.

Several risk factors were associated with disease occurrence including the animal county and animal species. Livestock in Isiolo county were more likely to be infected than those in Garissa and Wajir counties. This could be because of the semi-arid climatic conditions that characterize Garissa and Wajir counties coupled with the high environmental temperatures and low vegetation cover. *Echinococcus granulosus* eggs once shed, desiccate rapidly under such conditions. Wajir and Garissa counties are also characterized by very few dogs due to religious constraints (County Director of Veterinary Services, pers. comm.). Under similar environmental conditions, camels were more likely to be parasitized compared to the other livestock species. The findings in this study are similar to that of (Mbaya *et al*., 2014) in their study in Isiolo and Meru counties and may be because camels are mostly slaughtered in abattoirs while the small

stock especially, are slaughtered at home. Camels also take longer to mature and be ready for slaughter which gives chance for continuous infection over time and for the cysts to reach a detectable state at post-slaughter inspection.

About 110 samples did not amplify after PCR. This may have been brought about by their non-fertile, extremely calcified nature hence adequate DNA could not be retrieved.

5.2 Conclusions

- The proportions of CE in north-eastern Kenya was reported at 29.1% in camels, 14.4% in bovines, 9.9% in caprines and 8.2% in ovine. This is a region outside the well-studied Maasailand and Turkana regions.
- Garissa and Wajir counties are emerging CE foci due to the huge slaughter volumes and especially that of camels.
- Camel carcasses examined in this study had very high proportions of fertile cysts recovered.
- The *Echinococcus canadensis*, G6 and *Echinococcus granulosus*, G1 species were found to be dominant species in Isiolo, Garissa and Wajir counties.

5.3 Recommendations

- Further studies that include molecular characterization of *Echinococcus granulosus* should be carried out in livestock, humans and dogs in the non-endemic counties to determine prevalence and provide guidelines for effective prevention and control of this Neglected Tropical Disease.
- Dog population control coupled with health education should be conducted.
- Meat inspectors should be adequately trained to better identify cysts and parasites.

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APPENDICES

Appendix I. Proposal approval by Biosafety, Animal Use and Ethics committee of the University of Nairobi.

UNIVERSITY OF NAIROBI
FACULTY OF VETERINARY MEDICINE
DEPARTMENT OF VETERINARY ANATOMY AND PHYSIOLOGY

REF: FVM BAUEC/2019/227

Dr. Hellen Akoth Omondi
University of Nairobi
Dept. of PHP & Toxicology

12/06/2019

Dear Dr. Omondi,

RE: Approval of Proposal by Biosafety, Animal use and Ethics committee

The occurrence of cystic Echinococcosis and molecular characterization from livestock in Isiolo, Garissa and Wajir Counties, kenya

By Dr. Hellen Akoth Omondi Reg Number J56/ 8176/ 2017.

We refer to your MS.c proposal submitted to our committee for review and your application letter dated 17th April 2019.

We have reviewed your application for ethical clearance while identifying hydatid cysts during routine meat inspection at the abattoirs. The handling, collection and processing procedure of affected tissue within UON laboratory meets acceptable minimum standards of the Faculty ethical regulation guidelines.

We have also noted that registered veterinary surgeons will supervise the work.

We hereby give approval for you to proceed with the project as outlined in the submitted proposal.

Yours sincerely

Kaluwa.

Dr. Catherine Kaluwa, BVM, MSc, Ph.D
Chairperson,
Biosafety, Animal Use and Ethics Committee
Faculty of Veterinary Medicine.

Appendix II: Kenya Medical Research Institute, Centre for Microbiology Research laboratory use approval

KENYA MEDICAL RESEARCH INSTITUTE

Centre for Microbiology Research, P.O. Box 19464 - 00202, NAIROBI - Kenya
Tel: (254) (020) 2720794, 2720038, Nairobi , E-mail: cmr@kemri-nuitm.or.ke Website.www.kemri.org

18th February 2019

Dear Hellen,

RE: Permission to conduct MSc project in the Parasitology laboratory in CMR

Permission is granted for a period of six months to conduct your MSc project at the parasitology laboratory in Centre for Microbiology Research, KEMRI. This follows your application for a laboratory space in the centre. During your stay you will be working under the supervision and guidance of Dr. Cecilia Mbae and Mr. Erastus Mulinge.

