DISTRIBUTION AND GENETIC DIVERSITY OF CYSTIC ECHINOCOCCOSIS IN BUSIA AND BUNGOMA COUNTIES, WESTERN KENYA

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Distribution and Genetic Diversity of Cystic Echinococcosis in Busia and Bungoma Counties, Western Kenya

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DECLARATION

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

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DEDICATION

I dedicate this work to my wife Faith Mutwiri, and my three beautiful daughters; Natalia, Nina and Nyla for their presence in my life which has made it a beautiful adventure.

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AMOVA	Analysis of molecular variance
BLAST	Basic Local Alignment Search Tool
BPH	Benign Prostatic Hyperplasia
CDC	Center for Disease Control and Prevention
CE	Cystic Echinococcosis
CESSARi	Cystic Echinococcosis in sub-Saharan Africa Research Initiative
CL	Cystic Lesion
COX1	Cytochrome C oxidase 1
CT	Computed Tomography
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleoside triphosphates
EBI	European Bioinformatics Institute
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-Linked Immunosorbent Assay
EMBL-EBI	European Molecular Biology Laboratory -
ETB	Ethiopian Birr
FWA	Federalwide Assurance
GENtle	Software for DNA and amino acid editing
GPS	Global Positioning System
HCL	Hydrogen Chloride
HIV	Human Immunodeficiency Virus
ILRI	International Livestock Research Institute

IREC	Independent Ethics Review Committee
IWGE	Informal Working Group on Echinococcosis
JKUAT	Jomo Kenyatta University of Agriculture and Technology
KCl	Potassium Chloride
KEMRI	Kenya Medical Research Institute
KES	Kenya Shillings
KNHS	Kenya National Housing Survey
MEGA	Molecular Evolutionary Genetics Analysis
$MgCl_2$	Magnesium chloride
mtDNA	Mitochondrial Deoxyribonucleic acid
NACOSTI	National Commission for Science, Technology & Innovation
NAD1	Nicotinamide adenine dinucleotide
NADH	Nicotinamide adenine dinucleotide + hydrogen
NaOH	Sodium hydroxide
NCBI	National Center for Biotechnology Information
$(NH4)_2SO4$	Ammonium sulfate
PCR	Polymerase Chain Reaction
PE	Polycystic echinococcosis
pH	Potential for Hydrogen
RFLP	Restriction Fragment Length Polymorphism
Taq	*Thermus aquaticus*
TCS	A Java computer program to estimate gene genealogies
UK	United Kingdom

UOL	University of Liverpool
USA	United States of America
USD	United States Dollar
WHO	World Health Organization

ABSTRACT

Cystic Echinococcosis (CE) is a zoonosis caused by infection with the larval stages of the taeniid cestodes of the species complex *Echinococcus granulosus* sensu lato. It is prevalent among transhumant communities in East Africa, including those residing in northern Kenya. The distribution of the disease is enhanced by several factors, including livestock trade, which may facilitate the spread of CE to non-endemic areas such as Western Kenya. Poor condemnation of offal at the slaughterhouses potentially creates an indirect risk of disease spill-over to humans through dogs. This study: assessed the possible establishment of the CE life cycle by screening for the presence of human CE using ultrasound, examined dogs' infection levels with taeniid eggs and their potential role in contaminating the environment with intestinal parasites. The study also described intraspecific variance of *E. granulosus* s. s. and the parasites' haplotypes in livestock slaughtered in Western Kenya and conducted economic losses analysis through condemned offal. Eight sentinel sites in Bungoma County were purposively selected and a portable ultrasound scanner was used to screen for the presence of human CE**.** In the livestock package, a cross-sectional survey was conducted at slaughterhouses in Bungoma and Busia counties. A post-mortem visit was conducted on livestock sampled at the slaughterhouse, to screen carcasses for CE infection and the organ(s) infected. The DNA was extracted from the protoscoleces and nested PCR performed targeting NADH dehydrogenase subunit I (NAD1) and Cytochrome c oxidase I genes followed by sequencing. In the dog work package, sixteen ruminant slaughterhouses were selected in Busia and Bungoma Counties, and around each slaughterhouse ten homesteads owning free-roaming dogs were identified. A questionnaire was administered on dog management practices to the homestead owner and a faecal sample was collected from the dog's rectum. In Busia County the sampled dog were collared with a GPS tracker to assess their movement patterns. The faecal samples were examined microscopically following the zinc-chloride sieving-floatation technique for the presence of taeniid eggs and other canine intestinal parasites. Polymerase Chain Reaction – Restriction Fragment Length Polymorphism of NADH dehydrogenase subunit 1 gene and sequencing were used to confirm taeniid eggs identified during microscopy. Additionally, the Coproantigen-ELISA was used to detect the presence of taeniid antigen in a sub-set of the faecal samples. Economic losses were established by assessing the losses incurred from condemned offal at the slaughterhouses. In humans, 1002 participants were screened and sixty-seven participants (6.7%) had abnormal ultrasound findings and, of these, 7 (1.1%) had simple liver cysts/CL, as per WHO classification. In Livestock, a total of 153 *E. granulosus* s. s. cysts were sampled from livestock. In total, 135/153 cysts were genotyped as *E.* granulosus s. s. based on Restriction fragment length polymorphism (RFLP) of the Nad1 gene. Upon sequencing 11 *nad1* and 19 *cox1* haplotypes were identified in 120 and 122 sequences, respectively. This work serves as the first report of *E. granulosus* s. s. haplotypes in East Africa and will enhance the understanding of the genetic variability of the cestode sub-region. In dogs, helminths were detected in the 155 dogs sampled. They included hookworms (n=92; 59.4%), ascarids (n=15; 9.7%), and taeniids (n=1; 0.6%). Through Copro-PCR, 13 eggs extracted from the sample of the only taeniid infected dog were sequenced and identified as *E. canadensis* (G6/7) [n=1], *Taenia multiceps* [n=1], and *Taenia serialis* [n=6]; the remaining were

indeterminate. Of the 77 faecal samples tested for *E. granulosus sensu lato* (*s. l.*) with the Copro-ELISA, 64 (83.1%) were negative, 12 (15.6%) were positive, while 1 (1.3%) was suspicious. The human data results contribute to CE baseline data while providing insights on the implementation of ultrasound diagnosis in the field, as recommended by the WHO for targeted control of echinococcosis by 2030. The livestock data results serve as first report of *E. granulosus* s. s. haplotypes in East Africa and will enhance the understanding of the genetic variability of the cestode in the region. The dog data results indicate a relatively high carriage of zoonotic parasites by free-roaming domestic dogs in Western Kenya, which poses a risk to human and livestock populations. This study report for the first time a domestic lifecycle of *Echinococcus canadensis* and *Taenia multiceps* in Western Kenya, as well as a presumptive sylvatic cycle of coenurosis by *T. serialis*. The human study recommends routine screening of the population for CE in the health units as well as deployment of Ultrasound as a diagnostic tool to aid epidemiological investigations. The livestock study recommends sustained assessment of the significance of haplotypes variation in the control of CE in the region. Whereas, the measures to control infection by the parasite will require more understanding of its genetic diversity and how it affects transmission and pathogenicity. The dog study recommends development of an incountry Copro-antigen capacity to aid in surveillance programmes, a broader assessment of dog parasites with zoonotic potential, adherence to slaughterhouse management practices, and dog-ownership programmes to highlight the importance of deworming and restricted dog movements.

CHAPTER ONE

INTRODUCTION

1.1 Background

Cystic echinococcosis (CE) is a disease infecting dogs, humans, and livestock, caused by the tapeworm *Echinococcus granulosus* during its larval stage (CDC, 2022). Cystic echinococcosis is distributed worldwide causing a public health concern in regions that practice extensive livestock farming (Eckert *et al*., 2000, 2001; Garippa *et al*., 2004). It is an evolving and re-evolving disease in different regions of the world, but more particularly has impact in developing countries (Jenkins *et al*., 2005). There has been significant improvement achieved in the research and control of human CE, however, disease transmission has only been interrupted only in a few countries. Some of the islands which have shown some progress in reducing transmission include Cyprus, New Zealand, and Tasmania. At continental levels, however, *E. granulosus* control has been difficult, less effective, costly, and entails continued efforts over decades (Eckert *et al*., 2004).

Cystic Echinococcosis (CE) is a zoonotic disease caused by the tapeworm, *Echinococcus granulosus* sensu lato (s.l.), and is of worldwide public health importance (Thompson *et al.,* 2017) The disease causes considerable economic losses and public health problems in many countries (Budke *et al*., 2006; Eckert & Deplazes 2004) and more significantly in countries where livestock farming is heavily practiced. The species that cause human CE include *E. granulosus* sensu stricto (G1 to G3), *E. equinus* (G4) *E. ortleppi* (G5), and *E. Canadensis* (G6 to G10). (Agudelo *et al*., 2016)

The disease is mainly transmitted by canids and has an array of intermediate hosts, primarily ungulates e.g. sheep, goats, buffalo, horses, cattle, pigs, camels, and cervids (Nakao *et al*., 2013). The dogs maintain the parasite's life cycle and the domestic livestock serve as intermediate hosts (Benner *et al*., 2010). *Echinococcus* eggs exit from dogs/ carnivores through the faeces and may thus contaminate the environment (Adanir & Tasci, 2013). Therefore, animals get infected by grazing

within a pasture environment with dog faeces (Benner *et al*., 2010). Echinococcal eggs hatch within the intestine of the intermediate host, infiltrate the gut, and are transported via the blood to various body tissues and organs. They then progress to form metacestode cysts which ultimately cause severe pathological impairment. Humans get infected accidentally by ingesting taeniid eggs when taking in food or water with echinococcal eggs contaminant, or from handling dog faeces carrying taeniid eggs.

While there has been significant improvement achieved in research and control of human CE, disease transmission has only been interrupted in a few countries, and some islands, such as Cyprus, New Zealand, and Tasmania, have shown progress in reducing transmission. At continental levels, however, control of *E. granulosus* has been difficult, less effective, and costly as it entails continued efforts over decades (Eckert *et al*., 2004).

Africa has been found to have all the circulating species of *E. granulosus* s. l. (Romig *et al*., 2015; Romig *et al*., 2017) except for some countries in West Africa. The disease poses great challenges in countries practicing extensive livestock farming (Romig *et al*., 2011). All Arabic North African countries, including Algeria, Egypt Tunisia, Morocco, Libya, Mauritania, and Sudan show endemicity to *Echinococcus granulosus* (Eckert *et al.*, 2001). However, there is a paucity of data about the situation of CE in central and west African countries. In southern Africa, Zambia and Namibia have reported cases of CE, while scarce data are available from South Africa. (Wahlers *et al*., 2012; Deplazes *et al*., 2017; Romig *et al*., 2017) and Central Africa Republic (Develoux *et al*. 2011).

In East Africa, including Kenya, CE has been highly endemic in pastoral communities since the first cases were described by Ginsberg (1958), Wray (1958), Froyd (1960), and Nelson & Rausch (1963). In Kenya, CE disease is prevalent among the pastoralist and transhumant communities of Turkana and Maasai land (Wray 1958, French *et al*., 1982; Macpherson *et al*. 1984). Turkana holds the previous record for having the highest human CE incidence in the world, with a risk of 220 CE cases/per 100,000 inhabitants (French & Nelson, 1982). However, recent

studies have indicated that CE in Kenya may be more widespread than earlier anticipated. While in Western Kenya no records of CE have been reported, frequent movement of livestock for slaughter from endemic areas like Turkana, Maasailand, and West Pokot County into Western Kenya creates the potential for transmission of this disease.

In the early days, human hydatidosis was reported to be domiciled primarily in Turkana with high infection levels in the North West and North East Turkana and low infections in central and south Turkana, as well as among the lake-dwelling Turkana community, the Pokot and Maasai community (Macpherson *et al*., 1989). However, the opening up and growth of animal trade involving the movement of livestock from these regions of high incidence to areas of low incidence has led to an increased risk of animal-to-human disease spillover. Indeed, Kenya is continuously recording infections in livestock and dogs as evidenced in studies in Masailand (Addy *et al*., 2012); Central Kenya and its neighbourhood (Mbaya *et al*, 2014); Turkana, Masaimara, Isiolo and Meru (Mulinge et.al, 2018), highlighting the growing need to assess whether spillage of infections to human populations is also occurring. This, in turn, will contribute information on the distribution and pathogenicity of prevailing Echinococcus taxa in these regions, allowing for targeted and locally adapted prevention and control efforts (WHO, 2011).

Prevalence and diversity of CE in livestock in Kenya vary across different regions (Macpherson, 1985; Macpherson *et al*., 1989 Romig *et al*., 2011). A livestock postmortem survey conducted in Kajiado and Narok counties by Addy *et al*., (2012) reported CE prevalence of 25.8%, 16.5%, and 10.8% in cattle, sheep, and goats, respectively, which is higher compared to cases the prevalence of postmortem survey conducted in Meru and Isiolo counties where the prevalence of CE in cattle, goats, sheep, and camel was 1.92 %, 0.37%, 4.62%, and 6.94%, respectively (Mbaya *et al*., 2014). The Laikipia region, which sits on the leeward side of Mount Kenya, revealed a CE livestock prevalence of 11.8%, 1.5%, and 2.3% in cattle, sheep, and goats respectively (Gachengo *et al.*, 2017).

E. granulosus s.s and *E. Canadensis* (G6/7) are the most common species identified in Kenya among livestock, humans, and dogs (Romig *et al*., 2015; 2017; Mulinge *et al*., 2018). *E. ortleppi* and *E. equinus* are less common among livestock in Kenya (Romig *et al*., 2017). Kagendo *et al*. (2014), identified the first cases of *E. felidis* in lions and hyenas, while Mulinge *et al*. (2018) identified the species for the first time in dogs in Kenya (Mulinge *et al*., 2018).

Until recently, regions outside Turkana and Masailand were thought to be free from *E. granulosus* since there was no documented evidence until extensive surveys were conducted mainly in dogs and livestock, which are key players in sustaining the lifecycle of the parasite. Mbaya *et al*. (2014) reported a livestock prevalence ranging between 0.4 and 7% in central Kenya, while Mulinge *et al*. (2018) documented the presence of taeniid eggs in 11% of dog faecal samples collected in four regions in Kenya (Turkana, Maasai Mara, Isiolo, and Meru). Recent evidence has also documented the presence of *E. granulosus* in livestock slaughtered in Busia County in Western Kenya (Mutwiri *et al* unpublished). Western Kenya is a region that borders areas of high prevalence of CE and has experienced an increased importation of infected livestock, a factor that could lead to the potential establishment of the parasite life cycle in areas previously considered free from CE. Thus, animal movement along livestock trade routes poses significant challenges to the control of this disease.

Humans can serve as aberrant hosts when they become infected by ingesting tapeworm eggs excreted by infected carnivores. This occurs most frequently when individuals handle or contact infected dogs or other infected carnivores, or inadvertently ingest food or drink contaminated with faecal material containing tapeworm eggs (Torgerson & Heath, 2003). In the typical dog-sheep cycle, tapeworm eggs are passed in the feces of an infected dog and may subsequently be ingested by grazing sheep (Torgerson & Heath 2003; Lahmar *et al*., 2004). These eggs may then hatch into embryos in the intestine, penetrate the intestinal lining, and are then picked up and carried by the blood throughout the body to major filtering organs (mainly the liver and/or lungs). After the developing embryos localize in a specific organ or site, they transform and develop into larval echinococcal cysts in which numerous tiny

tapeworm heads (protoscolices) are produced via asexual reproduction (Zainab, 2013). According to Raether and Hänel (2003), infection with Ecchinococcus has been found in many different sites of the body, namely the brain, mediastinum/diaphragm, heart and blood vessels, spleen, colon, kidneys, Douglas pouch, Fallopian tubes, ovaries, peritoneal cavity, uterus, skeletal muscles, ocular muscles, subcutaneous tissues and bones (Eckert *et al*. 2001). These localizations are of interest not only for epidemiological reasons but also because of the controversial pathogenesis and diagnostic problems that may arise, sometimes leading to an unclear clinical diagnosis (Ahmadi & Badi, 2011).

Currently ultrasound is the best method to assess the prevalence of CE due to the peculiar biological features of this parasitic disease in humans, to its portability and its acceptance by communities throughout the world. Furthermore, US is superior to CT or MRI for staging cysts (Stojkovic *et al*., 2012) which further helps inform treatment options (Junghanss, 2008). These include the "Watch-and-Wait" method for uncomplicated inactive cysts (Brenetti, 2010), surgery, percutaneous treatments, and the use of chemotherapeutic agents for viable cysts. The choice of the optimal treatment option is guided not only by the cyst characteristics, but also by available medical and surgical expertise, healthcare facilities, and the patient's compliance with long-term monitoring.

Technological advancement and increased experience with sonography has aided the establishment of ultrasound as a clinically important, non-invasive, and widely acceptable diagnostic tool (Dietrich *et al*., 2009). US screening does not only guide therapeutic decisions even in remote underserviced areas that lack diagnostic facilities but also helps contribute to knowledge on the burden of such conditions. More often use of the US reveals incidental findings, which are generated in the course of seeking another condition or disease, where these findings may qualify as unsought information by the patient. Therefore, it is imperative that the patient is aware of this possibility before the procedure is conducted, and that alternative therapeutic processes to deal with them if encountered are put in place.

Since the identification of *Echinococcus granulosus* in Kenya in the mid-20th century by Ginsberg 1958, Wray 1958; Froyd 1960; and Nelson & Rausch 1963, few human screening initiatives have been conducted, primarily among the Turkana and the Maasai communities (Macpherson, 1989). Therefore, the objective of this study was to conduct a community ultrasound screening study for CE and other abdominal lesions in Bungoma County, western Kenya. This region represents the larger Lake Victoria basin ecosystem, a region with, concurrently, the highest rural human and livestock population densities in East Africa, operating in a mixed smallholder livestock production system (Fèvre *et al*., 2017). Furthermore, Bungoma County serves as an entry route for livestock from CE-high prevalence areas into Western Kenya, with consequent impacts on disease transmission.

Physical imaging is a more specific diagnostic tool for late-stage CE. However, early diagnosis of CE (when feasible) may provide opportunities for early treatment, more effective chemotherapy, and follow-up (Zhang *et al*., 2012). The World Health Organization (WHO) Informal Group on Echinococcosis has previously published an international consensus classification of ultrasonograms of hepatic cysts (Figure 3.6) A disadvantage of ultrasonography is that cysts in other sites (lung, brain, etc.) cannot be readily detected (WHO, 2003; Eckert & Deplazes, 2004).

Since in continental situations, *E. granulosus* control is more difficult, often less effective, and requires sustained efforts over many decades, the results of this study will serve as a model that promotes the need to prevent establishment of disease in previously CE free regions.

1.2 Problem Statement

While earlier studies on CE in Kenya have been done majorly in Turkana as well as Maasailand, there is evidence indicating a broader spread of the disease across other parts of Kenya (Mulinge *et al*., 2018; Mulinge *et al*., 2020; Nungari *et al*., 2020). However, there is no current data on CE in Busia and Bungoma in western Kenya. Slaughtered livestock originating from endemic areas present with CE suggesting possible entry and transmission of the disease through these routes. It hadn't been clear whether the movement of animals through routes of trade would introduce the

parasite in a new non-endemic foci. At the slaughterhouse level, livestock presenting with CE upon post-mortem remains unknown and this study sought to know the frequency of livestock CE in western Kenya. Data on molecular characterization of the *Echinococcus granulosus* s.l. is available for some regions in Kenya including Turkana, Maasailand, and Central Kenya (Romig *et al*., 2011; Addy *et al*., 2012; Mbaya *et al*., 2014; Mulinge *et al*., 2018; Mulinge *et al*., 2020; Nungari *et al*., 2020) however, there is no record for the disease in the expansive western Kenya region.

The plausibility for *canis familiaris* to contaminate the environment with parasites picked up from the slaughterhouses needed to be assessed in relation to facilitating infection of the zoonotic parasite through dog-human-livestock interaction.

In terms of global disease burden, loss due to CE is USD 1.6 - 3 million per year (Budke *et al*., 2006). This is close to that estimated for African Trypanosomiasis and Schistosomiasis, both of which receive far more attention than CE (WHO, 2004). Approximately 2.1 million people worldwide are infected with CE, which causes significant morbidity (Hotez *et al*., 2014). In the livestock industry, CE causes major losses as a result of condemnation of offal from animals at slaughter and a general reduction in livestock productivity, with more than USD 125,000 billion lost globally on an annual basis (Budke *et al.*, 2006). Earlier studies from across the world, for example in Ethiopia (Getaw *et al.*, 2002; Kumsa & Mohammedzein, 2012) and Iran (Harandi *et al*., 2012) have corroborated the significant losses attributed to CE. Kenya is thought to have a high new infection rate of CE in humans and animals when compared to many other countries, though there is yet no detailed record of economic losses, whether direct or indirect associated with CE in humans and livestock. This study will give the molecular diversity of *E. granulosus* s.l. and its distribution in Busia and Bungoma counties more clarity and documentation.

1.3 Justification

Recent studies have indicated that CE in Kenya is more widespread than earlier anticipated. By the time of conducting this study, there was no existing record of CE in Busia and Bungoma counties of western Kenya, but due to the movement of

livestock for slaughter from endemic areas like Turkana, and west Pokot County, there is a high potential for CE transmission into this new region.

Given that several genotypes of *E. granulosus* exist, and that these may influence biological characteristics of the parasite such as infectivity, pathogenicity, response to chemotherapeutic agents, host specificity, or antigenicity (Thomson & McManus, 2002), it is important to understand the genotypes currently circulating in this region. Determination of both the parasite's transmission dynamics and genetic diversity will be an essential tool for disease control and disease surveillance programmes. Molecular and immunological tools are now available and provide powerful means for unraveling aspects of *E. granulosus* that remain relatively unknown hitherto.

This study was impactful in determining the distribution of *E. granulosus* s.l. in Busia and Bungoma counties of western Kenya through employing available molecular approaches to characterize genetic variations of the parasites among the hosts. The outcome of this study is essential in pushing for greater consideration of mitigation methods for CE control.

1.4 General Objective

To determine the infection levels and genetic variability of *Echinococcus granulosus* across host species of the domestic cycle, and the economic losses of Cystic echinococcosis (CE) in Busia and Bungoma counties, western Kenya.

1.5 Specific Objectives

1. To determine the infection level of CE in humans, livestock, and dogs in Busia and Bungoma counties, western Kenya.
2. To determine *Echinococcus granulosus* s.l. species/strains in humans, livestock, and dogs in Busia and Bungoma counties, western Kenya.
3. To establish the genetic variability (haplotypes) of *Echinococcus granulosus* s.l. in Busia and Bungoma counties, western Kenya.

1.6 Research Questions

1. What is the infection level of Cystic echinococcosis in humans, livestock, and dogs in western Kenya?
2. What species of *Echinococcus granulosus* s.l. are spreading within humans, livestock and dogs in western Kenya?
3. What is the level of haplotype diversity for *Echinococcus granulosus* s.l. among the parasite's hosts in western Kenya?.

CHAPTER TWO

LITERATURE REVIEW

2.1 Distribution of *Echinococcus granulosus*

E. granulosus has a worldwide distribution (Ito *et.al.,* 2003), however, few regions in the world such as Ireland, Iceland, and Greenland are understood to be free of indigenous human CE (Budke *et al.,* 2006). *E. vogeli* and *E. oligarthrus* which cause Human polycystic echinococcosis are confined to Central and South America hence designated the name neotropical echinococcosis (Eckert & Deplazes, 2004). Only limited human infections with *E. oligarthrus* and *E. vogeli* have been recorded elsewhere, and all are suspected to be imported from Latin America (Torgerson, 2010).

Echincoccus granulosus, the causal agent of human hydatidosis, is primarily maintained through dogs and domestic ungulates. Consequently, human behaviour as well as husbandry practices and contact with definitive hosts influence human infections. (Eckert *et al.,* 2001). The annual incidence of human CE in some European hospitals varies from less than one to more than 8 per 100,000 population. Heightened disease levels have been reported in Eastern and Northern Africa (>3%) as well as in South America for instance in Uruguay which recorded an annual incidence of 9.2 per 100,000 population. (Eckert & Deplazes, 2004).

