PREVALENCE OF CYSTIC ECHINOCOCCOSIS AND DIVERSITY OF *ECHINOCOCCUS GRANULOSUS* INFECTION IN SHEEP IN OLOKURTO DIVISION, NAROK COUNTY, KENYA.

By

CORNELIUS TIAMPATI MANYUELE

(B. Ed, University of Nairobi)

I56/69591/2011

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF SCIENCE (APPLIED PARASITOLOGY)

UNIVERSITY OF NAIROBI

2014

DECLARATION

I declare that this is my original work and that it has never been presented for a degree in any other university

Signature…… Date……

Cornelius Tiampati Manyuele

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

Supervisors

Dr. D. Odongo
School of Biological Sciences
University of Nairobi

Signature…….. Date……

Prof. G.P. Kaaya
School of Biological Sciences
University of Nairobi

Signature…….. Date……..

ACKNOWLEDGEMENT

I thank the almighty God for the life and good health he has given me throughout my research work. I express sincere gratitude to my supervisors, Dr. D. Odongo and Prof. G.P. Kaaya for their tireless support, guidance and encouragement in the course of my work. I thank Mr. Erastus Mulinge and Dr. C. Mbae of the Centre for Microbiology Research (CMR) at Kenya Medical Research Institute (KEMRI), Nairobi, for their support, guidance and allowing me to use their reagents and facilities. I thank Dr. J. Wangigi (Veterinary officer in charge of Likia slaughter house) for allowing me access to the slaughter house. I appreciate the laboratory support of technologist in the School of Biological Sciences, University of Nairobi Mr. Peter Thimbu and Mr. Nicodemus Muia. I humbly appreciate my family members for their financial and moral support. I also appreciate the University of Nairobi for allowing me to pursue a Master's degree in Applied Parasitology. May God reward you all.

DEDICATION

I wish to dedicate this thesis to my family members and friends who supported me throughout my study.

LIST OF ABBREVIATIONS

AMREF	African medical research foundation
Bp	Base Pair
CMR	Centre for Microbiology Research
DNA	Deoxyribonucleic Acid
DNTPs	Deoxynucleoxide triphosphate
EDTA	Ethylenediaminetetra acetic acid
ITS	Internal transcribed spacer region
Kb	Kilo base
KEMRI	Kenya Medical Research Institute
M	Marker
MANOVA	Multivariate analysis of variance
Mm	Millimoles
MW	Molecular weight
NADH	Nicotinamide adenine dinucleotide hydride
PAIR	Puncture aspiration injection reaspiration
PCR	Polymerase chain reaction
RFLP	Restriction fragment length polymorphism
WHO	World health organization
µl	Microlitre

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Cystic Echinococcosis is a serious zoonotic disease caused by *Echinococcus granulosus* species complex. This research study was conducted to determine the level of infection of the disease in sheep in Olokurto division, Narok County, Kenya. The study area was divided into five locations and 180 sheep selected randomly from each location were inspected for infection. Sample collection was conducted in Likia slaughter house. The carcass of each sheep was inspected carefully for the presence of hydatid cysts, infection and the organs infected and the number of cysts was recorded. The sheep identification number and sex, and age as determined by dentition were also recorded. Hydatid cysts collected were preserved in 70% ethanol and transported to the laboratory for analysis. In the laboratory, cysts size and volume of hydatid fluid were measured, microscopic examination of hydatid fluid was performed to determine cysts fertility, and species identification was determined following restriction digests of amplified PCR products which targeted the NADH dehydrogenase subunit 1 (nad-1) gene. Overall prevalence was 16.0% (144/900), infection rates in the five sampling sites were significant ($P<0.05$), with the liver being the most infected organ (50.7%), followed by the lungs (36.8%), while mixed infections involving the liver and the lungs were detected in12.5% of the sheep sampled. PCR/RFLP results revealed that all strains were *E. granulosus* sensu stricto which means that the genotype is G1 (genotype1). The cysts were examined under the microscope to determine fertility, out of the 343 hydatid cysts collected and examined, 62.1% of the hydatid cysts were fertile, 35.2% were sterile while 2.7% were calcified. Lung cysts were found to be more fertile (73.02%) compared to liver cysts (53.4%). There was a direct relationship between age, number and size of hydatid cysts as the number and size of the cysts increase with increase in age of the sheep. Since all strains were G1 which is particularly pathogenic to humans, it is importance to control the disease