Please ensure you adhere to all the safety and regulations in the laboratory as the institute will not be liable for your safety. I hope the contribution of the KEMRI supervisors will be acknowledged appropriately during publishing or disseminating the findings of your research. I hope you will have a pleasant stay in KEMRI to achieve the objectives of your study.

Yours Faithfully,

Dr. Willie Sang,
Director, Chief Research Officer
Centre for Microbiology Research
Kenya Medical Research Institute

Cc.
Dr. Cecilia Mbae (CMR, KEMRI)
Prof. George Gitau (University of Nairobi)
Dr. Peter Gathura (University of Nairobi)
Dr. Bernard Bett (International Livestock Research Institute)

In Search of Better Health

Appendix III: Publication in the Journal of Helminthology

Journal of Helminthology

cambridge.org/jhl

Prevalence and genotyping of *Echinococcus granulosus* sensu lato from livestock in north-eastern Kenya

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Research Paper

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Cystic echinococcosis; *Echinococcus* species; livestock; Isiolo; Garissa; Wajir; Kenya

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Abstract

Cystic echinococcosis (CE) is a zoonotic disease of cosmopolitan distribution and caused by the larval stage of the dog tapeworm, *Echinococcus granulosus sensu lato* (*s.l.*). CE occurs in the wider African continent and in Kenya, notably in the Maasailand and Turkana regions; however, recent studies demonstrate its presence in other parts of Kenya. This study determined the occurrence of CE in livestock (camels, goats, sheep and cattle) in Isiolo, Garissa and Wajir counties, and characterized the species of *E. granulosus s.l.* present. An abattoir survey was used to determine the presence of CE in various organs in livestock. Polymerase chain reaction-restriction fragment length polymorphism and sequencing of the mitochondrial NADH dehydrogenase subunit 1 gene was used for genotyping. A total of 1368 carcasses from 687 goats, 234 camels, 329 sheep and 118 cattle were inspected for the presence of hydatid cysts. The overall proportion of infections was 29.1% in camels, 14.4% in cattle, 9.9% in goats and 8.2% in sheep. The liver was the most infected organ, while only the lung of camels harboured fertile cysts. Of the 139 cysts genotyped, 111 (79.9%) belonged to *Echinococcus canadensis* (G6/7) and 20 (14.4%) to *E. granulosus sensu stricto*. One and two cysts were identified as *Taenia saginata* and unknown *Taenia* species, respectively. There was a significant association between county of origin and species of the animal with occurrence of CE. This study reports, for the first time, the characterization of *Echinococcus* species in livestock from Garissa and Wajir counties, and the current situation in Isiolo county. The fertility of cysts in camels and frequency of *E. canadensis* (G6/7) in all livestock species indicate that camels play an important role in the maintenance of CE in the north-eastern counties of Kenya.

Introduction

Cystic echinococcosis (CE) is a cyclo-zoonotic disease of global importance that is caused by the larval stage of the dog tapeworm, *Echinococcus granulosus sensu lato* (*s.l.*). CE occurs as an infection in intermediate hosts, the herbivores and omnivores, and man as a 'dead-end host'. Dogs, other canids and felids are the definitive hosts that harbour the adult cestode in the small intestines (Thompson, 2017). CE occurs worldwide and is of great public health and socioeconomic importance in pastoral communities (Deplazes *et al.*, 2017). The burden of CE in humans is estimated to be between one and three million disability-adjusted life years, and, according to the World Health Organization, up to three billion US dollars is spent annually on the treatment of human CE cases and due to losses incurred in livestock industry (WHO, 2015).