In Kenya, incidences of as high as 5-10% have been recorded in some areas of arid northwest and northeast parts of Turkana County of Kenya (Macpherson, 1989). This community is composed of Nilotic nomadic pastoralists who rely on cattle, sheep, camels, and goats for their sustenance. The region presents a domestic cycle/dog-sheep-dog-cycle of *E. granulosus* (Nelson, 1963). The report by Macpherson *et al.* (1987) states that 3553 nomads in Turkana presented with hydatid cysts after screening with a portable ultrasound scanner or serology; 198 (5.6%) had liver or upper abdominal cysts. Among 2644 who were screened by both techniques, 174 (6.6%) of them had hydatid cysts detected by ultrasonography and 76 (2.9%) were infections were detected by serology. In comparison, ultrasonography was less

expensive, educationally valuable to the people, gave immediate results, and was more acceptable to the participants.

Rachel (2004) reported that between July 1981 and May 2002, 710 surgical cases of hydatid disease were documented in Kakuma Turkana; of these, 277 (39%) were males and 433 were females (i.e. a male: female ratio of approximately 2:3).

Macpherson *et al.* (1989), in their study on hydatid disease in pastoralists, realized a high infection level of hydatidosis by Ultrasound scanning. North-Western Turkana had a prevalence of 5.6% (198/3553) whereas North Eastern Turkana reported a prevalence of 2.1% (72/3462); Central Turkana had a prevalence of 0.3% (4/1508), similar to that of Southern Turkana (4/1361). Eastern Turkana, the area bordering Lake Turkana, showed no existence of disease among the 607 people who were scanned. The mean hydatid prevalence for the Turkana and the surrounding areas (Toposa, Nyangatom, Boran, Maasai, Hamar, and Pokot) at that time was 2.2%, while among the Turkana alone the prevalence of hydatidosis was 3.2%. The different prevalence in the different study areas in Kenya could be ascribed to varying environmental conditions, intensities of livestock stocking, and transboundary livestock migration (Njoroge *et al.*, 2002).

Both young (< 1 year old) and old (>75 years old) patients are vulnerable to infection with *E. granulosus* (Mcmanus, *et.al*., 2003). Inadequate husbandry and meat inspection practices, close contact between humans and dogs in the same environment, (Wachira *et al*., 1991), lack of proper awareness campaigns, and inadequate CE surveillance fundamentally influence the sustenance of transmission cycles to humans. Thus, CE remains among the top important zoonoses worldwide (Raether & Hänel, 2003).

Both Echinococcus vogeli and *E. oligarthrus*, are more prevalent in the Neotropics. Close to 200 cases of polycystic echinococcosis (PE) caused by *E. vogeli* have been recorded in more than 10 countries in South America (D'Alessandro & Rausch, 2008). The metacestodes of *Echinococcus vogeli* are typically found in the liver in humans, from here they spread to the peritoneal and pleural cavities, though other body parts may also be infected. The disease is thus classified into five forms:

infections occurring in the liver and the abdominal cavity (Type I), infections involving the hepatic insufficiency, liver and abdominal cavity (Type II), liver and chest cysts (Type III), mesenteric cysts (Type IV) and calcified cysts in the liver and lung (Type V). *Echinococcus oligarthrus* typically manifests itself as unicystic echinococcosis, a fluid-filled vesicle that is spherical and enlarges concentrically, and does not proliferate exogenously (D'Alessandro & Rausch, 2008).

In the northern hemisphere, Echinococcus multilocularis is widely distributed in carnivores. It is also well spread in parts of central Europe, North America, most of northern and central Eurasia, and spreading eastward to Japan (Eckert & Deplazes, 2004). In contrast to CE, all countries including China have a low prevalence of Alvelor Echinococcosis (AE) caused by *E. multilocularis* (Schantz *et al.*, 2003).

Cystic Echinococcosis (CE) disease is economically important due to its impact on disadvantaged pastoralist communities. It is neglected even in areas where it is reported as highly endemic and is caused by a highly diverse complex of species, usually assigned to "*Echinococcus granulosus*".

2.2 Life cycle and Transmission of Echinococcus

The *Echinococcus* species' life cycle presents with 3-6 mm adult dog tapeworms attaching in the intestinal lining of final hosts, which are carnivores such as dogs, wolves, or coyotes. On the other hand, herbivorous intermediate hosts present with echinococcal cysts, such as cattle, goats, and sheep. Other intermediate hosts range from pigs, camels, and horses, and in many parts of the world form part of the life cycle of echinococcus spp. (Torgerson & Heath, 2003; Moro & Shantz, 2008; Khilgiyaev, 2013).

Figure 2.1 depicts the classic life cycle that revolves around dogs and sheep, Echinococcal taeniid eggs are passed through the faeces of an infested dog and may consequently be taken up by grazing sheep (Torgerson & Heath 2003; Lahmar *et al.*, 2004). Within the intestine, these eggs may then hatch into embryos, and thereafter infiltrate the lining of the intestine and are transported by the blood to major filtering organs of the body (mainly liver and/or lungs). The forming embryos then localize in

definite organs or locations, where they get transformed and then mature into echinococcal cysts wherein plentiful tiny tapeworm heads (protoscolices) are formed via asexual reproduction (Zainab, 2013).

When the dogs ingest viscera containing echinococcal cysts with these protoscolices they get infected. This happens often due to the habit of feeding dogs with viscera, especially where the home slaughter of sheep and other livestock takes place. The protoscolices then peg onto the lining of the dog's intestines, grow and develop into mature adult tapeworms in between 40-50 days at which point they can produce eggs which are passed to the environment with the dog's faeces (Khilgiyaev, 2013).

Humans also act as intermediate hosts of the life cycle of the tapeworm. They may get the infection after taking in through the mouth of tapeworm eggs excreted by infected carnivores. This occurs often when individuals come in contact with dogs or other carnivores infested by the parasite, or quite unintentionally consume food or drink substances contaminated with tapeworm eggs (Torgerson & Heath, 2003).

The frequency at which carnivores feed on offal, the age of the intermediate host carrying the infection, and the levels of the parasites within the offal, all determine the infection magnitude in the final host (Bazalar *et al*., 1997). The final hosts and intermediate host immunity also play a big role in the parasites' transmission. When dogs for herding share space with pasture animals, dogs through their faeces may contaminate grazing, leading to the uptake of dog faeces by pasture animals leading to infection acquisition (McManus *et al*., 2003). Environmental temperature & environmental humidity can affect the survival of *E. granulosus* (McManus *et al*., 2003).

The parasite's shelf- life, and the regularity of treatment with antihelminthics, have a role in host infection. The Sheep once infected classically remains within the disease for life. In hosts such as dogs, the parasites can be cleared through treatment.

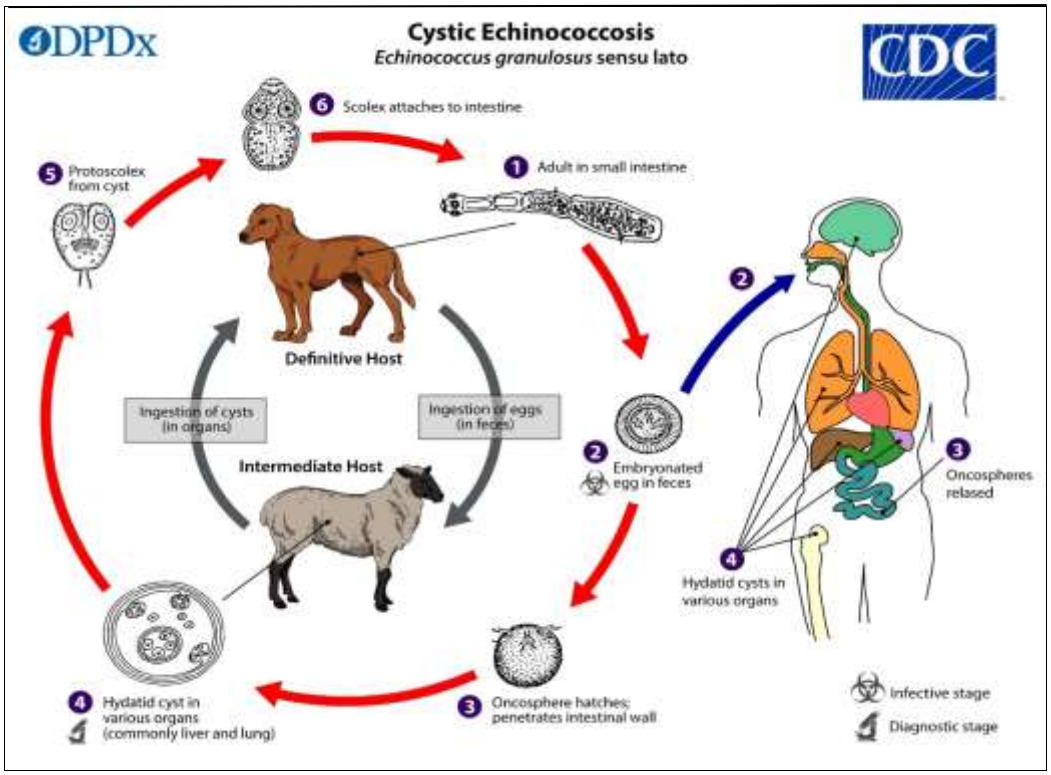

Figure 2.1: Life cycle of E. granulosus s.l. (retrieved from the CDC website on 11.11.2023). The adult *Echinococcus granulosus* (sensu lato) (2—7 mm long) [1.] Resides in the small intestine of the definitive host. Gravid proglottids release eggs [2] that are passed in the feces, and are immediately infectious. After ingestion by a suitable intermediate host, eggs hatch in the small intestine and release six-hooked oncospheres [3] that penetrate the intestinal wall and migrate through the circulatory system into various organs, especially the liver and lungs. In these organs, the oncosphere develops into a thick-walled hydatid cyst [4] that enlarges gradually, producing protoscolices and daughter cysts that fill the cyst interior. The definitive host becomes infected by ingesting the cyst-containing organs of the infected intermediate host. After ingestion, the protoscolices [5] evaginate, attach to the intestinal mucosa [6] and develop into adult stages [1] in 32 to 80 days. Humans are aberrant intermediate hosts, and become infected by ingesting eggs [2] Oncospheres are released in the intestine [3] and hydatid cysts develop in a variety of organs [4] If cysts rupture, the liberated protoscolices may create secondary cysts in other sites within the body (secondary echinococcosis) (https://www.cdc.gov/dpdx/echinococcosis /index.html).

2.3 Canine Echinococcosis

The domestic dog is the primary host that carries the adult stage of *E. granulosus*, but in some regions, carnivores from the wild take part in maintaining the Echinoccus life cycle (Eckert & Deplazes, 2004). Besides the many benefits that man gets from dog ownership, there are also associated likely health risks due to the possible passage of agents of diseases such as echinococcosis, toxocariasis, rabies, leishmaniasis, plague, chagas disease, and other bacterial infections (Chomel and Sun, 2011; Deplazes *et al*., 2011). *E. granulosus* infects flesh eaters of different types but the *Canis familiaris* (local canine) is the dominating definitive host where *E. granulosus* causes the intestinal type of echinococcosis. Canine echinococcosis is often innocuous as it does not instigate significant illness in the host carrying its adult stage, even when there is a substantial infection load (Eckert, *et al*., 2001). Young canines might have a big belly appearance in overwhelming infections which can lead to occasional obstruction of the digestive tract (Soulsby, 1982). Canines get infected when they consume hydatic cysts contained in offal with feasible protoscoleces (McManus *et al*., 2003). These protoscoleces evaginate and attach to the intestinal lining of the canine then transform to the adult phase in 4 - 5 weeks. When the worm achieves sexual maturity, eggs or gravid proglottids discharge themselves in the defecation leading to environmental contamination.

The parasite infiltrates profoundly in the villi of the epithelium inside the gut, yet often doesn't bring about any noteworthy pathology. Little features occur, for example, the epithelial cells become flat, minor cell penetration of the mucosa, and expanded generation of mucus. Circulatory antibodies might be produced as a result of the scolex discharging the excretory/secretory products. Although it is uncommon for canines and felines to take up the intermediate hosts position for *E.granulosus* (Pawlowski *et al*., 2001); simultaneous infection in canines as both intermediate host and the definitive host has been reported by Torgerson and Budke (2003). Enlargement of the abdomen and the presence of ascites and hyper-c-globulinaemia are some clinical features of dogs found with liver and peritoneum metacestodes (Haller *et al*., 1998).

Understanding dog population size, basic dog ecology and behavior, and infection rate with *Echinococcus* would be important for efficient hydatid control programs (Van Kesteren *et al*., 2013). The sensitivity and specificity of coproantigen ELISA is reasonable and can be used to screen individual dogs in populations. This method is advantageous in the sense that numerous samples can be examined by one person per day (Deplazes *et al*., 2003). *E. granulosus* infection can then be confirmed or excluded by a more sophisticated PCR test which is a highly sensitive and specific secondary test (Eckert & Deplazes, 2004).

2.4 Cystic Echinococcosis infection in the human body

Although *Echinococcus* most often affects the lungs and liver, infection in other organs has been observed in 20-30% of the patients. According to Raether & Hänel (2003), infection with *Ecchinococcus* has been found in many different sites of the body, namely the brain, mediastinum/diaphragm, heart and blood vessels, spleen, colon, kidneys, Douglas pouch, Fallopian tubes, ovaries, peritoneal cavity, uterus, skeletal muscles, ocular muscles, subcutaneous tissues, and bones. While some of these presentations are rare they show the possibility of infections with *Echinococcus* in almost all parts of the body**.** For example, a large intra-myocardial cystic mass was discovered in a transthoracic echocardiogram of a healthy man of 54 years from a rural area in Spain (David, *et.al*., 2007). In another case, a 45-year-old male previously reported to be well reported being generally unwell for 20 days, with dyspnea and fever. Within the tricuspid valve of the heart was a 3cm x 3cm round cystic structure revealed by transthoracic echocardiography. There were numerous calcified daughter cysts, which were agreeable for diagnosis as hydatid cysts. *E. granulosus* antibodies serology turned positive and confirmed the diagnosis (Rezaian & Aslani, 2008). In 2008, a six-year-old girl from a northern Saskatchewan indigenous community in Canada was diagnosed with a cerebral cystic hydatid (Himsworth *et al.,* 2010). A woman of 45 years visited a gynaecologist with an axillary region mass; on examination, a hard, semi-mobile, painless mass of 3cm diameter was found. Lymphadenopathy was revealed by ultrasonography and although occult breast cancer was suspected a pathological examination of a biopsy from the mass showed a hydatid cyst with germinative membranes (Ozsoy, *et al*,

2011). In a 35-year-old woman without any history of farming or raising livestock, a simple cyst with a well-defined border and intact thyroid was noted in the neck region by computed tomography (CT) scan. The patient underwent surgery and a left thyroid lobectomy was performed. Four cysts were removed from the thyroid, anterior neck, and submandibular region (Moghimi *et al.*, 2009). Another 30-year-old lady reported in hospital with unclear abdominal pain that had lasted for one year. While overall clinical assessment revealed no abnormality, a further systemic assessment showed a lump in the right hypochondrium. An abdominal ultrasound showed an upper kidney cyst, measuring 20 cm in maximum dimension. A CT scan of the abdomen confirmed the cystic feature on the right kidney. The rest of the abdominal viscera had no lesions. A verdict of rare echinococcosis of the kidney was passed based on the presence of a few daughter cysts at the bottom of the kidney (Shukla, 2011). A case report from Iran indicated a primary hydatid cyst situated in the left lobe of the thyroid gland (Moghimi *et al.*, 2009). A male of 44 years of age presented with a 4-week history of non-specific abdominal pain. Abdominal ultrasound showed an enlarged spleen and an abdominal CT revealed a splenic calcified hydatid cyst, without the involvement of any other abdominal organ (Vezakis *et al.*, 2012).

These reports confirm that *Echinococcus* cysts have been found in very unlikely sites in the human body. These localizations are key not only for epidemiological reasons but also would help unravel pathogenesis controversies and future diagnostic problems, which would otherwise lead to unclear clinical diagnosis (Ahmadi & Badi, 2011).

The growth dynamics of the cyst(s) are different between specimens from various parts of the world. For example, fast-growing cyst(s) in the liver and the abdomen is best known in the Turkana area of Kenya; on the other hand, slow-growing CE has been reported from Alaska. This could be as a result of variations in growth of parasitic cysts across different geographical regions due to haplotype changes. Statistics on echinococcosis provided in the highly referenced “sourcebook” edited by Eckert *et al*. (2001) show that 70-75% of patients with *E. granulosus* infection have hepatic involvement, while 17-22% of the patients have pulmonary

manifestations of the disease; other organ sites are usually only involved in 0.5- 5% of cases.

2.5 Diagnosis

Measurement of humoral and cellular responses in humans is a prerequisite for developing effective serodiagnostic tools. Future studies are needed to ascertain whether responses of cell-mediated immunity play a significant role in protecting the host against infections of *E. granulosus* or *E. multilocularis*. Some indirect evidence, however, suggests that these responses may be important. In a study on *E. multilocularis* in mice, the depletion of T cells enhanced the metastasis (Baron & Tanner, 1976, Zhang *et al*., 2008). Immunodiagnostic tests are quite dependable for AE diagnosis than for CE because more specific antigens to AE are now available for primary diagnosis or confirmation of imaging results (Eckert & Deplazes, 2004). During infection confirmation and monitoring of control programs for epidemiological studies, serological tools become potentially important. However, none of the diagnostic kits developed so far have been accepted by clinicians and, therefore, efforts should continue to develop diagnostics or improve existing ones (Zhang *et al.,* 2012).

Physical imaging is used as a diagnostic tool for late-stage CE infection. Opportunities for early treatment can be enhanced by early diagnosis of CE (when feasible) as it also enhances more effective chemotherapy, and follow-up (Zhang *et al.,* 2012). The Informal group of WHO for Echinococcosis published the international consensus on ultrasonograms and the classification of hepatic cysts. However when cysts occupy extra hepatic cysts e.g. in the brain and lungs they cannot be readily detected which is the main drawback of the ultrasonography (WHO, 2003; Eckert & Deplazes, 2004;).

Modern imaging techniques facilitate the visualization of cyst(s) at various sites of the body. The introduction of the WHO staging classification for a hepatic cyst(s) was a major advancement in international standardization (WHO, 2003). Hepatic and abdominal cyst(s) can now be categorized into stages CE1 to CE5 and, accordingly, the treatment strategy can be guided using this classification. The value of this

categorization of cysts was demonstrated in large prospective clinical and epidemiological surveys conducted in China (Yang *et al*., 2007). Polycystic Echinococcosis (PE) due to *E. vogeli* has a different clinical and imaging presentation in humans as compared to cases associated with *E. granulosus* from the cone of South America (D'Alessandro & Rausch, 2008).

DNA analysis of *Echinococcus* species can be done by Polymerase Chain reaction using protoscoleces or tissues. Three mitochondrial and one nuclear sequences from *E. oligarthus* are published in data banks(Eckert & Deplazes, 2004)

Prevalence of canine echinococcosis can be determined by examination of purgative samples or scrapings of the intestinal wall after necropsy, as well as identifying whole worms, eggs, and/or proglottids (Eckert *et al*., 1984; Allan *et al*., 1992; Craig, 1993).

2.6 Epidemiology and Genetic Diversity of *Echinococcus* spp.

This study intended to identify species/genotypes of *E. granulosus* as well as determine genetic variability and haplotype diversity of the parasite in livestock slaughtered in western Kenya. The livestock were mostly imported from neighboring counties to serve the need for meat protein in the counties of the former Western Province. The movement of animals through routes of the trade from north western Kenya to western Kenya potentially carries the risk of contaminating a new echo niche with the parasite and establishing a life cycle that may take many more years to disrupt. The ownership of trekked animals changed hands severally, which complicates the follow-up to the specific homestead the animal was sourced first time in the chain of trade. These results herein would therefore rightfully be superimposed on the counties of animal origin.

Bungoma livestock markets act as the gateway for animals arriving from west Pokot and Turkana into western Kenya. The slaughterhouses at Kimilili and Chwele are among the busiest and equally recorded high infections with CE among slaughtered animals. Fewer animals from west Pokot would get into Busia county directly through the Butula slaughterhouse.

One of the most important methods used for the genetic characterization of *E. granulosus* is the characterization of the nuclear *Nad1* gene. The *Nad1* gene is a single-copy gene that is located on the *E. granulosus* genome and is highly variable. This variability is due to the presence of many different single nucleotide polymorphisms (SNPs) throughout the gene.

E. granulosus, a cryptic species complex and the *E. granulosus s. s*. is the former sheep strain, Tasmanian sheep strain, and buffalo strain of *E. granulosus* previous classified as genotypes, G1–G3 respectively, but now insignificant because mtDNA sequence divergences are quite low and overlapping occurs among these genotypes (Vural *et al*., 2008; Sharbatkhori *et al*., 2009; Abushhewa *et al*., 2010). Therefore, the use of mtDNA haplotypes is more plausible in population genetics and phylogeographic analyses (Nakao *et al*., 2010a; Casulli *et al*., 2012; Yanagida *et al*., 2012).

In Africa at least five independent genotypes exist (Magambo *et al*., 2006), and these have very highly different biological characteristics e.g. infectivity to humans. The southern parts of South America have areas highly endemic to CE, other regions include the southern part of the former USSR, the Mediterranean coast, Kenya and Uganda, the Middle East, northern Africa, south-western Asia, Australia, and New Zealand. Of the population of the Tibetan Plateau, 6.6% have been found to have cysts whereas, in southern Argentina, it was 26.7 cases per 100,000 were found (WHO, 2005).

An epidemiological survey in east Libya reported an estimated incidence rate of surgical cases of *E. granulosus* to be at least 4.2 cases per 100,000. The majority of the cases were female patients and the prevalence of *E. granulosus* infections were from livestock (determined during post-mortem inspections at abattoirs), 20% in sheep, 13.6% in camels, 11% in cattle, and 3.4% in goats (Tashani *et al*. 2002). Livestock infection was age dependent, and largely more prevalent in females than in male animals. There were also size differences between the parasites isolated from humans and those from camels, but molecular analysis by sequencing of the cox1 gene in the *E. granulosus* protoscolex specimen indicated that irrespective of

whether the specimen was of human or animal origin, the sequences matched those of the *E. granulosus* common sheep strain (Tashani *et al*. 2002). Studies conducted on specimens obtained from humans in *E. granulosus* in Turkana (North West Kenya) and the Maasailand (South West Kenya), two geographical locations separated by a hydatid disease free-zone of 250-800 kilometers long, showed that cattle, sheep, goats, and humans served as intermediate hosts while definitive host role was served by the domestic dogs. Isolates of *E. granulosus* from various hosts in Kenya present with similar morphological and developmental characteristics both *in vitro* and *in vivo* (Wachira *et al*. 1993).

As the various species cannot be reliably identified at the cyst stage, and the necessary molecular tools are of rather recent development, very little epidemiological data are available (worldwide) on the medical and economic impact of the various *Echinococcus* species and genotypes. Most data available are from southern Europe, where the epidemiological pattern was shown to be highly complex. Sheep appear to be the main reservoir for the G1 strain of *E. granulosus* which is assumed to be the main genotype infecting humans. However, in Italy, the majority of the human patients examined yielded the G3 strain, which also occurs in water buffalo in Italy, but other host preferences remain unclear (Jenkins *et al*., 2005). Besides that, *E. canadensis* (G7) is also present in Italy in pigs, goats, and other livestock, but rarely seems to infect humans, while no records of *E. equinus* or *E. ortleppi* (which infect donkeys, horses, and cattle) are known to infect humans in that country (Jenkins *et al*., 2005; Varcasis *et al*., 2006; Busi *et al*., 2007; Varcasia *et al*., 2007). In Africa, initial studies showed a similarly confusing pattern and this led to the hypothesis that G1 might be the principal agent of human disease on the continent. However, a comprehensive picture of the importance of the various animal species in the transmission of the various *Echinococcus spp.* and strains is still lacking. This study, therefore, seeks to partially contribute to this hypothesis.

NADH dehydrogenase 1 (ND1) and mitochondrial cytochrome oxidase subunit 1 (COX1) genes sequences show ten diverse genetic types of *E. granulosus* (G1-G10), (Bowles & Mc Manus, 1993a; Bowles *et al*., 1994; Snabel *et al*., 2000).

The G1 *E. granulosus* sheep strain could be present in sheep, goats, cattle, and man, while the G6 *E. granulosus* camel strain has been found in camels and seldom in goats. The PCR-RFLP patterns and cox1 sequences of *E. granulosus* reveal a close relationship between the camel strain to a form of Eastern Europe *E. granulosus* found in pigs (Bowles, *et al*., 1992; Bowles & McManus, 1993b), and morphological evidence supported this relationship (Eckert *et al*. 1993). A much larger number of *E. granulosus* isolates have been examined by the rDNA PCR/RFLP approach than had been previously possible (Wachira *et al*., 1993).

An epidemiological survey conducted in Turkana County and also in the Maasai region, of 412 *Echinococcus* samples isolated from humans, sheep, goats, camels, cattle, or pigs and tested by specific PCR, hybridisation, and sequencing of genes, revealed conformity of 100% by all three methods (Dinkel. *et al*., 2003). The first evidence of *E. ortleppi* in Kenya was isolated from a pig and this was confirmed through sequencing of cox1 and nad1 genes. Of 117 human samples, 116 were of G1 genotype, and the remaining 1 isolate was assigned to genotype G6 of *E. granulosus*. The 1 G6 genotype was later confirmed by cox 1 and nad1 sequencing, and this became the first described case of a camel strain (G6) infection in humans from East Africa (Dinkel. *et al*., 2003).

Despite the high infection levels of CE in Turkana, and knowledge of history and community-based studies on *Echinococcus,* a gap exists regarding the genetic characterization of *Echinococcus* genotypes. This study therefore aimed at characterizing the various *Echinococcus* genotypes responsible for CE in Kenya and documenting their distribution.

2.7 Control and Treatment: Advances and Challenges

It is common knowledge that efficient meat inspection service, through establishing well-equipped standardized abattoirs, may act as an important monitoring system in the control of animal diseases. In addition, creating public awareness of the zoonotic importance of hydatidosis as well as control of stray dogs minimizes the risk of disease acquisition (Getaw *et al.,* 2002). A decrease of CE has been reported in studies where the breeders show meticulous and reduced self-slaughtering, better

general and sanitary attitude, adequate provision of safe drinking water, and enhanced deworming and control (antihelminthic treatment) of the dog population (Logar, *et al.*, 2008). It has been highlighted that in Ethiopia, where the majority of the pastoralists are Muslims and do not keep dogs, this religious aspect has indirectly contributed to a low prevalence in some regions (Erbeto, *et al.,* 2010). Providing portable ultrasound (US) screening scanners and a short Focused Assessment with Sonography for Echinococcosis course for general practitioners; together with the partnership between surgeons, radiologists, veterinarians, and paramedics as well as Albendazole administration, has aided in averting clinical complications which result from unrestricted growth of the cyst. It also helped minimize social and healthcare costs incurred by rural patients (Del Carpio *et al.,* 2012).

The WHO Professional consensus on CE and AE diagnosis and treatment in humans is based on image details at various stages which helps advise either of the subsequent decisions: surgery, anti-infective drug treatment, percutaneous treatment or watch and wait (Brunetti, *et al.,* 2010). Effective control for cysticercosis elimination in developed countries and elimination of echinococcosis has been reported for some island regions, namely, Iceland, Tasmania, and New Zealand (Carabin, *et al.,* 2005).

Efforts aimed at controlling and limiting disease transmission in areas of high endemicity would be highly aided by specifying variation of strains as a prerequisite, especially in regions of exhibiting behavioral association between livestock and humans (Raether & Hänel, 2003). Even though a highly effective recombinant vaccine against *E. granulosus,* EG95, has been developed, and some immune responses involved in infection have been addressed, there remain some unresolved questions regarding hydatid immunology whose answers may help improve vaccine design (Zhang *et al.,* 2008).

In Southern Africa, while CE is appreciated to be a clinical problem, there is not much documentation about the disease epidemiology. Additionally, no clear description of the disease spectrum has been documented and concerns exist that HIV-CE coinfection might lead to disseminated and severe disease presentation. This

would be particularly significant for a country with a high prevalence of HIV (Wahlers *et al.*, 2011). Despite decades of control efforts, the elimination of CE has only been achieved in a few places, and effective control requires long-term efforts (Craig *et al*., 2007). Moreover, treatment involves invasive procedures (percutaneous treatment techniques) of the abdomen or pulmonary as well as chemotherapy over a long period which may be costly and/or unavailable in remote areas (Buttenschoen *et al*., 2009).

2.8 Social Economic Impact and Disease Burden

The economic losses from CE can come from a variety of sources, including decreased productivity and income from affected livestock, increased veterinary costs, and lost income from tourism in areas where the disease is prevalent. Additionally, CE can also result in significant costs for human healthcare, including the cost of diagnosis and treatment, as well as lost productivity from individuals who are affected by the disease. The total economic impact of CE can vary depending on factors such as the level of infection in the population and the specific industries that are affected. Cystic echinococcosis is a serious public health problem in many countries, particularly in developing countries where the disease is endemic. The World Health Organization (WHO, 2001) has estimated that around 2 million people worldwide are affected by CE, with the majority of cases occurring in the Mediterranean region, Central Asia, China, and South America. The economic impact of CE is significant, with estimates of the total annual cost of the disease ranging from $1 billion to $3 billion globally.

In China, the economic burden of CE has been estimated to be around $1.2 billion per year (Li *et al*., 2013). A study by G. Li *et al*. (2013) found that CE resulted in decreased productivity and income from affected livestock, as well as increased veterinary costs. In addition, the disease also resulted in significant costs for human healthcare, including the cost of diagnosis and treatment, as well as lost productivity from individuals who are affected by the disease.

In the United Kingdom, the annual cost of CE in sheep has been estimated to be £3.3 million (Torgerson *et al*., 2008). The researchers found that the majority of this cost

was due to decreased productivity and income from affected animals. In Switzerland, the total cost of CE in humans was estimated to be around €5 million per year (Gottstein *et al*., 2009). A study by Gottstein *et al*. (2009) found that this cost was primarily due to costs for diagnosis and treatment, as well as lost productivity from individuals who are affected by the disease.

In Africa, CE is considered a neglected zoonosis with a significant economic burden on affected communities and countries. A study by Rudge *et al*. (2017) found that CE caused significant economic losses in pastoral communities in Ethiopia, due to decreased productivity and income from affected livestock, as well as increased veterinary costs. Another study by Mahamoud *et al*. (2018) estimated that the total economic impact of CE in the livestock sector in Tanzania was around $5.5 million per year, primarily due to decreased productivity and income from affected animals. A study by El-Sayed *et al*. (2016) found that CE also resulted in significant costs for human healthcare in Egypt, including costs for diagnosis and treatment, as well as lost productivity from individuals who are affected by the disease.

Overall, CE is a serious public health problem that results in significant economic losses globally. These losses come from a variety of sources, including decreased productivity and income from affected livestock, increased veterinary costs, and lost income from tourism in areas where the disease is prevalent. Additionally, CE also results in significant costs for human healthcare, including the cost of diagnosis and treatment, as well as lost productivity from individuals who are affected by the disease.

In conclusion, CE is a serious public health problem that results in significant economic losses worldwide. These losses come from a variety of sources, including decreased productivity and income from affected livestock, increased veterinary costs, and lost income from tourism in areas where the disease is prevalent. Additionally, CE also results in significant costs for human healthcare, including the cost of diagnosis and treatment, as well as lost productivity from individuals who are affected by the disease.

Cystic echinococcosis remains a neglected zoonotic disease even though its global burden of disease might be as high as that of other diseases such as Dengue, African Trypanosomiasis, Schistosomiasis, Onchocerciasis (Budke *et al.,* 2006). Cystic echinococcosis is estimated to cause 1, 009, 662 DALYs in humans, and about $141 million and possibly up to $2.1billion economic loss in the livestock industry (Budke *et al*., 2006)

Global human-associated losses when underreporting has not been factored in are estimated at USD 193,529,740, whereas losses after adjustment for underreporting is done are estimated at USD 763,980,979 (Budke *et al.,* 2006). In an Ethiopian study, high hydatidosis economic losses were estimated at an annual loss of USD 2013.94 (45,532.9 Ethiopian Birr), while the estimated bovine hydatidosis annual loss due to offal condemnation (lung and liver) and carcass weight loss in the same study areas was USD 1132.65 (25,608 ETB) (Getaw *et al.,* 2002). The total cystic echinococcosis annual economic loss incurred from goats and sheep slaughtered in Jimna town restaurants was valued at 149,312.8 USD (249,324 ETB) (Kumsa & Mohammedzein, 2012). In Iran the overall annual cost of CE was estimated at USD 232.3 million (95% CI USD 103.1-397.8 million), factoring in both direct and indirect costs. Human CE estimated costs were estimated at US$93.39 million (95% CI US$6.1–222.7 million), and livestock annual cost associated with CE was estimated at US$132 million (95% CI US$61.8–246.5 million) (Harandi *et al*., 2012). There is no clear account of the disease burden in most African countries, specifically in relation to human infection.

Cystic echinococcus in both humans and animals has major economic consequences (Togerson & Budke, 2003). Economic losses in the human sector arise from diagnosis, surgery, and chemotherapeutic treatment, hospitalization, recuperation, life impairment, and mortalities. On the other hand, economic losses associated with livestock are associated with a decrease in carcass weight, decreased milk output, and lowered fertility rates, as well as increase in the amounts of offal condemned. Estimating this burden from animals and human is critical in designing programs for the control of parasitic zoonoses (Budke *et al*., 2006).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area and Population

The study was done in two main counties of western Kenya – Busia and Bungoma (Figure 3.1).

Bungoma County sits within the former Western Province of Kenya. Its capital is Bungoma Town. It has an area of 3,023.9 km² and a population of 1,670,570, of which 812,146 are males and 858,389 females, as per the 2019 census (KNHS, 2019). Only 12.5% of this population live in urban or semi-urban areas, with the rest living in the rural parts of the county (KNHS, 1999).

Busia County borders Bungoma County to the north, Kakamega County to the east, and Siaya County and Lake Victoria to the southeast and south respectively. It is bordered to the south by Lake Victoria with some rivers pouring emptying into it. It has an area of 1,696.3 km^2 and a population of 743, 946 people (KNHS, 2009).

The main source of livelihood in this county is small-scale crop and livestock farming, though substantial fishing takes place near Lake Victoria in the southern part of the Busia county (Kristjanson *et al*., 2004). Busia and Bungoma counties border Uganda on the west, with frequent cross border trading of livestock, agricultural products, and manufactured goods taking place.

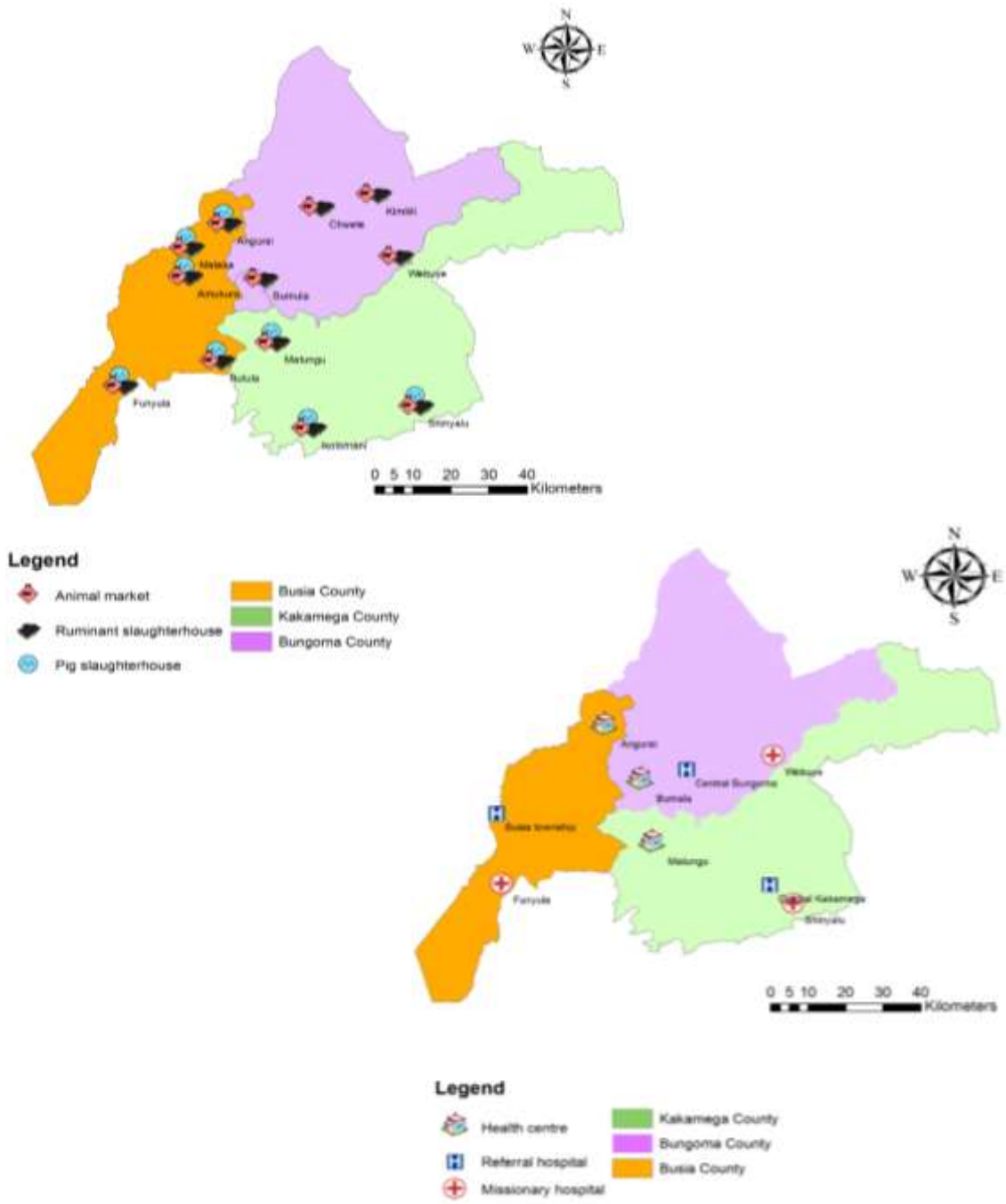

Figure 3.1: Maps of Western Kenya showing the sampling points

(Source: ZooLink, ILRI)

3.2 Study sites

For the dog and human work, the study was conducted in eight semi-urban sites in Busia County and a similar number in Bungoma County (Figure 3.1). In Busia County, the sites included: Amerikwai, Amukura, Bumala, Busia, Funyula, Malaba, Mudembi and Nambale. In Bungoma County, the study sites included: Tongaren (Naitiri), Webuye East (Misikhu), Webuye East (Wanaichi), Kimilili (Kamukuywa), Kimilili (Kimilili town), Kabuchai (Chwele), Kanduyi (Mayanja) and Bumula (Kimwanga). The Bungoma study area had a combined approximate population of 208, 685 persons. These sentinel sites were selected purposively, based on their proximity to slaughter houses, livestock markets health, or social facility where human congregate. For the livestock study, the same sixteen sites; eight from each county, had one slaughterhouse which included in the study for livestock post-mortem survey.

3.3 Selection Criteria

3.3.1 Inclusion Criteria

1. All carcasses of livestock slaughtered in the selected 16 slaughterhouses of Busia and Bungoma counties in western Kenya
2. All free roaming owned dogs in the homesteads of Busia and Bungoma counties
3. All humans in Bungoma county who consented for US scanning for CE

3.3.2 Exclusion Criteria

1. All livestock carcases not indicative of CE infection
2. All free roaming owned dogs from homesteads more than a kilometre away from the nearest slaughterhouse
3. All humans from outside of Bungoma county

3.4 Sampling Technique

This study involved the use of simple random sampling for carcasses of livestock slaughtered in the 16 selected slaughterhouses in Busia and Bungoma counties and a randomly selected dog from each of the ten randomly selected households around each slaughterhouse.

3.5 Sample Size Calculation

3.5.1 Sample Size Calculation for Human

Guided by the proportion confidence interval:

$$E = Z_{\frac{\alpha}{2}}\sqrt{\frac{pq}{n}}$$

Therefore, $n = Z^2_{\frac{\alpha}{2}}\frac{pq}{E^2}$

If the true proportion is not given, and we are guided by the Null hypothesis "The proportions of the infected and non-infected are equal", then we assume P = 0.5 and 1-P = 0.5.

In every County, the error is taken to be the prevalence as recorded in endemic areas such as in the Turkana region. With a 99.5% Confidence interval and being guided by a prevalence of 5%, (Nelson, 1986) from the studies conducted in Turkana, (unpublished data) n will be given by:

$$n = 2.81^2\frac{0.5 \times 0.5}{0.05^2} = 790$$

Therefore 790 people or more would undergo mass US scanning for CE.

3.5.2 Sample Size Calculation for livestock

Four slaughterhouses were identified as sampling points for this study per county. Therefore, a total 12 slaughterhouses were included for livestock infection and post-mortem survey for CE in the three counties of western Kenya. The number of

slaughterhouses was conveniently determined in the larger study (ZooLink) on the surveillance of zoonotic diseases in western Kenya. (http://www.zoonotic-diseases.org/project/zoolink-project/)

With 95% Confidence interval and being guided by the prevalence of 26% for cattle, 17% for sheep, and 11% for goats from the studies conducted in Masailand (Addy *et.al*, 2012), the average infection levels for livestock was 18%, n will be given by,

$$n = 1.96^2 \frac{0.18 \times 0.82}{0.05^2} = \quad 226$$

3.5.3 Sample Size Calculation for Dogs

With a target population size of dogs estimated to be equal to that of Siaya County (Muriuki *et al*., 2016) sample size calculation for dogs was determined as follows:

Target population, N, = 233, 973

Percentage estimated with the event of interest in the target population, p, = 20%

q = 1-p

Confidence interval, d, = ± 5%

Confidence coefficient = 95%

ICC = 0.1 as the best guess (for intra-cluster correlation of dogs around the same slaughterhouse)

Estimated design effect (DEFF) = 1 + ICC (cluster size – 1) = 1 + 0.1 (10 – 1) = 1.9

The sample size with an average number of 10 observations per cluster was:

$n = deff\ x\ \{{Npq}/{\frac{d2}{1.96*2}\ N - 1 + pq}\}$ = 350 dogs = Sampling 10 dogs for each slaughterhouse for the available 35 slaughterhouses would give us 350. However,

there were only 8 slaughterhouse sentinel sites therefore a proportionate number of 80 dogs for each of 4 slaughterhouses in each county were sampled.

Using a proportion of 8 slaughterhouses in each county, 80 dogs per county constituted our sample size. A faecal sample per dog was collected as well an interview with dog owner was conducted. The dogs in Busia County were collared and GPS-tracked.

3.6 Study Design

The study design involved a cross section study where the determination of presence or absence of disease was determined at one point in time.

A survey was done to collect 159 dog faecal samples from Bungoma and Busia counties, 192 livestock cysts samples, and 1002 humans screened for human hydatidosis. A survey for human CE was specifically conducted in the county of Bungoma which had a preliminary high incidence of livestock CE and was the transit route for livestock from Echinococcus endemic areas of Turkana and West Pokot. Eight (8) ruminant slaughterhouses in western Kenya were identified where a postmortem survey was conducted on livestock that came for slaughter. Around each slaughterhouse, 10 homesteads that kept dogs were traced for dog faecal sampling. From the preliminary results on the dog and livestock samples, Bungoma County was considered a potential area for human infection therefore, 8 sites, proximal to the dog study sites and slaughterhouses, were identified for a community screening of human hydatidosis. Questionnaires were administered to the dog homestead owner and a faecal sample was collected directly from the dog's rectum. The faecal samples were processed for microscopical examination of taeniid eggs and an aliquot was prepared for Copro ELISA technique for Echinococcus antigen detection.

3.7 Livestock Cysts Sample Collection and Storage

From 12 sentinel sites of Busia and Bungoma Counties (Figure 3.1), postmortem was conducted on livestock slaughtered. Livestock bio data regarding the origin, age and sex of the animal was recorded at the slaughterhouse. Hydatid cysts from livestock

were collected from the offal condemned at the slaughterhouse and stored in a sterile bag. The cysts were immediately transported in sterile tubes to the ILRI laboratories in Busia for further separation and partial processing (Figure 3.2).

They were preserved in 70% ethanol and stored at room temperature awaiting processing and by microscopy and molecular characterization which was done at the Kenya Medical Research Institute (KEMRI) Headquarters in the center for microbiology research (Figure 3.3).

Figure 3.2: Sample Isolation in the Laboratory for Subsequent Examination

Figure 3.3: Picking Protoscoleces from Cyst Samples at the KEMRI – CMR Laboratories

3.8 Human CE Community Surveillance

Before the start of the study, each site in Bungoma County (Figure 3.4) was visited to inform the local administration, including chiefs and assistant chiefs, about the study plans and seek their permission. Subsequently, the village elders were notified, who in turn assisted in preparing locals to participate in the intended exercise. Additionally, announcements were made by the chiefs in Barazas and religious gatherings regarding dates of screening for each of the sites.

Each site was visited once, on the scheduled day, by a research team which comprised of: an experienced sonographer, a clinical officer, a microbiologist, and one or two other research assistants. Posters with illustrative images of the parasite life cycle, as well as its detrimental effects on human and livestock, were used to create awareness about the ongoing ultrasonography session. These also served as

visual aids to highlight the risks of contracting the infection through contact with dog faeces and the consequences of humans and livestock infection.

The study rationale and what it entailed was explained to everyone who expressed interest in the study. In each site, a cubicle or a room enclosed with curtains for privacy was set up where the ultrasonographer and clinician would screen each patient in privacy. The participants were informed that in case of significant abdominal finding the images would be printed and clearly explained to them. In the case of a positive CE case, the ultrasonographer was to classify the case based on the WHO classification, in which case the patient was meant to be referred to a bigger hospital for surgery and treatment. After the awareness session the people who agreed to sign the consent form (Appendix IV) were admitted for the ultrasound screening. The children had the parents/ guardian sign an assent form on their behalf (Appendix VIII). All participants filled/responded to a questionnaire asking about their gender, age, place of origin, income level, and occupation (Appendix VI). If a participant had undergone any previous surgeries, this was noted together with the reason for the surgery.