Molecular phylogeny studies have classified *E. granulosus s.l.* into five species – namely, *Echinococcus granulosus sensu stricto* (*s.s.*) (G1, G3), *Echinococcus equinus* (G4), *Echinococcus ortleppi* (G5), *Echinococcus canadensis* (G6–8, 10) and *Echinococcus felidis* (Nakao *et al.*, 2013a, b). All these CE agents besides *E. canadensis* (G8 and G10) have been detected in Kenya (Romig *et al.*, 2017). The epidemiology of CE is well established in the traditionally known endemic areas of Turkana and Maasailand in Kenya (Dinkel *et al.*, 2004; Addy *et al.*, 2012; Mulinge *et al.*, 2018; Odongo *et al.*, 2018; Nungari *et al.*, 2019). Recent studies outside the known endemic areas have highlighted the differences in prevalence of CE, fertile cysts vs. calcified cysts, the most important intermediate host and the diversity of *Echinococcus* spp. (Mbaya *et al.*, 2014; Gachengo *et al.*, 2017; Kere *et al.*, 2019) – for example, the abundance of *E. ortleppi* in livestock from Meru and Isiolo (Mbaya *et al.*, 2014) and *E. canadensis* (G6/7) in dogs from Turkana (Mulinge *et al.*, 2018), despite being rare in characterized humans cases (Dinkel *et al.*, 2004; Casulli *et al.*, 2010; Mutwiri *et al.*, 2013). The differences could be due to sample size, cultural, slaughtering and livestock husbandry practices among the respective communities. Therefore, there is a need for further studies on CE in other regions outside the known endemic areas to understand the epidemiology of CE in those areas.

The epidemiology of CE in Garissa and Wajir counties is poorly understood due to lack of data, while some baseline data on livestock and dog infection do exist from Isiolo county (Mbaya *et al.*, 2014; Mulinge *et al.*, 2018). Garissa, Isiolo and Wajir counties are arid and semi-arid areas in north-eastern Kenya, whose residents largely depend on livestock keeping for their livelihood. These regions are characterized by the presence of many stray dogs, informal and home slaughtering of livestock and cultural conditions that facilitate CE transmission. The present study provides baseline data on the presence of species causing CE in livestock in Isiolo, Garissa and Wajir counties of Kenya.

Materials and methods

Study area

The study was undertaken in Isiolo, Garissa and Wajir counties, which are located in the north-eastern part of Kenya (fig. 1). The counties have a predominantly semi-arid and hot desert climate, with ample sunshine all through the year and a large land area with sparse vegetation. Rain falls infrequently and quite sporadically. Most regions are dry and receive less than 150 mm of rainfall annually, while the mean annual temperature is 28°C (KNBS, 2009). The study counties are predominantly rural and characterized by a large number of pastoralists, with the main economic activity being livestock production.

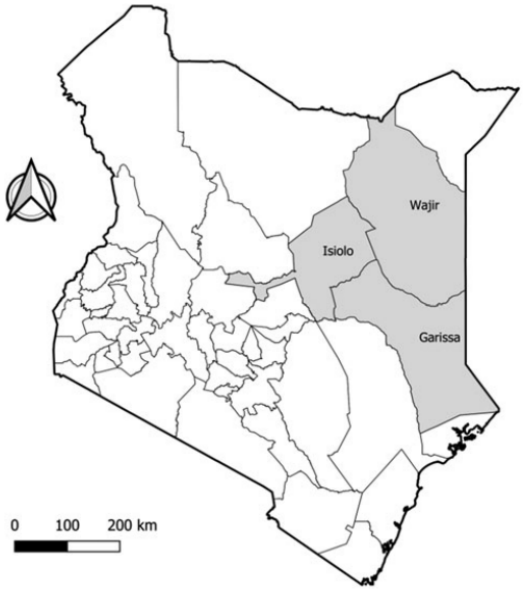

Fig. 1. Map of Kenya showing the study counties.

Collection and identification of hydatid cysts

During the months of October and November 2018, daily abattoir visits were made for one week in the main abattoirs per county in Isiolo, Garissa and Wajir, where carcasses and organs of cattle, sheep, goats and camels were inspected visually and physically through palpation and subsequent incision to establish presence of cysts. Data on total number of slaughtered animals, species, number infected with hydatid cysts and location of the cysts were recorded. Each cyst was labelled and stored separately in 70% ethanol in a plastic container while awaiting transportation to the Kenya Medical Research Institute (KEMRI), parasitology laboratory in Nairobi, for examination and further analysis.

Microscopic examination

The contents of all cysts were examined microscopically at 40× magnification for presence of protoscoleces. Fertile cysts were those with protoscoleces, while non-fertile cysts lacked protoscoleces or were calcified.

Deoxyribonucleic acid extraction

Crude DNA (lysates) were obtained by lysing of single protoscolex or cyst material in 0.02 M sodium hydroxide at 99°C for 10 min. In instances where the aforementioned process failed to yield sufficient DNA, extraction was performed on cyst material using the DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany) following the manufacturer's guidelines.

Polymerase chain reaction (PCR) and restriction fragment length polymorphism

A nested PCR was undertaken to amplify the NADH dehydrogenase subunit 1 (*nad1*) as previously described by Hüttner *et al.* (2008). In both PCR assays, the reaction mixture contained 2 µl of DNA, 1× DreamTaq™ Green Buffer (Thermo Fisher Scientific, Waltham, MA, USA) (20 mM Tris (hydroxymethyl) aminomethane hydrochloride (pH 8.0), 1 mM dithiothreitol, 0.1 mM ethylenediaminetetraacetic acid, 100 mM Potassium chloride, 0.5% (v/v) Nonidet P40 (Thermo Fisher Scientific, Waltham, MA, USA), 0.5% (v/v) Tween 20 (Thermo Fisher Scientific, Waltham, MA, USA), 0.2 mM deoxyribonucleotide triphosphates, 0.25 µM of forward and reverse primers, 2 mM magnesium chloride and 0.625 units of DreamTaq™ Green DNA Polymerase (Thermo Fisher Scientific) in 25 µl final volume. The conditions for amplification included prior denaturation at 94°C for 5 min, followed by 40 cycles of denaturation at 94 °C for 30 s, annealing at 55°C for 30 s and elongation at 72°C for 1 min, followed by final elongation at 72°C lasting 5 min. The *nad1* amplicons were digested, as earlier reported by Hüttner *et al.* (2009), using the restriction enzyme, *Hph*I (New England Biolabs, Ipswich, MA, USA). The total reaction mixture was 20 µl, including 7.5 µl distilled water, 2.0 µl of 10× CutSmart Buffer (New England Biolabs', Ipswich, MA, USA), 0.5 µl *Hph*I (five units) and 10 µl PCR product. The reaction mixture was incubated overnight at 37 °C and then separated on 3% agarose gel. From the samples, the *Echinococcus* species were detected by contrasting their banding patterns to those of already established patterns according to Hüttner *et al.* (2009). DNA sequencing was carried out on the *nad1* gene for samples whose restriction banding patterns were not identified. The secondary PCR product was first purified in accordance with the QIAquick PCR purification kit (Qiagen, Hilden, Germany) manual following the manufacturer's guidelines. The purified samples were carefully labelled and sent to Inqaba Biotec Laboratory, South Africa, for sequencing using the nested reverse primer.

Statistical data analysis

Data were first entered into MS Excel (Microsoft Inc., Sacramento, California, USA) and then imported to STATA 15.1 (StataCorp

LLC, College station, Texas, USA) for analysis. Initially, the data were checked for accuracy, coded and analysed using descriptive statistics. Proportions were determined for categorical variables and presented as a percentage of the overall number, along with a 95% confidence interval, where applicable. Univariable analysis using simple logistic regression was performed to determine unconditional associations between animal county and species with the presence of *E. granulosus*. A multivariable model with county of origin and animal species was built to allow estimation of coefficient. The area under the curve of the receiver operating characteristic was used to evaluate the overall performance of the model. *Echinococcus* and *Taenia* species sequences were carefully edited using GENtle version 1.9.4 (http://gentle.magnusmanske.de). The species were identified by comparing sequences with those available in the National Centre for Biotechnology Information database (NCBI) using the basic local alignment search tool (BLAST) (http://www.ncbi.nlm.nih.gov/BLAST/; Altschul *et al*., 1997).