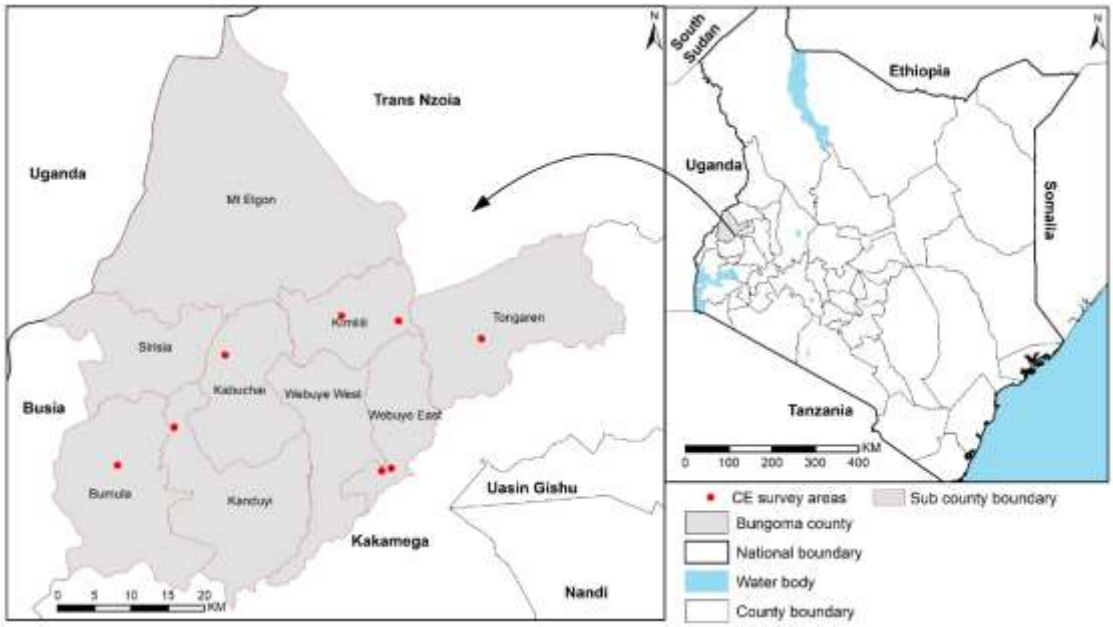

Figure 3.4: The Kenya Map showing the Sentinel Sites within Bungoma County

The ultrasound screening was conducted in a secluded room by an experienced sonographer using a portable ultrasound scanner which was generator powered to manage areas that did not have power or in case of electricity outages.

The findings were communicated to the participants and, when need arose, necessary counseling by the clinical officer and referral were done. All ultrasound images were anonymised before further analysis. These images were screened for the presence of hydatid cysts or other abdominal findings. Any hydatid cysts present were classified based on the WHO staging classification for hepatic cyst(s) (WHO, 2003). Specifically, these categorize hepatic and abdominal cyst(s) into stages CL or CE1 to CE5 (Fig. 3.5).

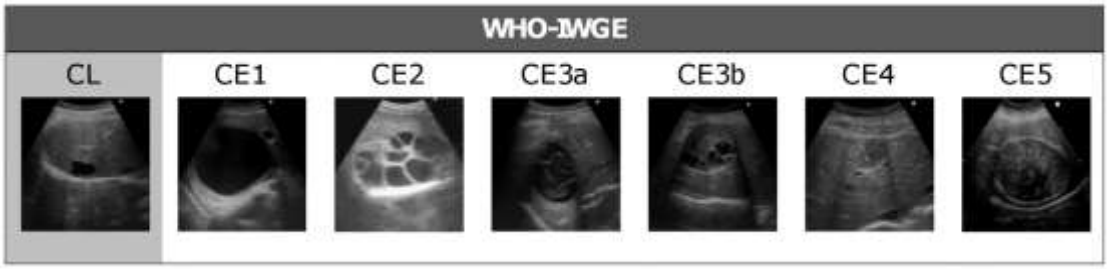

Figure 3.5: WHO Informal Working Group on Echinococcosis Standardized Ultrasound Classification of Echinococcal Cysts. CE1 and CE2 (active cysts), CE3a *and CE3b (transitional cysts), and CE4 and CE5 (inactive cysts) (Piccoli et al. 2014)*

3.9 Dog's Fecal Sample Collection

Dogs were randomly selected from households near the slaughterhouses (1 Km radius of the slaughterhouse). Only dogs with known owners were included. A single dog identified from each of the selected homesteads around the slaughterhouse was muzzled and a faecal sample collected directly from the rectum using a gloved lubricated finger. The faecal sample was transported to ILRI Busia laboratory and separated into two. One aliquot was placed in 0·3% PBS Tween buffer with 10% formalin for Coproantigen analysis and the other preserved in 70% ethanol for microscopy.

3.10 Laboratory Protocols

3.10.1 Isolation of Taeniids Eggs from Stool using Zinc Chloride Flotation Method

Dog faeces previously preserved in 70% ethanol after collection had the ethanol drained off and 2 grams or 2 ml of the faecal content transferred into 15 ml falcon tubes. The faecal content was concentrated by a slight modification of the zinc chloride floatation-sieving technique previously described by Mathis, *et. al.* (1996) to help recover any taeniid eggs present. The protocol involved draining off ethanol from the samples and washing the sample with 8 ml of distilled water. The rinsing water was then drained off to obtain faecal pellets. One part of the obtained pellets was mixed with four parts of the zinc chloride. After floatation, the top layer, which could have the taeniid eggs was collected and filtered using 50 µm sieve followed by a 22 µm sieve (Franz Eckert GmbH, Germany). The eggs were then washed off from the 22 µm sieve into a 15 ml falcon tube using distilled water. The samples were centrifuged, and the eggs were collected using Pasteur pipette and stored in 2 ml micro centrifuge tubes at 4°C.

3.10.2 Coproantigen Capture ELISA

The dog faecal samples were analyzed by coproantigen ELISA detection method described by Allan *et al.,* (1992). Approximately 3-5g of each dog faecal sample is added to in 10ml of 0.3% PBS Tween with 10% formalin in 35ml universal tubes. Each sample was homogenized using a wooden spatula, shaken, and centrifuged at 2500 r.p.m. (1125) for 5 minutes. The resultant supernatant was removed and stored at -80^{o}C for at least four days to remove the risk of infection with Echinococcus spp. (WHO/OIE, 2001). The ELISA uses a sandwich ELISA, which detects antigen between two layers of antibodies (i.e. the capture and conjugate antibody). The ELISA uses polyclonal antibodies derived from rabbit serum to detect Echinococcus spp. Rabbit 91 (Rb91) is the capture antibody and Rabbit 5 (Rb5px) is the peroxidase-conjugated antibody. Immunon 4HB 96 well ELISA plates (Fisher Scientific, Loughborough, UK) were coated with 100ul of the capture antibody (Rb 91), at a dilution of 1/1500, diluted in carbonate bicarbonate buffer (Sigma-Aldrich,

Dorset, UK, added to all wells except blamks). Cling film was used to cover the preparation and left overnight at 4ºC. The plates were washed three times (5 min each) with 0.1% PBS Tween buffer using a wash bottle. 100ul of 0.3% PBS Tween was added to each well except blanks. 0.3% PBS Tween was discarded and plate patted to dry. 50ul of foetal calf serum (Sigma-Aldrich, Dorset, UK) was added to each well except blanks and then 50ul of each faecal supernatant was added. Each well was mixed by pipetting up and down and then left covered for 1 hour at room temperature. The contents of the plates were discarded and washed three times (5 minutes each) with 0.1% PBS tween buffer using a wash bottle. 100 ul of conjugated antibody (RB5px) was added to each well (at a 1/1500 dilution in PBS/0.1% Tween), covered and left for 1 hour at room temperature. The contents of the plate were discarded and washed three times (5 minutes each) with 0.1% PBS Tween buffer using a wash bottle. 100ul of SureBlue® TMB substrate (Insight Biotechnology, Wembley, UK) was added to all wells and incubated for 20 minutes in the dark and then on a ThermoScientific Multiscan FC platereader at 620nm. A cut off was calculated from the mean+3Sd of control negative dogs. This came out to be 0.14. The positives were classified as either marginal positives (OD 0.14-0.17) or positives (OD 0.17 and above).

3.10.3 DNA Extraction from Cyst Material and Amplification

At the laboratory single cysts of protoscolices in distilled water in a Petri dish, were picked under low power microscopy and transferred into PCR tubes containing 10 µl 0.02 N NaOH and lysed at 99°C for 10 min (Nakao *et al*., 2003). The lysate was kept at −20°C for further analysis.

3.11 Polymerase Chain Reaction for Nad1 Gene

Two nested PCR assays targeting part or the entire NADH dehydrogenase subunit 1 gene (*nad1*) were used for genotyping of cyst materials obtained from the post-mortem samples as explained in section 3.4. The first nested PCR (entire *nad1* gene) was performed as described by Hüttner *et al*. (2009). In both PCR assays, the reaction mixture contained 2 μl of the DNA, 1 × DreamTaq Green Buffer (20 mM Tris-HCl) (pH 8.0), 1 mM DTT, 0.1 mM EDTA, 100 mM KCl, 0.5 % (v/v) Nonidet

P40, 0.5 % (v/v) Tween 20) (Thermo Scientific), 0.2 mM dNTPs, 0.25 μM of forward and reverse primers, 2 mM $MgCl_2$, and 0.625 units of DreamTaq Green DNA Polymerase (Thermo Scientific) in 25 μl final volume. The PCR cycling conditions were 5 min for initial denaturation at 94°C, 40 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 60 s, and a final extension at 72°C for 5 min. The reaction and cycling conditions that were used for the primary and secondary PCRs were the same except that 2 µl of primary PCR product was used as a template in the secondary PCR. For the *Nad* 1 PCR, the primers *Nad* A and *Nad* C were used in the primary PCR while *Nad* B and *Nad* D were used for the secondary PCR. (Table 3.1)

Table 3.1: *Echinococcus* spp. *Nad 1* primers and PCR Conditions

Region of *Nad1* gene	Name	Primers (5'-3')	Size (bp)	PCR conditions
5' region	*Nad* A	TGG AAC TCA GTT TGA GCT TTA CTA	1239	annealing temp 55°C, 40 cycles, 2 mM $MgCl_2$
	Nad C	ATA TCA AAG TAA CCT GCT ATG CAG		
3' region	*Nad B*	TAT TAA AAA TAT TGA GTT TGC GTC	1073	annealing temp 55°C, 40 cycles, 2 mM $MgCl_2$
	Nad D	TCT TGA AGT TAA CAG CAT CAC GAT		

3.12 Polymerase Chain Reaction for Cytochrome C Oxidase 1 (*Cox1*)

PCR based on cytochrome C oxidase 1 (*Cox1*) was performed on cyst lysate from the hydatid cycts obtained from the post-mortem samples as explained in section 3.4. The entire mitochondrial *cox1* was amplified in two overlapping parts, i.e. the five prime region, and the three prime region. This was because of the large size of the *cox1* gene (1608/9 bp). The five prime region was obtained in a nested PCR reaction in which forward primer *cox1* A and reverse primer *Efel D* were used in the primary PCR. The nested reaction was performed using forward primer *cox1* B and reverse

primer *cox1* walkLH (Table 3.2). In a 25 µl reaction the primary PCR consisted of 1 × PCR reaction buffer (50 mM Tris/HCl, 10 mM KCl, 5 mM $(NH4)_2SO4$, pH 8.3/ 25°C, Roche, Germany), 2 mM $MgCl_2$, 0.2 mM dNTPs, 0.4 µM of each primer, 0.625 units FastStart Taq DNA Polymerase (Roche, Germany) and 2 µl of cystic lysate. Similar conditions were used for the secondary PCR. The cycling conditions were 2 min of initial denaturation at 94°C, followed by 40 cycles of 94°C for 15s, 52°C for 30 s and 68°C for 1 min and a final extension at 68°C for 5 min. The 3' region of *cox1* gene was amplified in a nested PCR where in the primary PCR forward primer *Cox1 F* and *cox1* C were used. The nested PCR utilized forward primer *Cox1* G and reverse primer *cox1* D. PCR and cycling conditions for the 3' region were 5 min of initial denaturation at 94°C, followed by 40 cycles of 94°C for 30s, 55°C for 30 s and 72°C for 1 min and a final extension at 72°C for 5 min (Table 3.1). The secondary PCR products were purified using the High Pure PCR Product Purification Kit (Roche, Germany) following the manufacturer's protocol.

Table 3.2: *Echinococcus* spp. *cox1* Primers and PCR Conditions

Region of *cox1* gene	Name	Primers (5'-3')	Size (bp)	PCR conditions
5' region	*cox1* A	TTACTGCTAATAATTTTGTGTCAT	1132	annealing temp 52°C, 40 cycles, 2 mM $MgCl_2$
	Efel D	TGGATCACTAACATTAACACTAGA		
	cox1 B	GTTAGTTTTGACTGTACGTTTTCA	800	annealing temp 55°C, 40 cycles, 2 mM $MgCl_2$
	cox1 walkLH	ATCAACACATAAACCTCAGG		
3' region	*Cox* 1F	GTTGTCCTCGTCGTATTTTTCTAG	1323	annealing temp 55°C, 40 cycles, 2 mM $MgCl_2$
	Cox 1 C	ATACTTTAAAAAACTCCGTTAAGC		
	Cox 1 G	CTGTTTTGTTATTGGTTACGTTGC	1104	annealing temp 55°C, 40 cycles, 2 mM $MgCl_2$
	cox 1 D	CACAATTAAACAACCAGGTCAATG		

3.13 Restriction Fragment Length Polymorphism (RFLP)

PCR products (10 µl) for Nad1 were subjected to a restriction digestion using the enzyme *Hph*I and incubated at 37°C overnight. The digests were separated on 3.0% agarose gel for one hour, stained with ethidium bromide and photographed for documentation. The genotypes of the samples were determined by comparing their banding patterns to known ones (G1-G3, G5 and G6/7) as described by Hüttner *et al*., (2009).

3.14 Purification of PCR Products and Sequencing

For sequencing preparation both Cox1 and Nad1 PCR products were purified using Wizard® SV Gel and PCR Clean-Up kit according to the manufacturer's instructions. However, the final elution was done at 30 µl, instead of 50 µl in the protocol, to increase the amplicon concentration. The cox 1 and Nad 1 purified PCR products were shipped to Macrogen (Netherlands) and sequenced done using 23 ABI 3730XLS system. A consensus sequence was determined for each isolate and haplotype work analysis done using Aliview software.

3.15 Phylogenetic and Haplotype Network Analysis

GENtle software was used to view the Nad1 and Cox1 sequences and any detected misreads corrected manually. Two cox1 fragments were joined to obtain a complete gene concensus and using BLAST NCBI matched with existing sequences in in the GeneBank database.

DNA chromatographs for Nad1 and Cox1 sequences were viewed using GENtle v. 1.9 (Manske, 2003, University of Cologne, Germany) and manually edited in case of nucleotide base misreads. Isolates sequenced in two overlapping fragments were joined. Amino acid sequences were inferred from the nucleotide sequences by echinoderm mitochondrial genetic code (Nakao *et al*., 2000). Percentage divergence of DNA sequences were determined using Kimura 2-parameter model (Kimura, 1980) in MEGA v 6 (Tamura *et al*., 2013). Single locus and concatenated cox1 – nad1 haplotypes were estimated based on statistical parsimony (Templeton *et al*.,

1992) and network drawn at 95% connection limit using TCS v 1.8 (Clement *et al*., 2000). DnaSP v 5 (Librado & Rozas, 2009) was used to estimate the population indices: number of haplotypes, haplotype diversity and nucleotide diversity. Analysis of molecular variance (AMOVA), degree of genetic differentiation (pairwise fixation index – Fst) and neutrality indices of Tajima's D (Tajima, 1989) and Fu's Fs (Fu, 1997) were calculated using the Arlequin v 3.5 software (Excoffieretal, 2005). The identified cox1-nad1concatenated haplotypes and representative sequences of *E. granulosus sensu stricto* genotypes were aligned using Clustal Omenga in EMBL-EBI before being used to construct a maximum likelihood phylogenetic tree in MEGA v 6.

3.16 Data Management and Analysis

3.16.1 Livestock Cysts and Dog Faecal Samples

Data entry took place in the field or in the laboratory using Android GPS-enabled Kestrel tablets which were linked directly to a secure cloud server and a custom designed database. Any digital data (e.g. pictures, videos) of relevant lesions in both animals and human patients were collected in such a way as to preserve the anonymity of the patient. Basic clinical parameters were recorded on electronic forms. In the case of any participants with health conditions that required further evaluation, clinical evaluations were recorded on paper forms and summary information entered into the database. Questionnaire data was uploaded to the server on a daily basis, and unique identifiers used to link such data to laboratory outputs. Data cleaning procedures were performed before importing it for analysis into Stata 9.2 (Stata Corporation, Texas USA). Bivariate analysis using Fisher's exact chi-Square was used to measure the difference between different risks factors of *E. granulosus* transmission. Multivariate analysis using logistic regression models were carried out to control for confounding and to tease out the significant risks factors. Estimates of the parameters were considered statistically significant at $p < 0.05$.

3.16.2 Human Ultrasound Screening

Questionnaire data and ultrasound (US) findings were first recorded on paper-based forms at the site, and later entered manually into Microsoft Excel (Microsoft, Redmond, WA, USA). Data cleaning was then carried out to check for any errors that might have occurred during transcription.

Statistical analysis was conducted using Stata Statistical Software: Release 14 (College Station, TX: StataCorp LP). Descriptive statistics were performed to summarize variables and identify trends.

Regression analysis for the outcomes of interest were conducted. A causal diagram was developed to identify putative relationships between exposure variables of interest and outcomes. Continuous explanatory variables were checked for normal distribution; variables that were not normally distributed were transformed as needed. Subsequently, mixed logistic regression models were developed for each outcome variable, with site included as a random effect to account for spatial clustering within each sentinel site. Each explanatory variable was first screened for its unconditional association with the outcome variable; variables that were marginally significant ($p<0.2$) or considered confounding were retained for inclusion in the multivariable regression model. The intra-cluster coefficient was computed as the proportion of overall variation due to variation between groups.

3.17 Ethical Approval

This study was approved by the Institutional Research Ethics Committee (IREC Reference No. 2018-02; Appendix I) at the International Livestock Research Institute. ILRI IREC is accredited by the National Commission for Science, Technology and Innovation (NACOSTI) in Kenya, and approved by the Federalwide Assurance (FWA) for the Protection of Human Subjects in the United States of America. Approval to conduct this work was also obtained from the Kenyan Ministry of Health and the relevant offices at devolved government level, and sub-county medical and public health officers. (Appendix I, II, VIII, & IX).

CHAPTER FOUR

RESULTS

4.1 The Infection Level of CE in Humans, Livestock and Dogs in Western Kenya

4.1.1 Infection Levels of CE in Humans

A total of 1002 participants were screened for CE using a portable Ultrasound scanner. Out of this 438 were male and 654 were female. The particpants came from 8 sentinel sites which were close to slaughterhouses. These sentinel sites included: Chwele, Kamukuywa, Kimilili, Kimwanga, Mayanja, Misikhu, Naitiri and Wananchi. The screening took place over eight days, one day for each site. Some study sites, such as Kimilili, had a larger turn-out since they are larger towns with bigger populations (Table 4.1). Upon screening 935 participants did not show any abnormal ultrasound features but 67 had abnormal ultrasound presentations.

Table 4.1: Ultrasound Screening across the Sub-counties of Bungoma County

Ultrasound results	Selected sub-counties of Bungoma								
	Chwele	Kamukuywa	Kimilili	Kimwanga	Mayanja	Misikhu	Naitiri	Wanaichi	Total
Participants	83	102	218	155	124	75	123	122	1002
(M:F)	(21:62)	(33:69)	(111:107)	(45:110)	(28:96)	(22: 53)	(45:78)	(43:79)	(438:654)
normal	76	93	209	139	115	73	119	111	935
abnormal	7	9	9	16	9	2	4	11	67

1.88 times more women than men participated in the study, and this was consistent across the sentinel sites, except for Kimilili. The overall median age of the participants was 43 years (IQR=29-55); the median age was slightly lower in females (41) than in males (47). The median monthly income was 4000 KES (US$ 36.50), though this was lower in females (3100 KES; US$ 28.29) compared to males (4200; US$ 38.32).

Occupation data were available for 930 participants; farming was the most frequent occupation category (n=403; 43.43%), followed by paid occupation (n=215; 23.1%), businessmen (n=207; 22.3%); minor (n=78; 8.4%); and homestay (n=27; 2.90%) categories. Consequently, 430 (46.2%) and 500 (53.8%) of the participants were classified as being at high and low risk for human CE, respectively.

Previous surgeries were reported by 100 participants; 40, however, did not disclose the type of surgery. Of the remaining 60 participants who reported a previous surgery, the most frequent was caesarean section (n=29), followed by tubal ligation (n=8) and growth removal (n=3). Two participants reported previous removal of cysts, though details on the nature of the cysts were not provided.

Of the 1002 screened participants, 67 (6.7%) had abnormal ultrasound findings. Seven participants (0.7% of total, 10.44% of those with abnormal findings) - five females and two males - had simple liver cysts which were classified as Cystic Lesions (CL) following the WHO classification (Figure 4.1).

(a) (b)

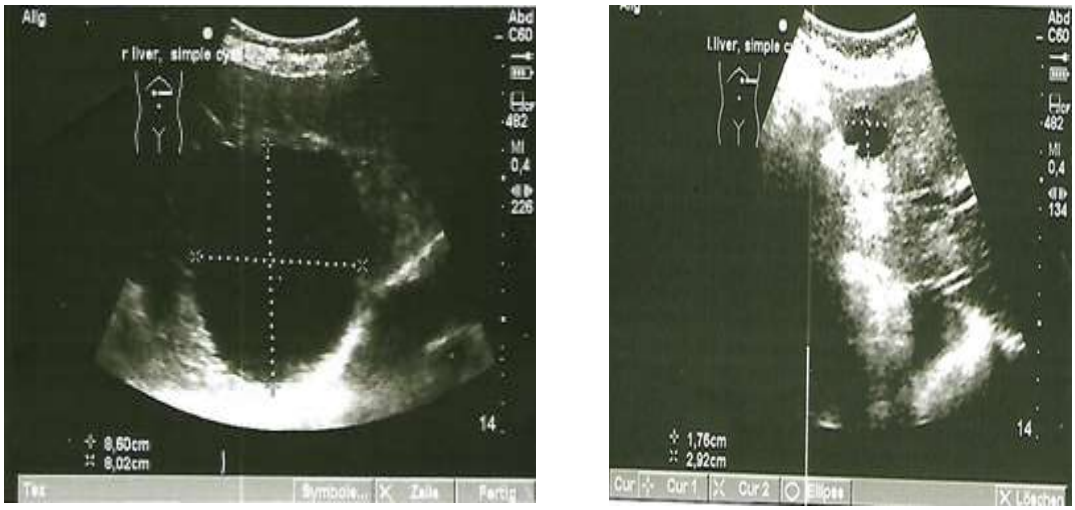

Figure 4.1: Cystic Lesions on some of the Study Participants (a) on the Right Liver and (b) on Left Liver

Other abnormal ultrasound findings included splenomegaly (n=14), ovarian cysts (n=14), uterine fibroids (n=10), polycystic kidneys (n=6), and benign prostatic hyperplasia (n=6). Abdominal ascites, cardiac insufficiency, cystic lesions in left scapular and lower abdomen, echogenic lesions in the left upper arm, and gall stones were each identified once. A rare case of umbilicus lymphoma (Sister Mary Joseph's nodule) was identified in a 40-year-old female, and two cases of hepatocellular carcinoma were identified. A possible ovarian tumour and uterine mass were also detected. These participants were all referred to an oncologist for further medical attention.

4.1.2 Regression Analysis of CE on Humans

Of the four explanatory variables identified in the causal diagram (Figure 4.2), age and income were continuous and therefore assessed for normality; while age was normally distributed, income had a right-skewed distribution and was therefore log-transformed.

The results of the unconditional associations between the two outcome variables and the explanatory variables are presented in Table 4.2, while the results of the final mixed logistic regression model for abnormal ultrasound findings are presented in Table 4.3. The variables gender and age were retained in the final model, regardless of their p-values, since they were identified as confounders (Figure 4.2). No variables remained significant in the multivariable regression model for simple liver cysts, so no final model is presented.

Table 4.2: Unconditional Associations between the Putative Risk Factors and the Two Outcome Variables - Abnormal Ultrasound findings and Presence of Liver Cysts - from 1002 Participants Screened in Eight Sentinel Sites in Bungoma County.

Explanatory variable	Outcome: Abnormal ultrasound findings			Outcome: Simple liver cysts		
	Odds Ratio	95% CI	p-value	Odds Ratio	95% CI	p-value
Gender						
Male	0.64	0.36-1.13	0.128*	0.75	0.14-3.89	0.73
Age	1.01	0.99-1.03	0.085*	1.09	1.04-1.15	<0.001***
Income_ln	0.65	0.49-0.86	0.003**	2.32	0.77-6.96	0.133*
Job risk						
Low	0.57	0.33-0.98	0.043**	0.86	0.17-4.28	0.85

*p<0.20; **p<0.05; ***p<0.001

Table 4.3: Final mixed logistic Regression Model for Abnormal Ultrasound findings Identified in 1002 Participants Screened in Eight Sentinel Sites in Bungoma County.