Results

Prevalence of CE in livestock in Isiolo, Garissa and Wajir counties

A total of 1368 carcasses were inspected in the three counties. The overall CE proportions per inspected livestock species were camels 29.1% (68/234), goats 9.9% (68/687), sheep 8.2% (27/329) and cattle 14.4% (17/118) (table 1). In Isiolo county, the proportions were 58% in camels (36/62), 15.3% in goats (39/255), 16.9% in sheep (10/59) and 17.2% in cattle (16/93). Garissa county recorded a species proportion of 10.4% in camels (13/125), 8.4% in goats (26/310), 3.6% in sheep (8/222) and 4.7% in cattle (1/21). In Wajir county, the prevalence rates were 40.4% in camels (19/47), 2.5% in goats (3/122), 18.8% in sheep (9/48) and 0% in cattle (0/4).

Location, number and viability of the cysts

A total of 295 cysts were recovered from 180 infected animals, and the liver was the major predilection site for the cysts with 72.9% (215/295), followed by the lungs at 26.4% (78/295), with the heart and masseter muscle each having a single cyst (table 1). The number of cysts per animal largely varied among the sampled livestock, ranging between one and 11 cysts (table 2). Fertile cysts 14/295 (4.7%) were found only in the lungs of camels from Isiolo county. The majority of the cysts were calcified 244/295 (82.7%) and these included all the cysts from sheep (table 3). The cysts identified were generally ovoid in shape with varying diameters, ranging between 2.5 mm and 10 cm.

Genotyping of cyst materials/protoscoleces

Genomic DNA or cyst lysate was obtained from 250 of 295 cysts, while the remaining 45 cysts were excluded for not being appropriate for DNA extraction. Crude DNA was obtained from protoscoleces originating from 22 samples and from cyst wall material of the remaining 228 samples, and included DNA extraction using the DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany) being undertaken on 195 cyst samples. Of the cysts tested by nested PCR, only 139 (55.6%) were PCR positive and, hence, genotyped up to the species level. Two species each of *E. granulosus s.l.* and *Taenia* were found. A total of 79.9% (111/139) cysts were identified as *E. canadensis* (G6/7) and 14.4% (20/139) as *E. granulosus s.s. Echinococcus granulosus s.s.* was found mainly in goats and cattle from Isiolo county, but was rare in all the species from Garissa and Wajir counties. *Echinococcus canadensis* (G6/7) was the most common species in livestock in all three counties, the only species in sheep and predominantly in camels (table 3). A total of 11 isolates were sequenced and included eight isolates with unknown restriction patterns whose representative sequences were deposited in GenBank. The *E. granulosus s.s.* isolate with accession number MT525963 showed 99.78% identity to reference sequence EF367333, cattle isolate from Morocco (Azlaf *et al*., unpublished data). *Echinococcus canadensis* (G6/7) sequences with accession numbers MT525964–MT525967 were 99.89–100% identical to reference sequences KX010873, KX010875 and KX010879 previously derived from cattle, goats and camels in Kenya (Addy *et al*., 2017). Three cysts (3/139) were identified as *Taenia* spp. One was *Taenia saginata* from a calcified liver cyst of cattle from Isiolo, with accession number MT535753, which showed 99.55% identity to the reference sequence AY684274 from Korea (Jeon *et al*., 2007). The other two *Taenia* spp., with accession numbers MT535754 and MT535755, were 99.66% identical to the reference sequence AB905200 from hyena in Ethiopia (Terefe *et al*., 2014). Both *Taenia* spp. isolates were calcified and located in camel liver from Garissa and Wajir, one of which occurred as co-infection with *E. canadensis* (G6/7). PCR products from 5/139 cysts could not be genotyped due to low yield of amplicon.