	Odds Ratio	95% CI	p-value	ICC
Site				1.67^{-9}
Gender				
Male	0.45	0.22-1.03	0.060*	
Age	1.01	0.99-1.04	0.241	
Income_ln	0.68	0.51-0.90	0.007**	

ICC=Intra-Cluster Correlation Coefficient

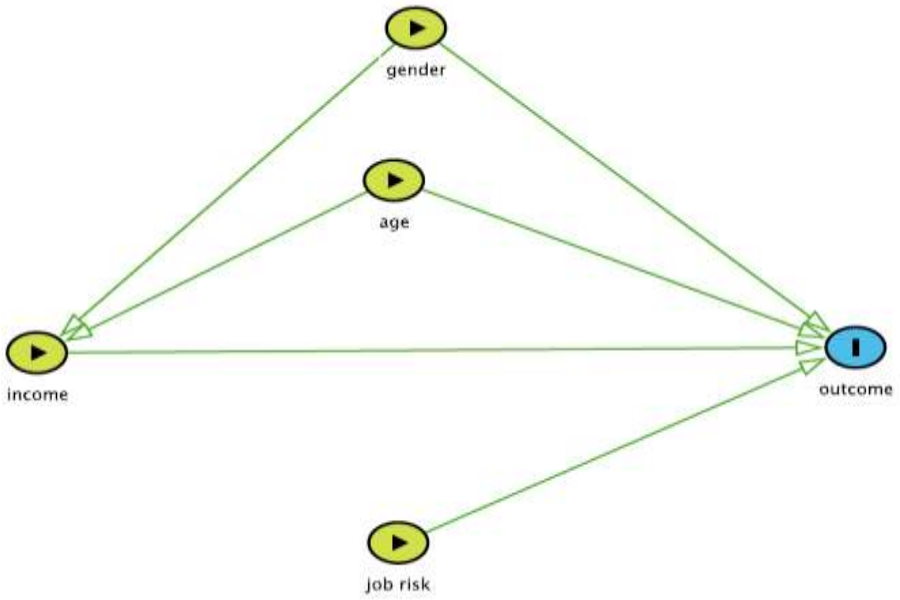

Figure 4.2: A causal diagram illustrating the putative relationships between the explanatory variables and the two outcomes of interest (presence of abnormal ultrasound findings and presence of liver cysts).

4.1.3 Infection Level in Dogs by CoproELISA Analysis

82 dog faecal samples (ZOL005629-ZOL005711) were analyzed by coproELISA method, 4 Positive controls (THR 369, TLD 476, TLD 197, TLE 275) and one negative control (MMEW 468) were used. 15/82 (18.3%) samples were positive of CoproELISA, (with 3 being marginally positive) and 67/82 (81.7%) were negative of coproELISA antigen.

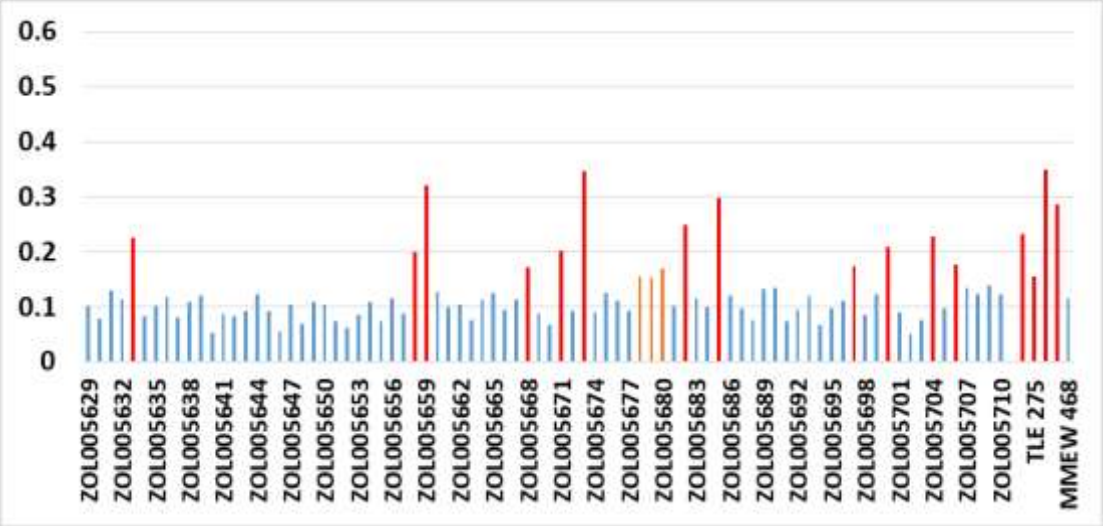

Figure 4.3: CoproELISA Data for Dog Faecal Samples. *Far right are 4 positive controls and one negative control*

4.1.4 Infection Levels and Haplotype Diversity on CE in Livestock in Western Kenya

A total of 192 livestock cysts were collected. Out of these 133 (72 %) were from Bungoma and 54 (28 %) from Busia County. Of the cysts condemned at the slaughterhouse 44/192 (22.9%) cysts were from the liver while 134/192 (69.8%) were from the lungs, 12/192 (6.25%) from the spleen, one from the tongue (0.5%) and one (0.5%) from the kidney. Of the 192 cysts, 153 (80%) had their DNA isolated and 135/153 isolates were successfully amplified for *nad1* mitochondrial gene giving a 88% amplification rate. The remaining 18/153 (12%) did not amplify so were removed. *Cox1* was amplified in two separate fragments, the front part and the back part. Out of 153 isolates, 134 (87.5%) amplified for the front part of *Cox1* gene and 131 (85.6%) amplified for the back part of *Cox1* gene. All the isolates typed as *Echinococcus granulosus sensu stricto* according to the band size and pattern on agarose gel electrophoresis (Figure 4.4). Each of the *E. granulosus* sensu stricto isolate was accurately identified using both nad1 and cox1 sequences using BLAST. Polymorphic sites were detected for the nad1 sequences and cox1 sequences. The

sequences then deposited into GenBank (accession nos.M27366680 –M2736690 for Nad1 and M2714523-M2714549 for Cox1).

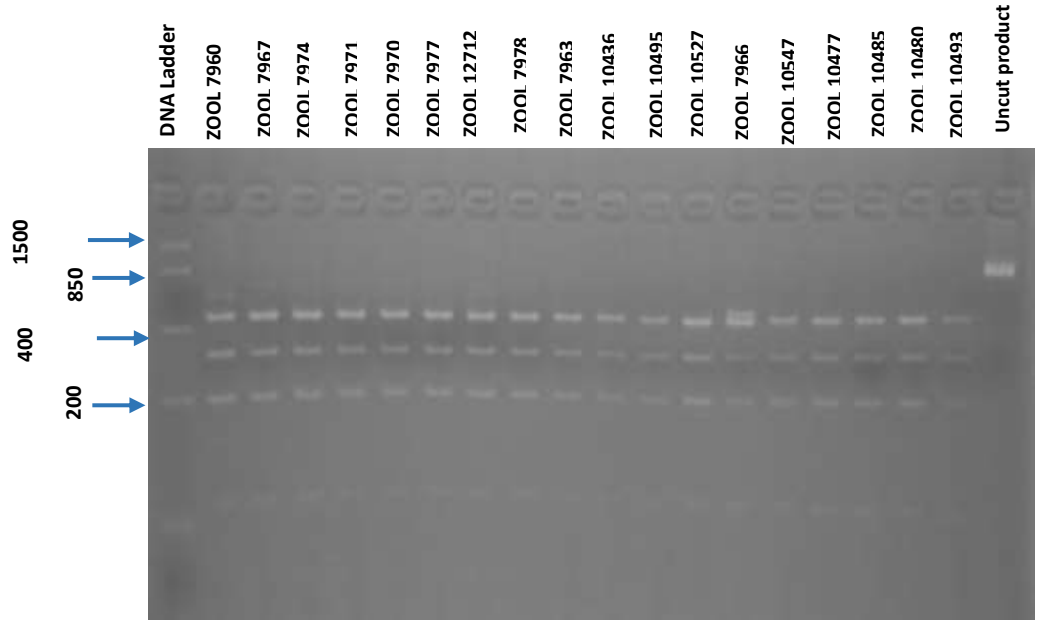

Figure 4.4: PCR RFLP for Nad1 Gene of *Echinococcus granulosus s.l.*

Considerable genetic variation was detected in both the *E. granulosus (s.s.)* nad1 and cox1 mitochondrial DNA sequences. The total number of polymorphic sites for *E. granulosus (s.s.)* sequences were 11 for Nad1 and 23 for Cox1, giving rise to 11 haplotypes for Nad1 (Table 4.4.) and 19 haplotypes for Cox1 (Table 4.5).

Table 4.4: *Nad1* Gene Nucleotide Mutations

	Nucleotide Position										
	24	**109**	**256**	**288**	**375**	**420**	**477**	**510**	**630**	**738**	**891**
REF_AF297617	**T**	**C**	**A**	**T**	**T**	**T**	**T**	**T**	**C**	**C**	**T**
MZ736680 (Hap 1)	-	-	-	-	C	-	C	-	T	-	-
MZ736681 (Hap 2)	-	-	-	-	C	-	C	-	T	-	-
MZ736682 (Hap 3)	-	G	-	-	-	-	C	-	T	-	-
MZ736683 (Hap 4)	-	-	-	-	-	-	C	-	T	-	-
MZ736684 (Hap 5)	G	-	-	-	-	-	-	-	T	A	-
MZ736685 (Hap 6)	-	-	-	-	-	-	-	-	T	A	-
MZ736686 (Hap 7)	-	-	-	-	-	-	-	-	T	-	-
MZ736687 (Hap 8)	-	-	-	C	-	-	-	-	T	-	-
MZ736688 (Hap 9)	-	-	-	-	-	-	-	C	T	-	-
MZ736689 (Hap 10)	-	-	-	-	-	G	-	-	T	-	-
MZ736690 (Hap 11)	-	-	G	-	-	-	-	-	T	-	-

In this study of the Nad 1 gene of cystic echinococcosis, 11 haplotypes were identified. The 11 haplotypes discovered in the study are a result of the genetic diversity of the parasite. Each haplotype is a distinct set of genetic markers that can be used to differentiate between different populations of the parasite. This genetic diversity is important for understanding the transmission and evolution of the parasite.

The presence of multiple haplotypes of the Nad1 gene in *E. granulosus* suggests that the parasite has a high degree of genetic diversity, which could be important for its survival in changing environmental conditions. This genetic diversity has also been used to develop molecular markers for the diagnosis and epidemiological control of CE.

Table 4.5: *Nad1* Gene Amino Acid Changes following Nucleotides Mutation

	Protein (Amino acid change)										
Nucleotide Position	**24**	**109**	**256**	**288**	**375**	**420**	**477**	**510**	**630**	**738**	**891**
REF_AF297617	**S**	**R**	**I**	**Y**	**G**	**F**	**C**	**Y**	**S**	**G**	**N**
MZ736680 (Hap 1)	-	-	-	-	G	-	C	-	**S**	-	-
MZ736681 (Hap 2)	-	-	-	-	G	-	C	-	**S**	-	-
MZ736682 (Hap 3)	-	G	-	-	-	-	C	-	**S**	-	-
MZ736683 (Hap 4)	-	-	-	-	-	-	C	-	**S**	-	-
MZ736684 (Hap 5)	S	-	-	-	-	-	-	-	**S**	G	-
MZ736685 (Hap 6)	-	-	-	-	-	-	-	-	**S**	G	-
MZ736686 (Hap 7)	-	-	-	-	-	-	-	-	**S**	-	-
MZ736687 (Hap 8)	-	-	-	Y	-	-	-	-	**S**	-	-
MZ736688 (Hap 9)	-	-	-	-	-	-	-	Y	**S**	-	-
MZ736689 (Hap 10)	-	-	-	-	-	L	-	-	**S**	-	-
MZ736690 (Hap 11)	-	-	V	-	-	-	-	-	**S**	-	-

Key: C = Cysteine; G = Glycine; I = Isoleucine; N = Asparagine; R = Arginine; S = Serine; Y = Tyrosine; F = Phenylalanine; V= Valine; L= Leucine. There were three changes in nucleotide sequence that caused change in amino acids at position 109, 256 and 420 from Arginine to Glycine, Isoleucine to Valine, and Phenylalanine to leucine respectively.

Table 4.6: *Cox1* Gene Nucleotide Mutations

	NUCLEOTIDE POSITION																							
	5	1	1	3	3	4	4	5	5	5	7	7	8	8	8	2	2	4	4	4	5	5	5
	1	2	9	1	2	0	2	0	6	9	5	5	0	1	5	5	5	5	6	6	0	1	9
Sequence ID	**1**	**1**	**1**	**2**	**5**	**2**	**3**	**9**	**1**	**4**	**2**	**9**	**0**	**0**	**5**	**1**	**2**	**1**	**4**	**5**	**0**	**1**	**2**
MN787528.1_Ref	C	C	A	T	A	C	T	A	A	G	T	A	C	C	C	C	T	T	A	T	A	A	C
MZ714523	-	-	-	-	-	-	C	G	-	-	C	-	-	-	-	-	-	-	-	-	G	-	-
MZ714524	-	-	-	-	-	-	C	G	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-
MZ714525	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	C	-	C	A	-	-	-
MZ714526	-	T	C	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-
MZ714534	T	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MZ714535	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	T
MZ714536	T	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	
MZ714538	T	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	T
MZ714527	-	-	-	-	-	T	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-
MZ714531	-	-	-	C	-	T	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-
MZ714532	-	-	C	-	-	T	-	-	-	-	-	-	-	T	-	-	-	C	-	-	-	-	-
MZ714533	-	-	C	-	-	T	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-
MZ714539	-	-	-	-	G	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	G	-
MZ714540	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MZ714541	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-
MZ714546	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	T	-	-	-	-	-	-	-
MZ714547	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	T	-	-	-	-	-	-	-
MZ714548	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-
MZ714549	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-

Cox 1 gene gave 19 haplotypes following some changes in the 1609 bp gene. The 19 haplotypes of the cystic echinococcosis *Cox1* gene refer to the 19 different variations of the cystic echinococcosis *Cox1* gene that were identified. Haplotypes are determined by studying the sequence of nucleotides in a gene region and then comparing them to the sequences from other individuals. This means that there may be 19 unique genetic variants of the parasite, which could be classified according to the differences in their genetic makeup.

Table 4.7: *Cox1* Gene Amino Acid changes following Nucleotides Mutation

	NUCLEOTIDE POSITION																						
	5	1 2	1 9	3 1	3 2	4 0	4 2	5 0	5 6	5 9	7 5	7 5	8 0	8 1	8 5	1 2	1 2	1 4	1 4	1 4	1 5	1 5	1 5
Sequence ID	**1**	**1**	**1**	**2**	**5**	**2**	**3**	**9**	**1**	**4**	**2**	**9**	**0**	**0**	**5**	**1**	**2**	**1**	**4**	**5**	**0**	**1**	**2**
MN787528.1_Ref	R	R	H	S	I	S	S	Y	S	T	R	G	A	F	S	I	S	L	N	S	V	N	A
MZ714523	-	-	-	-	-	-	S	C	-	-	S	-	-	-	-	-	-	-	-	-	V	-	-
MZ714524	-	-	-	-	-	-	S	C	-	-	-	-	-	-	-	-	-	-	-	-	V	-	-
MZ714525	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I	P	-	N	T	-	-	-
MZ714526	-	C	P	-	-	-	-	-	-	-	-	-	V	-	-	-	-	-	-	-	-	-	-
MZ714534	R	-	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MZ714535	-	-	-	-	-	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	V
MZ714536	R	-	-	-	-	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-
MZ714538	R	-	-	-	-	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	V
MZ714527	-	-	-	-	-	S	-	-	-	-	-	-	-	F	-	-	-	-	-	-	-	-	-
MZ714531	-	-	-	S	-	S	-	-	-	-	-	-	-	F	-	-	-	-	-	-	-	-	-
MZ714532	-	-	P	-	-	S	-	-	-	-	-	-	-	F	-	-	-	S	-	-	-	-	-
MZ714533	-	-	P	-	-	S	-	-	-	-	-	-	-	F	-	-	-	-	-	-	-	-	-
MZ714539	-	-	-	-	V	-	-	-	-	-	-	-	V	-	-	-	-	-	-	-	-	S	-
MZ714540	-	-	-	-	V	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MZ714541	-	-	-	-	-	-	-	-	-	-	-	M	-	-	-	-	-	-	-	-	-	-	-
MZ714546	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	I	-	-	-	-	-	-	-
MZ714547	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	I	-	-	-	-	-	-	-
MZ714548	-	-	P	-	-	-	-	-	-	-	-	-	-	-	-	I	-	-	-	-	-	-	-
MZ714549	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I	-	-	-	-	-	-	-

Key: A =Alanine; C = Cysteine; F = Phenylalanine; G = Glycine; H = Histidine; I = Isoleucine; L = Leucine; M = Methionine; N = Asparagine; P = Proline; R = Arginine; S = Serine; T = Threonine; V = Valine; Y = Tyrosine. There were 11 changes of nucleotide sequence arrangement that resulted to changes of amino acid sequence. Position 121 = Arginine to cytosine; position 191; Hisitidine to phenylalanine; position 325; Isoleucine to Valine; position 509, Tyrosine to Cysteine; Position 752 Arginine to Serine; position 759, Glycine to Methionine; position 800, Alanine to Valine; Position 1252 Serine to Proline; Position 1451, Leucine to Serine; Position 1465, Serine to Threonine; Position 1511 Asparagine to Serine, and Position 1592, Alanine to Valine.

Table 4.8: *Nad1* Haplotype Comparison with Existing Haplotypes in Gene Bank Datatbase

Haplotype/ Product	Accession No	Closest Match	Similarity	Authour	Status
Haplotype1_Nad_17	MZ736680	MN269992	99.78%	Ohiolei *et al*., 2019	Novel
Haplotype2_Nad_117	MZ736681	MN269992	99.89%	Ohiolei *et al*., 2019	Novel
Haplotype3_Nad_96	MZ736682	MN269992	99.89%	Ohiolei *et al*., 2020	Novel
Haplotype4_Nad_3	MZ736683	MN269992	100%	Ohiolei et.al., 2019	Not Novel
Haplotype5_Nad_130	MZ736684	MN269986	99.78%	Ohiolei et.al., 2019	Novel
Haplotype6_Nad_14	MZ736685	MN269986	99.89%	ohiolei et.al., 2019	Novel
Haplotype7_Nad_147	MZ736686	MN269986	100%	Ohiolei et.al., 2019	Not Novel
Haplotype8_Nad_10	MZ736687	MN269986	98.81	Ohiolei et.al., 2019	Novel
Haplotype9_Nad_47	MZ736688	MG642196	100%	Kinkar *et al*., 2018	Not Novel
Haplotype10_Nad_74	MZ736689	MN269980	99.89%	Ohiolei et.al., 2019	Novel
Haplotype11_Nad_1	MZ736690	MN269986	99.89%	Ohiolei et.al., 2019	Novel

The *NAD1* haplotypes were submitted to gene bank and given accession nos MZ736680 - MZ736690. Eight (8) out of 11 haplotypes were novel and had not been reported anywhere else. However 3 haplotypes had previously been reported in the works done Kinkar *et al*., 2018 and Ohiolei *et al*., 2019.

Table 4.9: *Cox1* Haplotype Comparison with Existing Haplotypes in Gene Bank Database

Haplotype/ product	Accession No.	Closest match	Similarity	Author	Status
Hap 1_cox_22	MZ714523	MK319771	99.81%	Ebi *et al*., 2018	Novel
Hap 2_cox_149	MZ714524	MK319772	99.81%	Ebi *et al*., 2018	Novel
Hap 3_cox_40	MZ714525	AB893250	99.81%	Ito *et al*., 2014	Novel
Hap 4_cox_42	MZ714526	MG672255	99.88%	Kinkar *et al*., 2018	Novel
Hap 5_cox_2	MZ714534	MG672286	100%	Kinkar *et al*., 2018	Not new
Hap 6_cox_32	MZ714535	MG672286	99.94%	Kinkar *et al*., 2018	Novel
Hap 7_cox_102	MZ714536	MG672286	99.88%	Kinkar *et al*., 2018	Novel
Hap 8_cox_147	MZ714538	MG672286	99.94%	Kinkar *et al*., 2018	Novel
Hap 9_cox_106	MZ714527	MG672193	99.94%	Kinkar *et al*., 2018	Novel
Hap 10_cox_5	MZ714531	MG672279	99.94%	Kinkar *et al*., 2018	Novel
Hap 11_cox_8	MZ714532	MK319807	100%	Ebi *et al*., 2018	Not new
Hap 12_cox_29	MZ714533	MK319807	99.94%	Ebi *et al*., 2018	Novel
Hap 13_cox_6	MZ714539	MG672248	99.94%	Kinkar *et al*., 2018	Novel
Hap 14_cox_12	MZ714540	MG672133	100%	Kinkar *et al*., 2019	Not new
Hap 15_cox_43	MZ714541	AB688137	100%	Konyaer *et al*., 2012	Not new
Hap 16_cox_28	MZ714546	AB893250	99.94%	Ito *et al*., 2014	Novel
Hap 17_cox_37	MZ714547	AB893250	99.94%	Ito *et al*., 2014	Novel
Hap 18_cox_88	MZ714548	AB893250	99.94%	Ito *et al*., 2014	Novel
Hap 19_cox_27	MZ714549	AB893250	100%	Ito *et al*., 2014	Not new

The Cox1 haplotypes were deposited to the Gene Bank were given accession Numbers MZ714523 - MZ714549. For these results only one accession number was used for each cluster of samples, therefore, serialization was not sequential. Fourteen (14) out of 19 haplotypes were novel and had not documented by any other study while 5 have previously been reported

CHAPTER FIVE

DISCUSSION

5.1 Infection Levels of Cystic Echinococcosis among Humans in Busia and Bungoma Counties, Western Kenya

This study conducted community Ultrasound screening for the presence of CE. These results contribute baseline data on field investigations for human CE while providing insights on the implementation of ultrasound diagnosis in the field, as recommended by the WHO for the successful targeted control of echinococcosis by 2030 [WHO 2020].

Most participants who underwent screening were between 29 and 55 years old. While some parents/guardians brought their young children and assented for their screening, the population of pupils in school largely missed out since the study was done during the school season. Additionally, the 29-55 years age group were more receptive and keener to participate in the screening, compared to the younger or more elderly age groups.

Many of the participants were farmers whose livelihood depends on subsistence farming. Indeed, their income was lower (median income of KES 3000 / US$ 27.10 per month), compared to that of the business and professional categories (KES 5000 / US$ 45.10 and 6250 / US$ 56.38 per month, respectively). The study observed that the levels of earning by the participants would not be sufficient to cater for their basic needs and routine medical check-ups, thus explaining their willingness to participate in the free ultrasound screening programme.

Cystic echinococcosis infections in patients were identified using ultrasound on the basis of pathognomonic signs by clinicians skilled in ultrasonography and radiology. The CE cysts were staged according to the WHO-IWGE classification (Brunetti *et al*., 2010). Pathognomonic signs of interest present in various forms and classifications, and in this study, the seven cases of cystic lesions detected by the ultrasonogram presented as unilocular fluid-filled cysts with a visible single wall and

were classified as cystic lesions of uncertain aetiology (referred to as CL in the WHO-IWGE classification). It was therefore not possible to form a definitive diagnosis as CL indicates an undifferentiated cystic lesion that requires further investigation before definitive decision on its parasitic nature is made. However, when a CL is detected on a scan in countries or regions where hydatid disease is endemic and serological results are pending or available, CE should be considered since the detection of CL cyst type is identical to the detection of a hydatid cyst not typical for echinococcosis (Brunetti *et al*., 2018).