Risk factors for CE in livestock

The variables that had significant association between positivity to *E. granulosus s.l.* infection in the final multivariable analysis were county of animal origin and animal species (table 4). The odds of an animal being positive for *E. granulosus s.l.* were lower for Garissa and Wajir counties as compared with Isiolo county. The odds of being positive for *E. granulosus s.l.* were higher for camels as compared to cattle, sheep and goats.

Discussion

The results of this study confirmed the presence of CE for the first time in the counties of Garissa and Wajir, and the current situation in Isiolo county. The proportions of CE from this study – 29.1% in camels, 9.9% in goats, 8.2% in sheep and 14.4% in cattle – were significantly higher than those of Mbaya *et al.* (2014) in their survey of Isiolo and Meru counties, where the prevalence were 6.9% in camels, 0.4% in goats, 4.6% in sheep and 1.9% in cattle. However, this study is comparable to that of Addy *et al.* (2012) and Nungari *et al.* (2019) from Maasailand, where infection rates were 10.8% goats, 16.5% in sheep and 25.8% in cattle, and 15.2% in goats, 14.9% in sheep and 14.2% in cattle, respectively. The findings that camels were the most infected species in this study confirms previous reports in Sudan (Elmahdi *et al*., 2004; Omer *et al*., 2010; Ibrahim *et al*., 2011) and Algeria (Bardonnet *et al*., 2003). Livestock in Isiolo county were more likely to be infected than those in Garissa and Wajir counties. This could be because of the arid climatic conditions that characterize Garissa and Wajir counties coupled with high environmental temperatures and low vegetation cover as compared to Isiolo county. Under similar environmental conditions, camels were more likely to be parasitized compared to the other livestock species. Camels had the highest CE prevalence rate, with the greatest number of fertile cysts and an odds ratio of about 12 for infection compared to sheep and goats, which had odds ratios of 0.89 and 0.51, respectively. The findings of this study simulate those of

Table 1. Proportion of CE and predilection site in the livestock species in Isiolo, Garissa and Wajir counties.

Livestock	Proportion infected (%)	Liver (*n*)	Lung (*n*)	Liver and lung (*n*)	Heart (*n*)	Masseter muscle (*n*)
Goats (*n* = 687)	9.9	61	7	0	0	0
Camels (*n* = 234)	29.1	40	18	9	0	1
Sheep (*n* = 329)	8.2	24	1	1	1	0
Cattle (*n* = 118)	14.4	14	1	2	0	0

n, number of animals.

Table 2. Number of recovered cysts per species in Isiolo, Garissa and Wajir counties.

	No. of recovered cysts			
Infected livestock species	1	2–5	6–10	>10
Goats (*n* = 68)	58	10	0	0
Camels (*n* = 68)	33	30	3	2
Sheep (*n* = 27)	22	5	0	0
Cattle (*n* = 17)	13	3	1	0

another study in Isiolo and Meru counties (Mbaya *et al.*, 2014) and elsewhere, as camels are usually slaughtered at an advanced age and, therefore, acquire and develop CE over a period of time (Elmahdi *et al.*, 2004). The analysis of comparable sample sizes from these counties is necessary to confirm with certainty the differences in the three counties.

Hydatid cysts have been reported in various internal organs, including the lungs, liver, kidney and heart (Varcasia *et al.*, 2007; Addy *et al.*, 2012). The majority of the cysts in this study were isolated from the liver, which is common since the liver is the first organ encountered by the migrating oncosphere after leaving the intestines. This finding agrees with observations in Maasailand, Kenya (Addy *et al.*, 2012; Odongo *et al.*, 2018; Nungari *et al.*, 2019) and in Sudan (Elmahdi *et al.*, 2004). These results, however, differed with reports from other regions in Kenya that found the lung as the most parasitized organ (Njoroge *et al.*, 2002; Kere *et al.*, 2019). There was multiple organ infection of mostly the liver and lungs, with one cyst recovered from the heart of a sheep and another from the masseter muscle of a camel. The multiple organ infection signifies the huge economic losses accrued from the condemnation of the organs at slaughter. The multiple organ infection also details the need of meat inspectors to be keen in conducting a thorough inspection of all internal organs and the carcass in general.