The CL cases normally present as unilocular anechoic, therefore lacking internal echoes and septa, and these patients are recommended not to be put on treatment until follow-up towards a definitive diagnosis is concluded. As such, the seven participants with CL were advised to undertake further investigations in a referral facility, as repeated check-ups would help differentiate a potential CE cyst (CL) from a non-parasitic one. The positive predictive value for serological tests for CE is known to be low (Torgerson & Deplazes., 2009), so serology was not done. Since this study was time bound, continued follow-up is being done by the county referral hospitals.

Age was unconditionally associated with the presence of simple liver cysts, whereby the odds of having a simple liver cyst increased by 1.09 with every 1-year increase in age equivalent to a 2.37 (1.09^{10}) increased odds of having simple liver cysts with every 10-year increase in age. Hydatid disease is a chronic infection; it takes time to develop in humans and may persist in body organs for many years (Spruance 1974). It is therefore not surprising that the odds of infection in a CE prevalent area would increase with age, and this should be kept in mind when developing future targeted surveillance strategies.

Over the years, Western Kenya has had a high worm burden, particularly of soil transmitted helminths and schistosomiasis among preschool (Masaku *et al*., 2020) and school-going children. The Kenyan Ministry of Health, with the support of the WHO, has been implementing a school-based deworming programme using albendazole every three months, and recent studies in Kenya indicate a continuous

decline in the incidence of *A. lumbricoides*, hookworms, and *T. trichuria* among children in Kenyan public schools (Nikolay *et al*., 2015; Okoyo *et al*., 2016; Gonclaves, 2017; Mwandawiro *et al*., 2019). While continuous uptake of anti-helminthic drugs in the region may have suppressed the incidence of parasitic diseases, there is currently very low evidence that regular anthelmintic treatment would have an effect on CE. The movement of animals from Turkana and Pokot to western Kenya results in an ongoing risk for creation of a new CE econiche. The concern as to whether or not competent parasitic reservoirs have been established to advance the lifecycle is therefore fragile but viable. Our earlier investigations on the ecology of domestic dogs in this region have confirmed that domestic dog scavenging behaviour is associated with proximity to abattoirs dealing in meat from at-risk regions (Muinde *et al*., 2021), such that all elements are in place for the parasite to become established. It is therefore possible that minimal interventions like continuous deworming of dogs, livestock and humans may be interrupting or delaying the establishment of a new disease focus, and this should be investigated further.

Incidental findings of other ultrasound-detectable conditions was expected, and our study therefore implemented an information and referral protocol for these cases. Such findings create a challenge because the underlying causes may have significant health implications, and be of great concern to patients (Booth *et al*., 2010). Participants had consented for disclosure and referral of possible incidental findings. Arrangements were in place to hand over any confirmed CE case(s) to the Cystic Echinococcosis in sub-Saharan Africa Research initiative (CESSARi) for follow-up and treatment in collaboration with county hospitals. For other lesions, we committed to providing counseling through the clinician and a referral to a county hospital for follow-up. I therefore did not provide any compensation since I could not follow up on their undertakings.

Incidental abdominal findings included mild to moderate splenomegaly, uterine fibroids, ovarian cysts, polycystic kidneys, and benign prostatic hyperplasia (BPH). Western Kenya is considered endemic for malaria (Noor *et al*., 2012), and splenomegaly might be linked to the high incidence of malaria and *Schistosoma*

mansoni (Booth *et al*., 2004; Bashir *et al*., 2019), or malaria and invasive bacteria co-infections (Gómez-Pérez, 2014), in the study area.

In the multivariable logistic regression model, income was statistically associated with abnormal ultrasound findings, whereby the odds of having abnormal ultrasound findings decreased as the monthly income increased. This corroborates earlier findings in the same region which reported an inverse relationship between economic power and the risk of infection by an array of pathogens (de Glanville *et al*., 2019). In this study, a considerable proportion of the study population lived below the international poverty margin as their earnings were less than US$ 1.90 (Klasen *et al*., 2016), and would therefore not afford to sustain a treatment process, including surgery, hospitalization and chemotherapy, required for a CE case. Indeed, the minimum treatment costs for a case of human CE in a Kenyan government facility is between US$ 600 (Odero, 2015) and US$ 1000 (Zeyhle, personal communication), depending on the severity of the case and cyst location. A single case of CE in a population without insured healthcare may compromise the already meager resources of the household. Cystic echinococcosis remains a neglected tropical disease (Brunetti *et al*., 2011) mostly affecting the world's poorest; efforts to control CE (WHO, 2010) must therefore be accompanied by concomitant efforts to improve their standard of living.

Gender was marginally associated with abnormal ultrasound findings, whereby males tended to have lower odds of having abnormal ultrasound findings, compared to females. However, several of the abnormal findings were naturally "female-related", such as uterine fibroids and ovarian cysts, which are majorly linked to increased production of oestrogen (Reynolds, 2007), and female endocrine disorders, respectively. Positive abdominal findings also increased with an increase in age, and among the elderly participants, there were reports of BPH (in men) as well as simple liver cysts.

The portable ultrasound machine was a valuable resource in such a poor resource setting region. Nonetheless, power outages and power generator breakdowns often delayed the completion of scheduled activities, highlighting the challenges of

working in such field conditions. The use of ultrasound has not been fully considered a field deployable tool or a point of care option, both due to limited supply and unavailability of expertise. However, a point-of-care tablet-based ultrasound system has been used successfully to perform abdominal ultrasounds in a separate field investigation in western Kenya (Straily, 2021), further illustrating the potential of such tools. The American College of Gastroenterology proposes observation with expectant management for simple liver cysts (Marreroet *et. al*., 2014) and thus may require use of extra ultrasound diagnostic tools to aid the management of CLs.

This was a short study designed as an initial assessment of the potential establishment of a new CE focus in a potentially at-risk population living near abattoirs where many *Echinococcus granulosus* cysts in livestock had been identified (Falzon *et al*., 2021) and roaming dogs are abundant (Muinde *et al*., 2021).

5.2 *Echinococcus granulosus* in Dogs in Busia and Bungoma Counties, Western Kenya

In this study, 47% (70/148) of the participants reported deworming their dogs, though fewer did so regularly. The high frequency of hookworms identified in the sampled dogs could be due to this poor uptake of deworming practices, with a consequent heightened risk of zoonotic disease transmission. This study sought to determine the infection levels of taeniid cestodes and other parasites infecting dogs in western Kenya using various targeted diagnostic techniques. The study also sought to explore whether the movement behaviour of dogs that live in homesteads near slaughterhouses is associated with possible *Taenia* spp. and *E. granulosus* s. l. parasite burden. This, in turn, could shed light on the potential role of *Canis lupus familiaris* (domestic dogs) in contaminating the environment with infectious parasites and the potential propagation of a novel parasitic life cycle of CE given the reported interactions between dogs and livestock.

Dogs' infection with *Echinococcus* spp. and *Taenia* spp. has been reported previously from different parts of Kenya (Mulinge *et al*., 2018; Mulinge *et al*., 2020; Nungari *et al*., 2020). However, no study had been done in western Kenya, a region that lies between the two *Echinococcus* spp. prevalent regions of Turkana in the

north and Maasailand in the south. The dog infection with *E. canadensis* (G6/7) reported in this study is a notable finding, further supporting the emergence of this disease through the establishment of a life cycle of *E. granulosus s. l.* in the western Kenya region. Mulinge *et al*. (2018) reported finding of *E. canadensis* (G6/7) from dogs in Turkana, Maasai Mara, and Isiolo regions of Kenya, as well as *E. granulosus s. s.*, *E. felidis* and *E. ortleppi*. Similarly, Kagendo *et al*. (2014) reported *E. granulosus s. s.* and *E. felidis* from the sylvatic cycle in Kenya (lions and hyena). Most recently, Nungari *et al*. (2020) reported the presence of *E. equinus*, *E. felidis,* and four *Taenia* spp. *(T. hydatigena*, *T. multiceps T. ovis,* and unknown *Taenia* spp.*)* from dogs in Kajiado west in Maasailand, Kenya. This study records the second instance finding of *E. canadensis* in dogs (Mulinge *et al*., 2018) from a region previously considered non-endemic due to a lack of reports on *Echinococcus* spp. and *Taenia* spp. infection.

The finding of eggs of *T. multiceps* indicates the presence of coenurosis in livestock in western Kenya, while *T. serialis* in dog faeces as confirmed through sequencing may indicate the presence of a sylvatic cycle in the region, triggered potentially by dog predation of rabbits or wild hares. These zoonotic parasites pose the risk of coenurosis in humans (Deplazes *et al*., 2019). Mulinge *et al*. (2020) have reported seven different *Taenia* spp. among domestic dogs from other regions in Kenya. The establishment of a parasitic life cycle in western Kenya was indicated by the infection of the Mayanja dog with *E. canadensis* (G6/7) and *Taenia* spp. as shown by the Copro-PCR method. This study also confirms that mitochondrial DNA markers aid in demonstrating multi-species infection of taeniids in individual canids as shown in other studies (Štefanić *et al*., 2004; Xiao *et al*., 2006; Zhang *et al*., 2006; Schurer *et al*., 2014). *Echinococcus granulosus s. l.* in western Kenya would be maintained through a putative synanthropic life cycle involving dogs and sheep/cattle, similar to what is experienced in the Turkana region of East Africa. The same is reported in studies in Algeria (Kouidri *et al*., 2012) and Libya (Buishi *et al*., 2005).

This study would partially guide other studies in countries and regions with undocumented dog parasite-transmitted diseases. The study is timely as over the last few years, an increasing live animal trade of ruminants destined for slaughter and

location consumption has developed in the study region, with significant movements of animals into western Kenya originating in more northerly regions of the country (Watson & Binsbergen, 2008) where these helminths are known to be endemic (Griffith *et al*, 2020).

The close dog-human-livestock interactions in western Kenya, coupled with unsafe disposal of dog faeces, facilitate disease transmission between dogs, ruminants, and to humans. Dogs act as definitive hosts of several helminthic worms with the potential to contaminate the environment by shedding worms or eggs which subsequently mature in the soil (FAO, 2014).

The Coproantigen ELISA technique detects the presence of a specific antigen in a sample. It involves the use of capture and a detection antibody where the intestinal release of metabolic products by the parasite is immunologically detected, even in instances where eggs are undetected in the faeces (Allan and Craig, 2006). The presence of 18.3% coproantigen-ELISA positive dogs in Busia, even without concomitant detection of taeniid eggs by flotation and microscopy, may denote previous infections that had not been treated, though one must note that the Copro-antigen method does not discriminate between *Echinococcus* spp. and *Taenia* spp. infections. The Copro-antigen technique has been successfully used for surveillance of infection levels in dogs in Brazil, 11% (de la Rue, 2008), Chile, 3.5-11.7% (Acosta-Jamett *et al*., 2010), Peru, 46% (Moro *et al*., 1999), Uruguay, 4.3% (Irabedra and Salvatella, 2010), Turkey, 8.9% (Guzel *et al*., 2008), and China 18% in 2008 and 15.9% in 2009 (WHO, 2011). Since the Copro-antigen protocol is not yet locally available in Kenya, this study was only able to apply this diagnostic technique to a small sub-sample of the surveyed dog population after export of the processed samples. A more extensive surveillance effort is undoubtedly necessary, and the development of a local Copro-antigen ELISA surveillance protocol on dog faeces would aid effective surveillance toward eventual control of CE.

The density of the definitive hosts is key to transmission efficiency. The continuous growth in the dog population in Kenya (Kitala *et al*., 2001), as well as their roaming / straying nature, has become an impediment in the control of diseases for which they

are vectors. The home range of the dog, together with its infection intensity, is epidemiologically significant in determining the dispersal distance of the parasite, thus facilitating transmission. The dogs in this study had a core home range of 0.4 ha and a median daily travel distance of 13.5 km. The dogs may roam to scavenge for food, or when accompanying owners in their daily engagements (Muinde *et al*., 2021). All these sites that dogs interact with can therefore act as potential point sources for spread of emerging parasites such as *E. granulosus s. l.* in this environment, with the potential for further spread through dog movements to neighbouring areas.

Analysis of movement data indicated that some dogs visited the slaughterhouse, and these dogs had higher odds of being copro-antigen positive, though these results were only marginally significant. Other studies indicate that dogs can get infected with CE in a slaughterhouse disposal area where condemned organs are readily available (Ajlouni *et al*., 1984; *Mulinge et al*., 2018). Some of the smaller slaughterhouses in the study area did not have secured condemnation pits, providing dogs with potential access to infected offal as food. Cases of home slaughter were reported, and two-thirds of the slaughter done at home was not inspected by an authorised meat inspector, posing a risk for disease transmission. Some slaughterhouses did not meet the standard operational requirements and lacked appropriate facilities and hygiene, and owned dogs could easily access them to scavenge raw offal. Improved slaughterhouse infrastructure coupled with adherence to regulations governing condemnation is therefore key legislative requirements for effective control of CE.

Formalised and effective surveillance for *E. granulosus s. l.* the transmission would require frequent Copro-antigen surveys of dogs (Craig *et al*., 2015). Though Copro-ELISA is a common protocol, the lack of established capacity in East Africa restricts routine and cost-effective surveys. Building capacity for Copro-ELISA would allow for the establishment of baseline testing, followed by the more sophisticated Copro-PCR and sequencing methods that help in species specificity. Promulgation of education programmes on the control of echinococcosis and uninterrupted deworming of dogs with praziquantel is also recommended to disrupt the establishment of the life cycles of CE and other *Taenia* spp. Such surveillance and

control programmes could complement the National Rabies control strategy (Bitek *et al*., 2018) geared towards the elimination of dog-mediated human rabies, through the implementation of an intersectoral approach of dog population treatment and management leading to the coordinated control of multiple zoonoses.

5.3 Infection Levels and Haplotype Diversity of *Echinococcus granulosus* s.s. in Livestock in Western Kenya

The identification of haplotypes for *E. granulosus* s. s. in Kenya is the first of its kind in the east Africa region, considering the historical prevalence levels and the epidemiological distribution in Kenya, this report is poised to significantly influence future CE studies which may design control programms. Addy *et al*. (2017) conducted studies on haplotypes of *E. ortleppi* and *E. canadensis* G6/7 but there has been missing link for *E. granulosus* s. s. haplotypes which this study fills. Through the *Nad1* PCR-RFLP, all the isolates were identified as *E granulosus s. s.* (Figure 4.3) which confirms the dominance of the species in Kenya and the diminishing presence of other members of *E. granulosus s. l.* among the livestock from Northwestern Kenya. Other studies in Kenya have noted the presence of three species of the parasite as being present in cattle from other regions in Kenya *E. granulosus s. s, E. ortleppi* (Addy *et al*., 2012; Mbaya *et al*., 2014) and *E. Canadensis* (Mbaya *et al*., 2014), It is also indicated that fertility of the cysts might favour transmission in Kenya whereby in cattle mostly fertile cysts are of *E. ortleppi*, therefore having *E. granulosus s. s.* may have helped reduce the level of transmission in western Kenya. The absence of *E. canadensis* (G6/7) species in the region despite the parasite being present among dogs (Mulinge *et al*, 2018) in Turkana though in low numbers, needs further investigation. Moreover, studies in Sudan demonstrated overwhelming infection of cattle with the *E. Canadensis* G6/7, 98 % and scant infection with *E. ortleppi* at 2% (Omer *et al*., 2010). Further in the Southern parts of Africa, in his work in western Zambia, Banda *et al*. (2020) reported 100% of cattle infections to be of *E. ortleppi*. These differences in the presentation of cattle infection in Kenya shows interesting epidemiology of the parasite with Kenya harbouring the most diverse presentation in one country.

E. granulosus s. s., is a synanthropic species of tapeworm whose domestic life cycle is maintained by dogs and sheep in large pastoral areas worldwide. The minor involvement of other intermediate hosts e.g. goats, cattle, and camels has been proven via molecular epidemiological surveys (Romig *et al*., 2015; 2017). In Kenya the population of cattle infected with *E. granulosus* s. s. is high and the involvement of cattle in perpetuating the lifecycle is possible where some infections exhibit fertile cysts of *E. granulosus s. s.* among infected cattle. Just like the tapeworm became widespread through anthropogenic movements of dogs and sheep, through pastoralism in Eurasia and the European colonisation, the continued movement of animals across borders and within countries (as happening in Kenya) may continually propagate the spread of *E. granulosus* into new Eco niches.

5.4 Conclusion

In determining the infection of CE in humans this study reports that human evidence is not definitive, as this is a slow disease, however, with the current continued risky practices huuman infections may be realized soon.

In determining the infection of CE in livestock this study reports infection of *Echinococcus granulosus* s.s. among livestock imported from Turkana and west pokot into Busia and Bungoma counties, western Kenya. The expression of the various *Nad1* and *Cox1* gene haplotypes of E. granulosus is the first report in Kenya, and show extensive disersity of *E. granulosus* s.s. among livestock slaughtered in Busia and Bungoma counties, western Kenya. The study also confirms *Cox1* gene is more diverse than the *Nad1* gene of *Echinococcus granulosus* s.s.

In determining the infection level of Canine echinococcosis in dogs, this study confirms dogs pick up the parasite from condemned offal while presence of circulating antigens of *E. granulosus* s.l. by coproELISA is prove of infection in dogs in Busia County, while, the molecular identification of a dog infection by *Echinococcus* spp supports the plausibility of a life cycle of *E. granulosus* s.s. in Bungoma County. Infection of a dog with a mixed infection of *E. Canadensis*, *Taenia* multiceps and *Taenia serialis* confirm establishement of life cycle of the parasite in Bungoma County, western Kenya.

5.5 Recommendations

1. This study recommends control of *E. granulosus* transmission through frequent coproantigen survey of dog faeces, which in turn calls for development and validation of an incountry protocol for coproELISA
2. There is need for controlled livestock movement to seal off continued importation of disease from regions of high parasites density, improved slaughterhouse infrastructure to keep off dogs acess to offals, and increased surveillance through efficient diagnostic systems.
3. Promulgation of education programmes on control of echinococcosis and continuous uninterrupted deworming of dogs with praziquantel are recommended to disrupt establishment of lifecycle of *E.granulosus* s.l.
4. There are grounds for a larger study to be undertaken in this population that would potentially involve the routine screening of the population attending health units. Consideration of the US by health services as a field deployable diagnostic tool to aid epidemiological investigations is important as it would help in early detection of a range of conditions with high disease burden.

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APPENDICES

Appendix I: IREC Approval Letter

23 March 2018

Our Ref: ILRI-IREC2018-02

International Livestock Research Institute P.O. Box 30709 00100

Nairobi, Kenya.

Dear Prof. Eric Fevre & Titus Mutwiri,

Re: Cystic Echinococcosis and other zoonotic parasites in western Kenya: distribution and genetic diversity

Thank you for submitting your request for ethical approval to the ILRI Institutional Research Ethics Committee (ILRI IREC). ILRI IREC is accredited by the National Commission for Science, Technology and Innovation (NACOSTI) in Kenya.

This is to inform you that ILRI IREC has reviewed and approved your study titled *'Cystic Echinococcosis and other zoonotic parasites in western Kenya: distribution and genetic diversity'*. The approval period is March 23, 2018 to March 22, 2019 and is subject to compliance to the following requirements:

- Only approved documents will be used;
- All changes must be submitted for review and approval before implementation;
- Adverse events must be reported to ILRI IREC immediately;

- Access and Benefits Sharing (ABS) requirements, where applicable;
- Submission of a request for renewal of approval at least 30 days prior to expiry of approval period; and
- Submission of an executive summary report within 90 days upon completion of the study.

Please do not hesitate to contact ILRI IREC on

ILRIResearchcompliance@cgiar.org for any clarification or query.

Yours Sincerely,

Dr Silvia Alonso

Chair, ILRI Institutional Research Ethics Committee

Documents received & reviewed:

- Research Compliance Form & IREC Form
- Protocols for the work on Echinococcosis
- Consent Form (Human parasites, Ultrasound)
- Child Assent Form (Human parasites, Ultrasound)
- Questionnaire (Household & Abattoir)
- Hydatid Cyst Sample Collection Forms

Appendix II: Ilri Institute Biosaftety Committee Approval

ILRI

Our Ref: ILRI-RC001 17/IBC/001/CR 1th May, 2017

International Livestock Research Institute,
P.O. Box 30709-00100,
Nairobi, Kenya.

Dear Prof Eric Fevre

RE: Cystic Echinococcosis in western Kenya: Distribution and Genetic Diversity

This is to inform you that the ILRI IBC has reviewed the risk assessment for the study titled **"Cystic Echinococcosis in western Kenya: Distribution and Genetic Diversity"** and granted approval for implementation as per the research protocol and other related documents submitted. The approval period is from 1st May, 2017 to 30th January, 2020.

This approval is subject to compliance with the following requirements:

- Only documents submitted, reviewed and approved by the ILRI IBC shall be used.
- All changes (amendments, deviations, violations) shall be submitted for review and approval by ILRI IBC before implementation.
- Adverse events or unexpected occurrences, related to the study must be reported to the EOHS office immediately.
- All safety requirements as per the risk assessment and protocol submitted shall be adhered to.
- All staff working on the project shall be trained.

Should you need any further clarification, contact ILRI EOHS.

Yours Sincerely,

Dr. Vish Nene
ILRI IBC CHAIR

Appendix III: Consent Form for Homestead Owner for Dog and Human Parasites Research

Project name: Zoonoses in Livestock in Kenya

Current research: Zoonotic helminthic parasites in western Kenya

Invitation to participate and description of project

You are invited to participate in a research study that is conducting surveillance for Cycstic echinococcosis and zoonotic parasites in Western Kenya. Cystic echinococcosis also referred to as Hydatid disease is caused by a dog tapeworm. And it infects livestock and human. Zoonotic helminthic parasite are worms that infect animals and humans and may pose huge public health problems. The include parasites like Schistosoms, Trichuris, Ascaris, and hookworms. We therefore plan to collect dog faecal sample around homesteads that are within proximity of selected slaughterhouses in Bungoma, Busia Counties. At the same time, we shall also collect faecal sample from one person preferably a child from the same homestead. The faecal sample collected from the dog in your homestead will be tested for presence of *Echinococcus granulosus* eggs at our laboratories in Busia and Nairobi. Through this work we will better understand how common hydatid disease pose as a risk is in this region as well as determine the infection level of helminthiasis among human in western Kenya. This information will then help us make better recommendations on disease control and prevention strategies.

In order to decide whether or not you would like to participate we would first like to tell you what your participation will involve, and the possible risks and benefits of participating. We would like to stress that your participation is entirely voluntary. This work is being carried out by independent research workers at ILRI, and our partner institutions. While we work in partnership with government institutions, this research work is being carried out independently and does not represent any government body.

Description of study procedures

If you decide to participate, the following things will happen:

Short questionnaire, health screen and sampling

a. We will also ask you questions about your dog and its habits, its movements, feeding, treatment and vaccinations.
b. We will ask you or about the sanitary measure practiced in your homestead in regard to water availability, frequency of hand washing, how often do children wear shoes, etc.
c. We will collect a faecal sample from your dog and we will request you to help in this activity by soothing the dog as we muzzle it for sample collection
d. We will ask a child in your homestead preferably above 8 years to provide a stool sample. In the event that they cannot provide a sample at that time we shall leave the faecal pot behind and come for the sample at a later time/day
e. Lastly, we will ask you some questions about yourself, including your name, age, family status, work and contact information.

This should last 20-30 minutes.

Confidentiality

Any personal data disclosed during this interview will remain confidential to the research team, and will only be used for the purposes of this project. Your identity will remain anonymous throughout. Although we will collect your personal details, these are solely for the purpose of contacting you in case the need arises, and will be kept separate from any answers you give to the questionnaire. Personal identifying information, such as your name and telephone number will be securely kept and destroyed once the data collection has finished.

We may record your answers to the questionnaire electronically using a phone or tablet. This will be transmitted securely over the internet to computers at ILRI. Your answers and medical details will be given codes so that you cannot be identified from our database. All data is kept securely, and only the research team will have access to

the database. We will not disclose any of your information unless we are legally required to do so; for example in the case that we identify a reportable disease, we are obliged to notify the Kenyan authorities. This is important to ensure that disease does not spread further, and to protect both you, and your family's, well-being.