Based on the fertility rates of cysts, it can be postulated that camels are potential intermediate hosts that facilitate the transmission cycle of CE in the north-eastern region of Kenya. All cysts from the other livestock species were non-fertile and mostly calcified. The role of camels in the transmission of CE is difficult to explain as they are rarely slaughtered at home compared to small ruminants. However, transmission could have been due to the improper disposal of offal at abattoirs/slaughter slabs that was observed in this region and has been reported in Turkana and Maasailand (Mulinge *et al.*, 2018). All cysts recovered from sheep were calcified possibly due to the rare occurrence of *E. granulosus s.s*, a trend contrary to other studies in Kenya (Mbaya *et al.*, 2014; Gachengo *et al.*, 2017; Odongo *et al.*, 2018; Nungari *et al.*, 2019) that reported high cyst fertility in sheep. Indeed, calcified cysts accounted for 82.7% of all samples in this study – a common trend in recent studies in Kenya – 32.9% in Maasailand (Nungari *et al.*, 2019) and 80% in Turkana (Zeyhle, unpublished data). The sampling procedure of cysts employed in recent studies in Kenya is responsible for the characterization of many calcified cysts; however, the cause of calcification remains unknown (Nungari *et al.*, 2019).

Echinococcus canadensis (G6/7) was the most frequent species isolated, which is contrary to previous studies in Kenya (Dinkel *et al.*, 2004; Addy *et al.*, 2012; Mbaya *et al.*, 2014; Odongo *et al.*, 2018; Nungari *et al.*, 2019). However, it should be noted that 75% of the genotyped cysts originated from camels, the primary intermediate host for *E. canadensis* (G6/7), and confirms the findings of Mbaya *et al.* (2014) where 36 out of 39 camel's cysts belonged to *E. canadensis* (G6/7). In this study, the proportion of camel cysts that belonged to *E. canadensis* was 94.5%. Although goats are considered important in the maintenance of the life cycle of *E. canadensis* (G6/7), especially in the absence of camels (Varcasia *et al.*, 2007; Addy *et al.*, 2012), in this study goats infected with *E. canadensis* (G6/7) were almost equal in number to those infected with the *E. granulosus s.s.* The calcification of cysts and low prevalence of CE in goats in the current and a previous study in Isiolo (Mbaya *et al.*, 2014) indicate that goats may be playing a minor role in the maintenance of CE in these areas. Moreover, all the cysts from sheep in this study were *E. canadensis* (G6/7) and calcified, despite examining more sheep (329) compared to the previous study (65) (Mbaya *et al.*, 2014). These findings confirm the suggestion that sheep are not suitable hosts for *E. canadensis* (G6/7) (Dinkel *et al.*, 2004; Varcasia *et al.*, 2007; Omer *et al.*, 2010).

The most notable difference between this study and those of high-prevalence regions is the rare occurrence of *E. granulosus s.s.* Although previous studies in Kenya have confirmed sheep as the main intermediate host of *E. granulosus s.s.* where cysts attained high fertility rates (Dinkel *et al.*, 2004; Addy *et al.*, 2012; Odongo *et al.*, 2018; Nungari *et al.*, 2019), in this study, no cyst from sheep presented this species. The analysis of more cysts from sheep in this region is required. In cattle, the majority of the cysts recovered were of *E. granulosus s.s.* and all were non-fertile, showing that cattle are poor hosts for this species. *Echinococcus ortleppi* is known to occur in cattle and has been reported in Kenya in cattle, goats and sheep (Addy *et al.*, 2012; Mbaya *et al.*, 2014; Nungari *et al.*, 2019). None of the samples from this study presented this species, although the low number of cattle sampled does not permit further conclusions.