The samples taken from your homestead will be tested at the field lab in Busia (International Livestock Research Institute/Department of Veterinary Sciences Zoonosis Lab) for dog and human parasitic infections. We will also store the samples at the laboratory for further research after the initial tests are completed. Some of these samples may be sent to international laboratories at a later date for further testing, and some may be used to screen for other infections. You will not be able to be identified from the samples that you provide, and we will only use them to look for infectious diseases. Research results from your individual sample will not be returned to you. After the project is completed, anonymous samples may be kept in a long-term storage facility at ILRI or elsewhere and used for further research. As the storage will be anonymous, you will not be personally identifiable, and it will not be possible to report back to you the results of any future studies. However, if we find infection in the dog sample and I particular *Echicnococcus granulosus* we will come back and ask for your permission to screen you during a human cystic echinococcosis mass screen activity using the ultrasound machine.

Anonymous data obtained through this project will be shared with other projects and study results will be published in journals or shared with government stakeholders.

Risks

All health checks will be carried out by trained medical staff. There are no risks involved with the dog faecal sample collection. The dog may experience some little discomfort for a short period during sample collection which will be done directly from the rectum. If you experience any problems during the study, please alert the research team, who will allow you to take a break. You are also free to withdraw from the study at any time.

Benefits

The general health screen will be provided at no cost to you. If any problems are identified during the examination, advice will be provided.

We will not report research results back to you individually. You will not directly benefit from the research, but we will hold a community meeting to discuss the research at a later date. Government departments will also be informed of the research results, and we hope that this will help them to make better decisions that bring benefits to both livestock and human populations in western Kenya.

Participation and withdrawal

We would like to emphasise that your participation is voluntary. If you consent and then later decide that you would like to withdraw, you are free to do this at any point.

If you later decide that you do not want that the samples taken from you, or your information, to be used in the study, you can contact the study team and ask that your contribution be withdrawn. Contact details are provided below. However, contribution can only be withdrawn for a limited time, since the anonymization process will make it impossible to identify an individual's answers/samples after that time.

If there is anything that you have not understood, please feel free to ask questions. You are welcome to ask us to go over any aspect of this form again before you decide whether or not to participate. You are also welcome to ask about other aspects of the study overall.

Authorisation

I confirm that I have read (or someone has read to me) this form, and I have understood the purposes of the research, what my participation will involve, and any risks of the research. I agree to participate in the project described.

Name of homestead owner……

Signature / thumb print……..……

Date……

Signature of Investigator……..……

Contact details:

If you have any further questions about this project, or you have a research-related problem, please contact a member of the study team.

Investigator name …..……

Investigator contact number ……

Investigator: Titus Mutwiri **Tel:** 020 422 3000

If you have any concerns about the way that the research has been conducted and would like to speak to an independent member of the Institutional Research Ethics Committee, please contact:

Ephy Khaemba (Manager, Research compliance and Environment Health and Safety)

ILRI, Old Naivasha Road, PO Box 30709-00100, Nairobi

Tel: 020 422 3375

Appendix IV: Household Questions

Section 1

Demographic questions

1. Date_____(This will Auto Insert)
2. Interviewer

    i. Patrick Muinde
    ii. Titus Mutwiri
    iii. Maurice Karani
    iv. Kelvin Momanyi
    v. Allan Ogendo
    vi. Joseph Ogalo
    vii. Laura Falzon

3. County name

    i. Busia County
    ii. Bungoma County

4. Area (Location) also take GPS coordinates (coded into the form

    i. Sub-county
    ii. Sub-location
    iii. Village

5. Respondent

    i. Head of the household
    ii. Other adult
    iii. Minor (below 18yrs)

6. Gender of the respondent

    i. Male
    ii. Female

7. Number of people living in the in the household:_____
8. Do you own a dog ?

    Yes

    No

9. If yes, how many dogs do you own (the total number of dogs they claim responsibility of taking care of)

    i. Number of males
    ii. Number of females

10. What are the reason for you keeping a dog? (The assumption is that all the dogs are treated the same way)

    i. Hunting
    ii. Security (home guarding)
    iii. Herding (Herd dogs)
    iv. Pet
    v. Other (Specify)

11. Do your dogs have an access to the outside of the household compound?

    i. Yes
    ii. No

12. Do the dogs have an access to human living areas?

    i. Yes
    ii. No

13. On a typical day, how many hours is your dog kept outside the home compound?

    i. Less than 2 hours
    ii. 2 hours – 6 hours
    iii. More than 6 hours

14. Do the dogs accompany you when going out (for work, visits, etc)

    i. Yes
    ii. No

    If yes, how often?

    iii. Rarely (<25% of the time)
    iv. Sometimes (>25% of the time)

15. Do you see other dogs apart from yours in your compound?

    i. Yes
    ii. No

    If yes, how often?

    iii. Sometimes/Occasionally
    iv. Frequently

    If yes, how many dogs in a day?

16. Which livestock species do you keep in your homestead?

    i. Goats
    ii. Sheep
    iii. Cattle
    iv. Pigs
    v. None of the above

17. Can the dogs access where the livestock live? (Note: kindly observe)

    i. Yes
    ii. No

18. How do you dispose dogs fecaes?

    i. Leave it
    ii. Dispose into the garden
    iii. Dispose into the toilet
    iv. Bury in the soil
    v. Other (specify)

Section 2.

Possible transmission factors of Echinococcus

1. Do you carry out home slaughter for your livestock?

    i. Yes
    ii. No
    iii. Not applicable

2. If yes, are the carcasses inspected by a meat inspector?

    i. Always
    ii. Sometimes
    iii. Never
    iv. Not applicable

3. How regularly do you feed your dogs (any food given to the dog)?

i. Daily
ii. Every couple of days

iii. Occasionally
iv. Never

4. In a month, approximately how much do you spend on buying dog food (or taking care of the dog)_____

5. Do you feed your dogs with internal organs from livestock (e.g. offals)?

i. Sometimes
ii. Always
iii. Never

6. If yes above,where do you source the offals from?

i. Own slaughter
ii. Abattoir
iii. From the butchery
iv. Other

7. How do you prepare these offals before giving to the dogs?

i. Fed raw
ii. Cooked (Boiled, fried)
iii. Roasted

8. In those internal organs (offals), have you ever seen any cysts? Show a picture

i. Yes
ii. No

9. What do you do oftenly with the meat containing the cyst(s)?

i. Cooked and consumed in the household

ii. Cooked and fed it to dogs
iii. Fed to dogs raw
iv. Chopped off the infected area and consume the remaining portion in the house
v. Chopped off the infected area and fed the rest as raw to dogs
vi. Chopped off the infected area and fed the rest as cooked to the dogs
vii. Discarded everything
viii. Buried/Burned

10. What do you do to carcasses of dead animals within your household?

i. Bury/Burn
ii. Skin and eat/sell
iii. Feed to dogs
iv. Discard
v. Other (specify)

11. Do the dogs accompany cattle, goats and sheep when grazing?

i. Yes
ii. No

12. Do the dogs defecate where the livestock graze?

i. Yes
ii. No
iii. Don't know

13. Do any of the family members play with the dogs?

i. Yes
ii. No

14. Do you deworm your dogs?

    i. Yes
    ii. No

15. If yes, how frequently do you deworm them?

    i. Monthly
    ii. After every 3 months
    iii. Every 6-12 months
    iv. Never
    v. Other (Specify)

16. If 15 yes, which drug do you use? (have a list of common dog dewormers)
17. Have you vaccinated your dogs in the last 12 months?

    i. Yes
    ii. No

    How much did you pay for it?

Section 3.

Proximity and access to a slaughter house/ slab

1. Do your dogs visit the slaughter house/ slaughter slabs?

    i. Yes
    ii. No
    iii. Don't know

2. If yes above, how oftenly do they go to these slaughter houses?

    i. Occasionally

ii. Frequently
iii. Don't know

Section 4

Awareness of echinococcosis amongst household members

1. Have you ever seen a "proglottid" (*Echinococcus*) in dog faeces? (show Picture)

    i. Yes
    ii. No

2. If yes, do you know what these "proglottids" are?

    i. Yes
    ii. No

3. When you see the "proglottids' in the dogs faeces; what actions do you take? (Multiple selection)

    i. Nothing
    ii. Bury the feaces
    iii. Dispose the feaces
    iv. Deworm the dog
    v. Other (Specify)

4. Are the offals/organs from livestock infected with cysts safe for consumption by humans and dog?

    i. Yes
    ii. No
    iii. Don't know

Section 5

Collaring of the dog

5. What is the age of this dog? (The dog to be collared)

i. Young (less than 1 year)
ii. Adult
iii. Old (more than 5 years)

6. What is the sex of the dog to be collared?

i. Male
ii. Female

If male, is it castrated?

i. Yes
ii. No

If female, is it spayed/Neutered?

i. Yes
ii. No

7. Take fecal sample and scan the barcode

Section 6

Household questionnaire for soil transmitted helminthes (STH) infection

1. Age
2. Gender
    a. Male
    b. Female

3. GPS coordinates
4. No of people in homestead
5. No of toilets and/or pit latrines in the homestead (observe for pit latrines)
6. Type of toilet used
    a. House
    b. Latrine
    c. Bush/field defecation
7. Are the toilets shared
    a. Yes
    b. No
8. How often do you clean the toilet/ pit latrine

    a. Every time after use
    b. Once or twice a day
    c. Few times in a week
    d. When we see faeces on top of toilet
    e. We don't clean

9. Your pit latrine floor is made of what material
    a. Wooden
    b. Cement
    c. Earthen
10. Where do children defecate
    a. Toilet
    b. Pit latrine
    c. Playground/ bush
11. If playground/bush/field, what do you do with the faecal material left by
    a. Leave it
    b. Discard in toilet/pit latrine
    c. Throw it away but further from the homestead
12. Do you see faeces in toilet facility?
    a. Yes
    b. No

c. Sometimes

13. Ever observed sewage pooling from latrine
    a. Yes
    b. No
    c. sometimes
14. Type of water source
    a. Borehole
    b. Taped
    c. Spring
    d. Rain
    e. River
15. Is water available all the time
    a. Yes
    b. No
16. Is water treated?
    a. Yes
    b. No
    c. Sometimes
17. Do you use the same water for washing, cooking, and drinking
    a. Yes
    b. No
    c. Sometimes
18. Do you boil water before drinking
    a. Yes
    b. No
    c. Sometimes
19. Frequency of washing hands
    a. Very rare (e.g. once in two days)
    b. Sometimes (e.g. once or twice in a day)
    c. Most often (more than three times in a day)
20. What do you use for washing hands?
    a. Water only

b. Water and soap sometimes
c. Water and soap always

21. Frequency of wearing shoes (observation)
    a. I don’t wear shoes
    b. Sometimes
    c. Every time
22. Ever dewormed yourself
    a. Yes
    b. No
    c. Can’t remember
23. If Yes, when was that
    a. Less than three months ago
    b. More than three months ago
    c. More than 6 months ago
24. Faecal sample taken (Barcode)

Appendix V: Distribution and Genetic Diversity of Human Cystic Echinococcosis in Western Kenya

Principal Investigator : Eric Féver

Investigators : Titus Mutwiri, Laura Falzon, Fredrick Amanya, Joseph Ogola, Alan Ogedo, Maurice Karani, Loreen Alumasa, Eberhard Zyhle

INFORMED CONSENT

Distribution and genetic diversity of cystic Echinococcosis in western Kenya

INTRODUCTION

You have been selected to participate in this study because you are a resident of western Kenya and you may help us obtain more information regarding the disease Cystic Echinococcosis which is caused by a dog tapeworm. This parasite is found in all parts of the world, but in Kenya it has been found to be very common, compared to other countries in Africa. This disease can cause severe illness and may sometimes even lead to death in both humans and animals. The disease in human, when caught in the early stages, can be cured using drugs. However, at advanced stages, the disease can only be cured through surgical procedures.

If you agree to participate in this study we request to conduct an ultrasound imaging procedure during a mass screening activity that shall be conducted in your community. In case you are found having hydatid cysts within your body organs you may be referred to another organization for appropriate advice and possible surgery, however in this case all the information will be explained to you by the team of researchers conducting this study.

Taking part in this research study is your choice. If you can, please read all of the following information. If you cannot read, please listen to the person explaining this information to you very carefully. Ask the investigator, to explain to you any words, terms, or sections that are not clear to you. You should also ask any questions that you have about this research study. Do not sign this consent form or let anyone sign it on your behalf unless you understand all the information in it and have had all your questions answered to your satisfaction.

If you decide to take part in this research study, you will be asked to sign this form and you will be given a copy of the signed form. You should keep your copy for your records. It has information, including important names and telephone numbers, which you can use in the future either to ask questions, or seek clarifications.

This research study has been reviewed and approved by the International Livestock Research Institute (ILRI) Ethical Review Committee. This group consists of clinicians, veterinarians, molecular scientists, sonographers, and other relevant nonmedical personnel, and they review human and animal research studies to ensure that they are safe and that the people who take part in them are safeguarded from any harm.

PURPOSE OF STUDY

The purpose of doing this study is to determine the infection levels of *Echinococcus* in humans,. Titus Mutwiri, will conduct the study under the supervision of Prof Eric Fèvre, Prof Ann Muigai, & Prof Kithinji Magambo, alongside other researchers who include Dr. Laura Falzon, Dr. Patrick Muinde, Dr. Jospeh Ogola, Dr. Alan Ogendo, Dr. Maurice Karani, and clinicians Fred Amanya and Lorren Alumasa. This study is essential because hydatid disease is a major concern in Kenya, particularly in those individuals living in this western Kenya where movement of livestock from hydatid endemic regions take place which can enhance propagation of the parasites life cycle through domestic dogs

PROCEDURES TO BE FOLLOWED FOR SURGERY

After agreeing to participate in this study, you will be asked to sign this form. In case of human infection where need for an surgical operation is identified, this would be carried out by experienced doctors with permission from the ministry of Health. During the surgery, we shall collect the hydatid cyst/s which contain the parasite that causes hydatid disease, and preserve the cystic material for further analysis at the ILRI Laboratories in Busia. The project may not pay for the surgical procedure but will organize through the consortium of Cystic Echinococcosis in Sub Saharan Africa Research Initiative (CESSARi) how such surgeries will be conducted at the referral hospitals through the ministry of health. It is highly recommend that surgery is done so as to stop the chronic illness. After the surgery, you will be put on the necessary treatment to avoid further infections. Depending on the outcome of this study these samples may be used for further research.

Your participation in this study does not affect your medical care, and members of this research study will have no contribution to decisions made by your doctors. Any significant finding developed during the course of this study will be provided to your hospital management, but no personal/ identifying information will be provided unless upon your request.

BENEFITS

We will conduct our research under the ZooLinK arm of the Zoonoses in Livestock in Kenya. This will be of benefit because we will try and find out how different types/strains of *Echinoccocus* present themselves both in humans and livestock, and this will assist in designing appropriate control programmes for this disease. Results from the specimen analysis will be made available to the

clinicians managing you to allow for appropriate action. You will also be provided consultation at the screening point for treatment of any other infections detected by this analysis**.**

Results obtained from this study can help in further understanding and developing ways of preventing hydatid disease, and hopefully in future the developing a vaccine against it. This could be of help to many people in Kenya and in other parts of the world.

WHOM TO CONTACT

If you have problems or questions about this study at any time, please call: Titus Mutwiri +254 20 422 3329 or contact the International Livestock Research Institute Nairobi or Busia

COSTS

There will be no costs to you associated with participation in this research study. This study is supported by International Livestock Research Institute.

PAYMENT

You will not be paid or given any incentives for participation in this study.

Confidentiality

Any personal data disclosed during this interview will remain confidential to the research team, and will only be used for the purposes of this project. Your identity will remain anonymous throughout. Although we will collect your personal details, these are solely for the purpose of contacting you in case the need arises, and will be kept separate from any answers you give to the questionnaire. Personal identifying information, such as your name and telephone number will be securely kept and destroyed once the data collection has finished.

We may record your answers to the questionnaire electronically using a phone or tablet. This will be transmitted securely over the internet to computers at ILRI. Your answers and medical details will be given codes so that you cannot be identified from our database. All data is kept securely, and only the research team will have access to the database. We will not disclose any of your information unless we are legally required to do so; for example in the case that we identify a reportable disease, we are obliged to notify the Kenyan authorities. This is important to ensure that disease does not spread further, and to protect both you, and your family's, well-being.

The results from your individual ultrasound images will be communicated to you after the procedure. Anonymous images shall then be kept in a long-term storage facility at ILRI or elsewhere and used for further research.

Anonymous data obtained through this project will be shared with other projects and study results will be published in journals or shared with government stakeholders.

Any results from the project to be published in regional and international journals or presented at appropriate scientific meetings will seek scientific clearance from the Publications Committee of ILRI.

PARTICIPANT'S STATEMENT

I have read this consent form and have discussed with the research assistant the procedures described above. I have been given the opportunity to ask questions, which have been answered to my satisfaction.

I understand that my participation is voluntary. I also understand that if, for any reason, I wish to discontinue participation in this study at any time, I will be free to do so, and this will have no effect on my future care or treatment by my physicians or this hospital. I understand that I will not be given any money to participate in the study.

If I have any questions concerning my rights as a research subject in this study, I may contact the ILRI Ethical Review Committee at +254 20 422 3329. I have been fully informed of the above-described study, and I hereby consent to the procedures set forth above and I have received a signed copy of this consent form.

I understand that as a participant in this study my identity and my medical records and data relating to this research study will be kept confidential.

_____ _____

Date Name Participant's Signature

I have fully explained to _____ the nature and purpose of the above-described study and the risks that are involved in its performance. I have answered all questions to the best of my ability.

_____ _____

Date Principal Investigator or Representative's Signature

Appendix VI: Protocol for Community Surveillance for Human CE by Ultrasonography in Western Kenya

Principal investigator : Eric Févre

Co-investigators : Titus Mutwiri, Eberhard Zyhle, Laura Falzon, Kithinji Magambo, Anne Muigai

Clinicians : Fredrick Amanya, Lorren Alumasa

Patient Preparation.

- The ultrasound examination is most effective if the patient has been NPO for at least 6 hours.
- This allows the biliary system to be distended and easily imaged by the sonographer.
- When a patient is fasting there is a decreased opportunity for gas to accumulate within the colon since gas prohibits the passage of the sound and thus limits visualization of abdominal structures.
- If the patient is able to consume liquids and the pancreas is not well-visualized, the administration of 32 oz. of water may be given to fill the stomach and duodenum to better delineate the pancreas.

Patient Position.

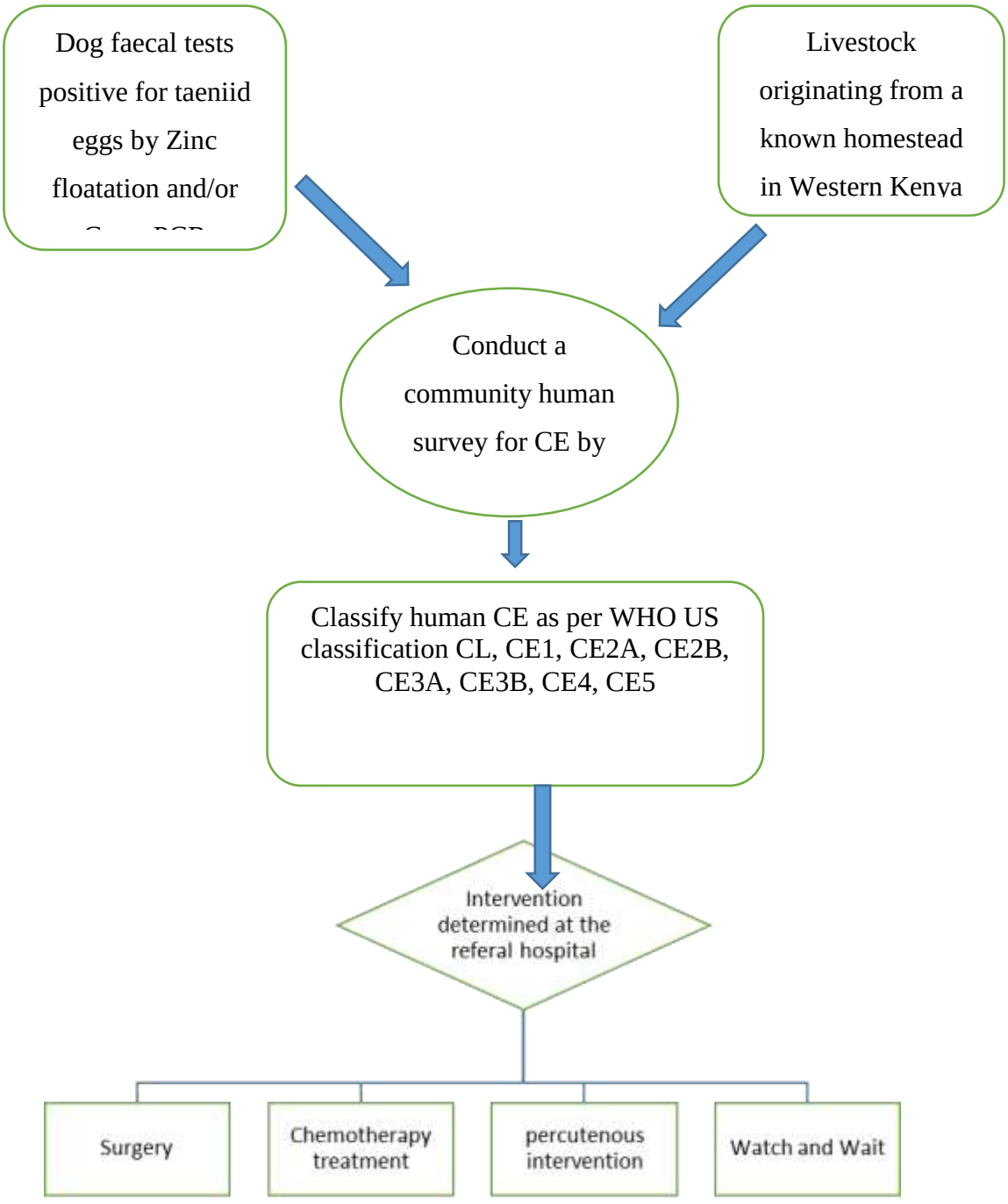
- The position of the patient for the general abdominal scan is usually supine for the initial images.
- The patient is then rolled into various degrees of obliquity to better demonstrate the biliary system, pancreas, liver, kidneys, or spleen.
- If the scanning plane is oblique, the sonographer indicates the change of position on the documented image without specifying the exact degree of obliquity.
- The same would apply if the patient were in a lateral, upright, or prone position.

Sectional Anatomy

- Ultrasound of the abdomen is generally performed in at least two image planes, transverse and longitudinal.
- It is not unusual for the sonographer to alter these imaging planes or change the patient position if adequate visualization is not obtained.

Patient Care Protocols

- It is the responsibility of the sonographer to ensure that patients are afforded the highest quality care possible during their ultrasound procedure.
- This entails identifying the patient properly, ensuring confidentiality of information and patient privacy, providing proper nursing care, and maintaining clean and sanitary equipment and examination rooms.


Dog faecal tests positive for taeniid eggs by Zinc floatation and/or
Livestock originating from a known homestead in Western Kenya
Conduct a community human survey for CE by
Classify human CE as per WHO US classification CL, CE1, CE2A, CE2B, CE3A, CE3B, CE4, CE5
Intervention determined at the referal hospital
Surgery
Chemotherapy treatment
percutenous intervention
Watch and Wait

Appendix VII: IREC – Institutional Research Ethics Form 2 (In-Depth)

This form is to be submitted to ILRIResearchcompliance@cgiar.org **along with any relevant documentation** after completing all sections and all endorsements (see Annex for instructions).