The *Taenia* species found in this study were calcified and could not be differentiated morphologically from hydatid cysts. Bovine cysticercosis is rare in Kenya, even in areas with high CE prevalence (Zeyhle, pers. comm.). In Isiolo, a prevalence of 4.95%, 30.69% and 6.93% was reported in cattle by meat inspection, antigen and

Table 3. Conditions of cyst and *Echinococcus/Taenia* species from livestock isolated in Isiolo, Garissa and Wajir counties.

Animal spp. (no. of cysts genotyped)	Nature of cyst	Isiolo county				Garissa county				Wajir county			
		E. canadensis (G6/7)	*E. granulosus* (G1/3)	*T. saginata*	*Taenia* spp.	*E. canadensis* (G6/7)	*E. granulosus* (G1/3)	*T. saginata*	*Taenia* spp.	*E. canadensis* (G6/7)	*E. granulosus* (G1/3)	*T. saginata*	*Taenia* spp.
Goats (*n* = 15)	Calcified	6	7	0	0	1	0	0	0	0	0	0	0
	Viable and no protoscoleces	0	1	0	0	0	0	0	0	0	0	0	0
Sheep (*n* = 7)	Calcified	4	0	0	0	2	0	0	0	1	0	0	0
Cattle (*n* = 13)	Calcified	5	2	1	0	0	0	0	0	0	0	0	0
	Sterile	0	5	0	0	0	0	0	0	0	0	0	0
Camels (*n* = 99)	Calcified and degenerated	43	3	0	0	2	1	0	1	9	1	0	1
	Viable and no protoscoleces	23	0	0	0	1	0	0	0	0	0	0	0
	Fertile	14	0	0	0	0	0	0	0	0	0	0	0

Table 4. Multivariable logistic regression association of cystic echinococcosis with animal species and county of origin in Isiolo, Garissa and Wajir counties.

Variable	Category	Odds ratio	95% CI	*P*-value
County	Isiolo	Reference		<0.001[a]
	Garissa	0.05	0.02, 0.11	<0.001
	Wajir	0.17	0.08, 0.38	<0.001
Species	Cattle	Reference		<0.001[a]
	Goats	0.51	0.20, 1.26	0.144
	Camels	11.84	5.03, 27.89	<0.001
	Sheep	0.89	0.29, 2.69	0.834

[a]Overall *P*-value. CI, confidence interval.

antibody enzyme-linked immunosorbent assay (ELISA), respectively (Onyango-Abuje *et al.*, 1996). Similarly, a high prevalence of cysticercosis by antigen ELISA was observed in western Kenya, possibly due to cross-reaction with other common *Taenia* species such as *Taenia hydatigena* (Fèvre *et al.*, 2017). An unknown *Taenia* spp. was identified in two camels and was 99.66% identical to a *Taenia* spp. isolated from hyena in Ethiopia (Terefe *et al.*, 2014). Although this *Taenia* spp. was first isolated from hyena, it was found to be phylogenetically close to *Taenia solium*. The host range of this *Taenia* spp. and its pathogenicity to humans remains unknown. Domesticated animals were suggested as possible prey for hyena in Ethiopia (Terefe *et al.*, 2014); however, the involvement of camels in the transmission of this species is not clear as both cysts were calcified. Further studies to understand the epidemiology of this *Taenia* spp. are required.

In conclusion, this study reports, for the first time, the characterization of *Echinococcus* species in livestock from Garissa and Wajir counties, and the current situation in Isiolo county, Kenya. The fertility of cysts in camels and dominance of *E. canadensis* (G6/7) highlights the importance of camels in the maintenance of CE in livestock in the north-eastern counties of Kenya. This study provides an opportunity for further studies in humans and domestic dogs to understand fully the epidemiology of CE in these regions.

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Conflicts of interest. None.

Ethical approval. The study complies with the University of Nairobi regulations. This study was approved by the Department of Veterinary Services, Kenya, and permission was obtained from the Directors of Veterinary Services of Isiolo, Garissa and Wajir counties, Kenya.

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