IREC ref no. **IREC use only**

POINTS TO NOTE:

- This form is valid, unchanged, for one year.
- If minor changes are required then the **IREC FORM 3 MINOR AMENDMENT** (see website for form - www.ilri.org/ethicscommittee) should be completed and sent to the IREC committee for consideration.
- The committee requires the form to be submitted to the IREC committee at least 1 month before the start of the research activity to enable sufficient time for it to be approved.
- The ILRI IREC committee follows international standards for research ethics and follows the principles of research ethics (autonomy, beneficence, non - maleficence and justice).

Approval Type Requested:	X the box which applies. Note that 'Conditional' applications should be followed up by 'Final' applications at project activity planning stage		
Conditional (e.g. project proposal stage)		Final (e.g. project activity planning stage)	X
If previous conditional approval received give the IREC ref. number			

1. Title of research project & activity:	Overall project title & research activity to which this form refers (Max. 30 words)
Cyctic Echinococcosis and other zoonotic parasites in western Kenya: Distribution and genetic diversity	

2. Team, location & grant code under which research activity falls:	e.g. BT02 NBO WEL004
DAD054	

3. Principal Investigator (PI): (Person ultimately responsible for this research activity)	*Prof Eric Fèvre – Programme Leader +254 20 422 3329*	Home Tel. Mobile Tel.	
4. ILRI staff responsible & role:	*Titus Mutwiri – Doctoral student +254 721 653 089*		
5. Names or description of others involved in conducting the research:	Laura Falzon – Post Doc and field supervisor Patrick Muinde – Research Assistant Fredrick Amanya – Clinical Officer Allan Ogendo – Veterinarian Joseph Ogola – Veterinarian Samuel Njoroge – Laboratory manager *Lorren Alumasa – Clinical Officer* *Maurice Karani – Research Assistant* ***Other staff from ZooLinK Suite of projects may also be involved in some instances*** ***Eberhard Zhyle an expert in ultrasonography will be involved***		

6. Overall objectives of this research activity:	*(Provide numbered list)*

1. To determine the prevalence of zoonotic parasites in humans from homesteads around slaughterhouses in western Kenya.
2. To perform a community surveillance for human CE by ultrasound scanning in western Kenya
3. To establish the genetic variability (haplotypes) of human CE in Western Kenya.
4. To determine the economic impact of human CE in Western Kenya.

7. Regulatory Requirements / Guidelines:	*Are you in compliance with local regulatory requirements (give details & info. on any approvals required)*
The study is partly operated under the ZooLinK suite of projects (ILRI-IREC 2017-08)	

8. Anticipated benefits:	*1. Anticipated benefits to research participants and their community* ***2.*** *Anticipated benefits to other communities*

1. An improved knowledge of the prevalence and distribution of Echinococcus and other zoonotic parasites in humans, shall allow for more informed decisions regarding disease control and prevention, in turn helping to reduce the overall burden of these diseases.
2. Trained workforce on diagnostics protocols for Echinococcus and other zoonotic parasites.

9a. Anticipated risks and their management	*1. Anticipated risks to research participants* *2. Anticipated risks to personnel involved in conducting the research* *3. Anticipated risks to animals, environmental sustainability, economy or development*

	4. Procedures taken to minimise & manage these risks

1. Research participants may be found (by the screening protocol) to have a different or more serious problem/infection, diagnosable by ultrasound, which they did not anticipate. Such cases will be automatically referred to higher level hospitals within the public health care setting.
2. Research participants are knowledgeable on laboratory protocols and field activities to be conducted – any risk will be handled as any risk that would occur in the field or laboratory. No major risks are anticipated
3. Basic Good laboratory practice shall be followed so as to minimize any risk in the laboratory

9b. Care and protection of research participants	*Regarding 'risks to research participant' in Section 10, Detail here:* *1. Any <u>medical care or treatments to be provided</u>* *2. Any rewards and compensations for research participants* *3. Provisions for compensation/indemnity in case of adverse effects*

N/A (No risks anticipated)

We do not anticipate providing ancillary care for research participants. The participants will be made aware of possibility of finding hydatid cysts prior to giving consent and that often surgery is the available option for treatment. AMREF which has been conducting surgical procedures in Turkana for decades will be requested to conduct operations to remove cysts to minimize cost. The county government will be requested to offer support through the County executive committee once the actual cases are identified

10. Research activity details / protocol	*1. Study type / design (incl. use of 'counterfactuals', if relevant & site selection)* *2. Justification for number of participants (e.g. power / sample size calc.)* *3. Plans for data management & analysis* *Attach to application any relevant documentation (e.g. study, sampling, or data management protocols)*

1. Surveillance for zoonotic parasites in human and dogs at homestead level

   Eight slaughterhouses will be identified from each county, and from around each slaughterhouse 10 homesteads keeping at least one adult free-roaming owned dog will be identified. From each homestead one person and preferable the head of the homestead (unless in instances where the head in unavailable) will have the study explained to them in details. If the head of the homestead agrees to participate in the study they will be asked to sign the consent form to allow the field team collect faecal samples from the dog, an adult or a child within the homestead

   a. One member of the family and who most often closely relates with the dogs within the homestead will be asked to provide a faecal sample, after an individual consent form has been signed. In the case of a child providing the faecal sample the immediate parent or in the absence of the parent the closest guardian/relative will provide assent for the child to provide a faecal sample. Faecal pots/ polypots will be provided to the person who is to provide the sample and instructions given on faecal collection so as to avoid self-contamination. In some cases where the person is not ready to give the sample immediately, the faecal pot will be left behind and they will be advised to collect the faecal sample later.
   b. One dog from the homestead will have a faecal sample collected directly from the rectum using a lubricated gloved finger. The dog will be leashed and muzzled during the procedure and a homestead member close to the dog will be available to sooth the dog during the process.
   c. An electronic in a tablet questionnaire will be administered to collect information about the dog - human interaction. Both samples obtained from humans and dogs will be transported to the ILRI Busia laboratory for analysis of zoonotic parasites.

2. Surveillance for human CE at Community level:

In this study a community refers to homesteads within an area surrounding an abattoir. The community surveillance process will be conducted under the supervision of a clinician.

a. Inclusion criteria

A community will be considered for inclusion into the study if it meets the following conditions as per ongoing sister studies on livestock CE and canine echinococcosis in western Kenya

i. If the livestock CE data collected from the abattoir level shows an "indigenous infection" with CE among the livestock brought for slaughter then the community around that slaughterhouse will be earmarked for mass screening of human CE. i.e if the livestock is suspected to have acquired infection locally/ from within the community.

ii. If the dog test results obtained at homestead level (No 1 above) show presence of taeniid eggs by microscopy and later confirmed as *Echincococcus granulosus* infection by CoproPCR, then people living within that community will be tested for human CE infection through mass ultrasound screening.

b. Human mass ultrasound screening for CE

Human ultrasound scanning to be conducted by an experienced sonographer and a clinician. There will be provision for informed consent to ensure participants make informed choices about the procedure, the meaning of a positive or negative test result, and any appreciable risks and potential harms, and benefits before undergoing screening. Participants will be explained about the risk of a false positive test result and the procedures that may follow. If Hydatid cysts will be identified during the procedure they will be be reported and classified as per the WHO U/S classification and appropriate advice on intervention

will be given as per WHO recommendations. This may include available *treatments* for uncomplicated hepatic cystic echinococcosis (CE) include *surgery*, medical therapy with albendazole (ABZ), *percutaneous* interventions and the *watch-and-wait* (WW)

The aim to do conduct 100 ultrasound scans per day using a portable ultrasound scanner.

3. Human social economic impact of CE

Economic impact in humans will be assessed by determining both direct and indirect costs in humans. Direct estimated costs for human will include costs for: standard care procedures, clinical tests/diagnosis, surgical interventions, chemotherapeutic treatment, medical care and hospitalization. A cost per patient estimate will be calculated using the average normal cost of care in the referral county hospital. Indirect costs comprising human productivity losses will be determined, and the age and gender of the patients will be used for estimation of these productivity losses in humans.

4. Disability Adjusted Life Years

Disability Adjusted Life Years for CE in western Kenya will be calculated as the sum of the Years of Life Lost (YLL) due to premature mortality in the population and the Years Lost due to Disability (YLD) for people living with CE or its consequences. Therefore, DALY = YLL + YLD. The basic formula for YLL will be: YLL= N×L, where N = No. of deaths, and L = standard life expectancy at age of death in years. On the other hand, YLD for CE during the study period will be given by the number of incident cases in that period multiplied by the average duration of the disease and a weight factor that reflects the severity of the disease on a scale from 0 (perfect health) to 1 (dead). The basic formula for YLD will be: YLD = I × DW × L, where: I = number of incident cases; DW = disability weight; L= average duration of the case until remission or death (years).Genetic work that will be done

Samples collected in the field and or parasites retrieved from cysts following surgery

will be transported to Busia ILRI Laboratory for processing, preservation and molecular characterization as approved in the protocol ILRI-RC 001 17/IBC/001/CR

5. Data Management

Data entry shall take place in the field or laboratory using Android GPS-enabled Kestrel tablets which will be linked directly to a secure cloud server and a custom designed database. Any digital data (e.g. pictures, videos) of relevant lesions in both animals and human patients will be collected in such a way as to preserve the anonymity of the patient. Basic clinical parameters shall be recorded on electronic forms. In the case of any participants with health conditions that require further evaluation, clinical evaluations shall be recorded on paper forms and summary information shall be entered into the database. Questionnaire data shall be uploaded to the server on a daily basis, and unique identifiers shall be used to link such data to laboratory outputs. Data cleaning procedures will be performed before importing it for analysis into Stata 9.2 (Stata Corporation, Texas USA). Bivariate analysis using Fisher's exact chi-Square will be used to measure the difference between different risks factors of *E. granulosus* transmission. Multivariate analysis using logistic regression models will be carried out to control for confounding and to tease out the significant risks factors. Estimates of the parameters will be considered statistically significant at $p < 0.05$. The information will be presented using tables, graphs and charts. The identification of cox1 haplotypes and the drawing of their networks will be done by TCS 1.2 program using statistical parsimony

11. Recruitment & involvement of research participants and informed consent	1. *Means of recruitment (incl. inclusion & exclusion criteria, withdrawal criteria)* 2. *Means by which full information about the research activity will be conveyed to communities / participants* 3. *Details of the informed consent process (attach to application relevant forms)*

The slaughterhouses previously identified for the ZooLinK study (ILRI-IREC 2017-08) will be used as focal points for this study. Ten homesteads around each slaughterhouse will be identified on condition that they keep a free roaming dog. A single dog per homestead will be identified and a faecal sample collected directly from the rectum after the dog owner signs the consent form. One person from the homestead, who is mostly close to the dogs will be required to sign a consent form and then have their faecal sample collected too, in case the sample will be provided by a minor assent will be sort from the parent or guardian . A questionnaire will be administered to the said person (or the guardian in case the participant is a minor)

For the Community surveillance on CE the village chiefs and elders will be called for a meeting and enlightened on the aim of the study. They will be requested to inform the villagers on the proposed Ultrasound scanning process intended to run for one week. After the chiefs and elders pass the information we shall conduct the scanning procedure one location at a time accompanied by the local chief and the village elder in charge of that location. Before scanning we shall explain the process to the villagers and those who consent will be examined using a portable US scanner to check for any cysts in the liver, the lungs and other possible organs

12. Protection of privacy & confidentiality	*Detail the following:* 1. *Measures taken to ensure privacy (during data collection & after)* 2. *Who will have access to data & in what form, plan to anonymise the data etc.*

Personal information will not be given to anyone without written permission by research participant. Information obtained from participant will be identified using a unique indentification number, hence individual names and medical record will not appear on any study paperwork or samples. A code will be used to link the indentification number with name. The codes will be locked up and will only be accessible to the investigators involved in this study. This information will be backed up in computer files that will be accessible only to the investigators using a password. If any publication arises from this work it will have prior authorizatton by

the Publications Commmittee of ILRI and we will exclude any information that could be used to identify any participant. Any results from the project will be published in regional and international journals or presented at appropriate scientific meetings

13. Feedback to communities & participants	*How will research results be made available to research participants and the communities (detail for each type of results – e.g. survey, sample results)*

Ultrasound images and Laboratory results from the specimen analysis will be made available to the clinicians managing the patient to allow for appropriate action. You will also be provided consultation at the County hospital for treatment of any other infections detected by our analysis. For this reason the samples will not be anonymized at this initial stage. Survey results obtained from this study can help in further understanding and developing ways of preventing hydatid disease. This could be of help to many people in Kenya and in other parts of the world.

14. Proposed (future) commencement date:	December 2017	Duration:	18 months

15. Details on site facilities, equipment & skills of technical staff	*Explain the various technical facilities required / in place for the research (e.g. labs, field stations) & the skills and any relevant training required e.g. medical qualifications for human sampling, training of enumerators for household surveys etc.)*
The ILRI Busia laboratory will be used for laboratory protocols, The field work involving livestock and dogs shall be done with the coordination of the ZooLinK Veterinarians based in Busia. The human component shall be coordinated by Eberhard Zyhle (ultrasound) and the ZooLinK clinicians based in Busia. The Laboratory Technologists will conduct the laboratory analysis. See section 5 above.	

16. Instruments	*List relevant research instruments attached: e.g. consent form, questionnaire, interview guide, protocols – study, sampling, data management.*
1. Sampling plan	5. Consent form
2. Questionnaire	
3. Protocols	
4. Data Management plan	

17. Other ILRI approvals required?:	*1. Research activities involving animals require an IACUC form (this also covers the Ethical issues relating to research on animals)* *2. Research activities involving biohazardous substances must be approved by the IBC*	
	Required? (X if yes)	Reference (if already received)
Institutional Animal Care & Use Committee (IACUC)		**X** IACUC reference no. Ref No 2017-10.

Institute Biosafety Committee (IBC)		X ILRI-RC 001 17/IBC/001/CR

18. Endorsements:	*Main signatures in the order below signify that all necessary obligations/requirements have been satisfied.*		
Signature of Principal Investigator *:		Date:	
Signature of Program Leader :		Date	
Signature of DDG-R (IS or BioS) :		Date:	

* I certify that the any revision to this protocol will be forwarded to the IREC for review using the IREC amendment form and revised protocol will not be implemented until IREC approval has been obtained.

IREC USE ONLY

Date received by IREC:		**Received by:**	
Application number:			

IREC review actions:	*(Reference can be to an e-mail, description of actions or edited version of form)*	
Reference:	**Requested by**	**Action (what / by whom):**

	(name):	

IREC FINAL APPROVAL:					
Signature:		**IREC position:**		**Date:**	

ANNEX

The sign-off procedure is as follows

N.B. This process / chain should be indicated by FORWARDING e-mails through the process

Principal Investigator (PI): This is be the person (researcher) who takes responsibility for ensuring that the experiment runs as stated in the protocol - and that animals are cared for as they should be. The PI should be the person who is touch with the field staff and can answer and explain all questions about the way the protocol is implemented and why. They will be based wherever the experiment is taking place.

↓

Program Leader: This is the person who is directly responsible for the research project under which the PI is working and is likely to be the supervisor of the PI. In some cases PI and program leader will be the same person.

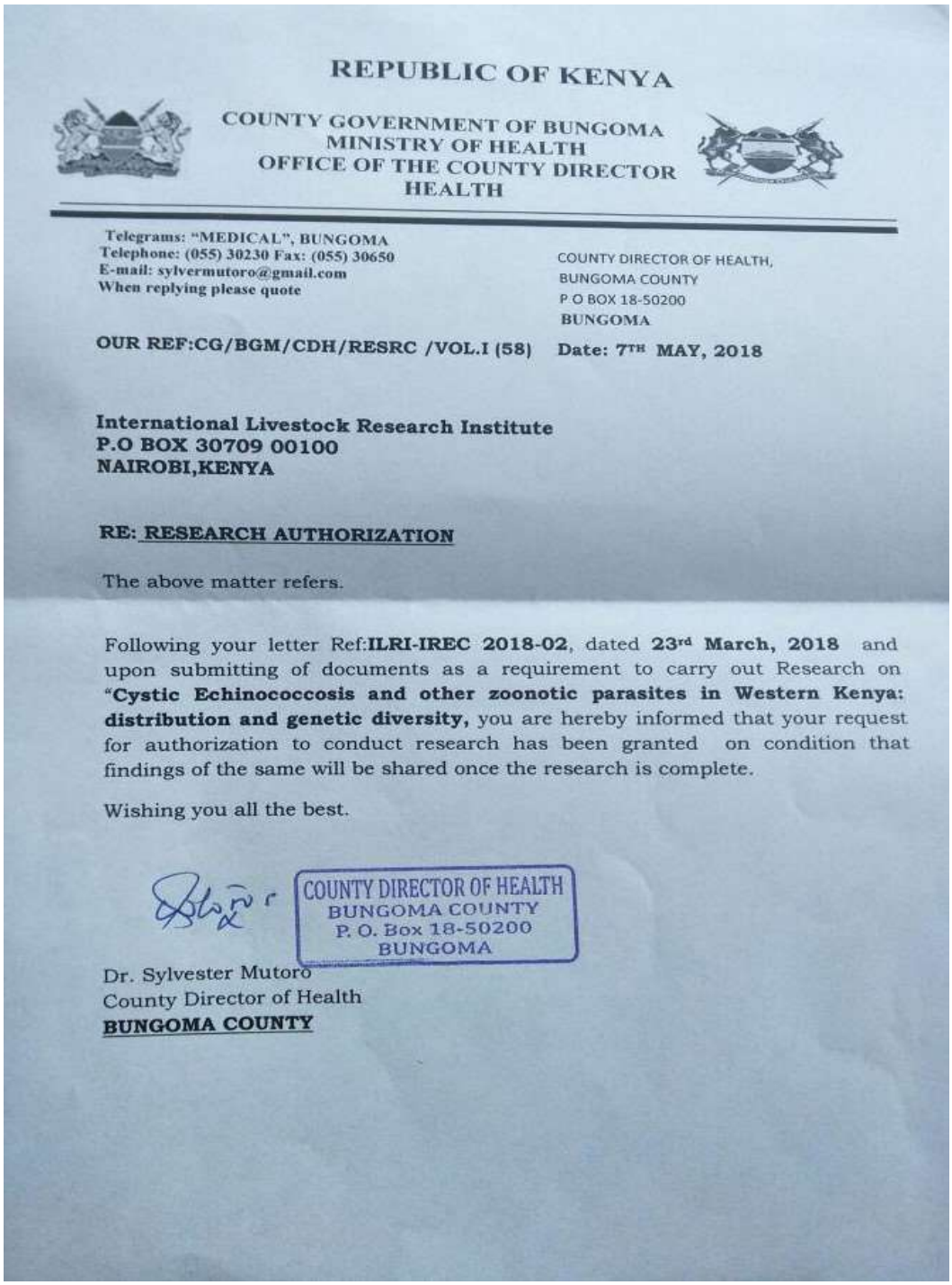
↓

Deputy-Director General Research - Integrated Sciences / BioSciences: DDG under which the Program, project and PI falls.

Approval of Scientific Merit (DDG-Research Approval Required):

The IREC assumes that the proposed research has been reviewed and approved on the basis of scientific merit by the relevant DDG-R, evidenced by their signed-off or his/her designee (in the “Endorsements” section).

Appendix VIII: Bungoma County Approval Letter



REPUBLIC OF KENYA

COUNTY GOVERNMENT OF BUNGOMA
MINISTRY OF HEALTH
OFFICE OF THE COUNTY DIRECTOR
HEALTH

Telegrams: "MEDICAL", BUNGOMA
Telephone: (055) 30230 Fax: (055) 30650
E-mail: sylvermutoro@gmail.com
When replying please quote

COUNTY DIRECTOR OF HEALTH,
BUNGOMA COUNTY
P O BOX 18-50200
BUNGOMA

OUR REF:CG/BGM/CDH/RESRC /VOL.I (58) Date: 7TH MAY, 2018

International Livestock Research Institute
P.O BOX 30709 00100
NAIROBI,KENYA

RE: RESEARCH AUTHORIZATION

The above matter refers.

Following your letter Ref:**ILRI-IREC 2018-02**, dated **23rd March, 2018** and upon submitting of documents as a requirement to carry out Research on **"Cystic Echinococcosis and other zoonotic parasites in Western Kenya: distribution and genetic diversity,** you are hereby informed that your request for authorization to conduct research has been granted on condition that findings of the same will be shared once the research is complete.

Wishing you all the best.

COUNTY DIRECTOR OF HEALTH
BUNGOMA COUNTY
P. O. Box 18-50200
BUNGOMA

Dr. Sylvester Mutoro
County Director of Health
BUNGOMA COUNTY

Appendix IX: Published Manuscript I

PLOS GLOBAL PUBLIC HEALTH

RESEARCH ARTICLE

Findings of a community screening programme for human cystic echinococcosisin a non-endemic area

Titus Mutwiri[1,2,3]*, Japhet Magambo[4], Eberhard Zeyhle[4], Anne W. T. Muigai[1], Lorren Alumasa[2], Fredrick Amanya[2], Eric M. Fèvre[2,5], Laura C. Falzon[2,6]*

1 Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya, **2** International Livestock Research Institute, Nairobi, Kenya, **3** Kenya Methodist University, Nairobi, Kenya, **4** Meru University of Science and Technology, Meru, Kenya, **5** Institute of Infection, Veterinary and Ecological Sciences, University of Liverpool, Liverpool, United Kingdom

* titusmutwiri@yahoo.com (TM); laura.falzon@liverpool.ac.uk (LCF)

Abstract

Cystic Echinococcosis (CE) is a zoonosis caused by infection with the larval stages of the taeniid cestodes of the species complex *Echinococcus granulosus sensu lato*. It is prevalent among transhumant communities in East Africa, including those residing in northern Kenya. The movement of livestock from these regions of high incidence to areas of low incidence creates an indirect risk of disease spill-over to humans. To assess possible establishment of the CE life cycle outside known endemic regions, we used a portable ultrasound scanner to screen for the presence of human CE in Bungoma County of western Kenya, an area which imports substantial numbers of cattle for slaughter from neighbouring pastoralist regions. Eight sentinel sites were purposively selected based on their proximity to slaughterhouses handling animals introduced from pastoralist regions, and necessary permissions to conduct the study were sought. Regression analyses were conducted to identify risk factors associ- ated with the presence of abdominal and cystic lesions (CL). In total, 1002 participants were screened; of these, 654 (65.3%) were female and the median age was 43. Farming (n = 403; 43.4%) was the most frequent occupation, followed by professional (i.e. on regular sal- ary) (n = 215; 23.1%), and business (n = 207; 22.3%) categories. Sixty-seven participants (6.7%) had abnormal ultrasound findings, of these, 7 (1.1%) had simple liver cysts/CL, as per WHO classification. As such, their outcome was inconclusive and they were not put on treatment but advised to attend follow-up investigations in a referral health facility. Other abnormal findings included splenomegaly (n = 14), ovarian cysts (n = 14), uterine fibroids (n = 10), polycystic kidneys (n = 6), and benign prostatic hyperplasia (n = 6). Age was uncondi- tionally associated with the presence of presumptive CL. These results contribute to CE baseline data while providing insights on the implementation of ultrasound diagnosis in the field, as recommended by the WHO for targeted control of echinococcosis by 2030.

OPEN ACCESS

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Appendix X: Published Manuscript II

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Original Article

The potential role of roaming dogs in establishing a geographically novel life cycle of taeniids (Echinococcus spp. and Taenia spp.) in a non-endemic area

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ABSTRACT

Introduction: Cystic Echinococcosis (CE) is endemic in humans and livestock in many pastoral communities in Kenya. The distribution of the disease is enhanced by several factors, including livestock trade, which has allowed for the spread of CE to non-endemic areas such as western Kenya. Dogs' roaming behaviour, with consequent contamination of the environment with intestinal parasites, could then lead to parasite establishment. This study examined dogs' infection levels with taeniid eggs and their potential role in contaminating the environment with intestinal parasites.

Methodology: We selected sixteen ruminant slaughterhouses in Busia and Bungoma Counties, and around each slaughterhouse we identified ten homesteads owning free-roaming dogs. We administered a questionnaire on dog