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1.General introduction

Cystic echinococcosis infection caused by larval stage of *Echinococcus granulosus* species complex is of medical and veterinary importance. Currently, five strains of *E. granulosus* are known in Africa: *E. granulosus* sensu stricto, *E. equines*, *E. ortleppi*, *E. canadensis* and *E. fidelis*. *E. granulosus* sensu stricto is the most common sheep strain (Addy *et al*., 2012). Apart from *E. granulosus* sensu lato, other species include *E. multilocularis*, *E. oligarthus* and *E. vogeli*. There appears to be very limited genetic variation within *E. multilocularis* and no information available to show the variability of either *E. vogeli*, or *E. oligarthus* (McManus and Thompson, 2003). Based on the current information on species, strains and genotypes, Africa has the highest incidence in the world (Romig *et al.*, 2011). The disease occurs in humans, wild herbivores, wild carnivores and livestock. The adult worm is present in the canids and the larval forms in wild herbivores, livestock and humans. The disease is potentially dangerous and found in many parts of the world due to its ability to adapt to many hosts and occupy a wide geographical area. High incidences of infection by *E. granulosus* often coincides with rural grazing areas where dogs are able to ingest organs from infected wild and domestic animals, approximately 2-3 million human cases occur worldwide annually. In Africa, the prevalence is higher in the northern part such as in Sudan, Egypt and Ethiopia. The adult worm is about 3 to 6 mm long and resides in the small intestines of the definitive hosts. Eggs are released through mature proglottids in definitive host and can survive for about a year as they are highly resistant to environmental stress. Man can get infected by accidentally ingesting eggs from contaminated

food, water, handling live canids and canid's faeces. In the intermediate host, eggs hatch to release oncospheres in small intestine, which penetrate intestinal wall and get into blood the circulation and are then carried to various organs such as the liver, brain, lungs and eye tissue. In the body, once the oncospheres reach the organ of choice they form cysts which contain protoscolices that then develop into adults in the intestines of definitive hosts which eventually produce eggs to complete the cycle. Infection with adult stage of the parasite is asymptomatic and non-pathogenic to the canid host, while in human and herbivore hosts, it exerts pressure on organs adjacent to the slow growing masses filled with hydatid fluid leading to tissue damage. Lung infection results in breathing problem and chest pain, liver infection results in abdominal pain. The hydatid cysts can break suddenly resulting in sudden death of the animal. The prevalence of hydatidosis varies from place to place but is most prevalent where livestock farming is practiced, especially in arid and semi-arid areas. Such communities also keep dogs to help in herding of their livestock and guard at night (Schwabe, 1969). According to a study conducted in Kenya, in cattle, hydatidosis has been reported to be about 30%, 13% in sheep and 15% in goats (Gathura and Kamiya, 1990). The disease has also been reported in wild carnivores and herbivores but their role in perpetuation of the disease in nature remains to be clearly understood (Macpherson *et al.,* 1983). In Olokurto division in Narok County, no research concerning the prevalence and diversity of the disease in the area has ever been carried out, hence a gap in knowledge.

1.1.1 Etiology

Echinococcus species is a cestode parasite that infects a wide range of vertebrates including humans. Classification of the causative agents of cystic echinococcosis has been a big challenge for many years as a result of limited morphological description and lack of evidence for geographical or ecological segregation of the parasite as all were conventionally assigned to *E. granulosus* (Thompson and MacManus, 2002). Further studies on the parasite have led to the development of a strain concept that differs from other groups of the same species in gene frequencies (Thompson and Lymbery, 1988). They use canids as definitive hosts and a wide range of ungulates as intermediate hosts. Based on current information on species, strains and genotypes of cystic echinococcosis, Africa has the highest diversity of these parasites (Romig *et al*., 2011). The most important species infecting humans and livestock in Africa include *E. granulosus* sensu stricto (common sheep strain G1), *E. equines* (horse strain), *E. ortleppi* (cattle strain), *E. canadensis* (camel/pig strain) and *E. fidelis* (lion strain) (Omer *et al*., 2010). In East Africa, the parasite presents a complex pattern of infectivity with more than one strain occurring sympatrically in different or the same livestock species. Based on the limited number of studies conducted so far, differences in strains composition among different regions seem to exist. This uncertainty depicts a complex epidemiology of the disease which is not fully understood (Romig *et al*., 2011).

1.1.2 Life cycle of *Echinococcus granulosus* species

The disease occurs in humans, wild herbivore, wild carnivore and livestock, the adult worm is present in canids and the larval form in wild herbivore, livestock and humans. The adult worm is

about 3 to 6 mm long and resides in small intestine, eggs are released through mature proglottides in definitive hosts. The eggs are accidentally ingested by herbivore host during grazing and man can get infected by ingesting eggs from contaminated food, water or through handling live canids and canid's faeces. In the herbivore host eggs hatch to release oncospheres in the small intestines which penetrate intestinal walls and enter the blood streams and eventually enter organs such as the kidney, liver, lungs, spleen, brain and bone marrow. In humans the eggs hatch in the duodenum and the oncospheres penetrate the intestinal walls and enter the blood circulation and are then carried to various organs such as the liver, brain, lung and eye tissue. In the intermediate hosts, once the oncospheres reach the organ of choice, they form cysts. The canids acquire the infection by feeding on intermediate host organs that contain hydatid cysts which contain protoscolices, the protoscolices then develop into adults which eventually produce eggs to complete the cycle (Fig.1).

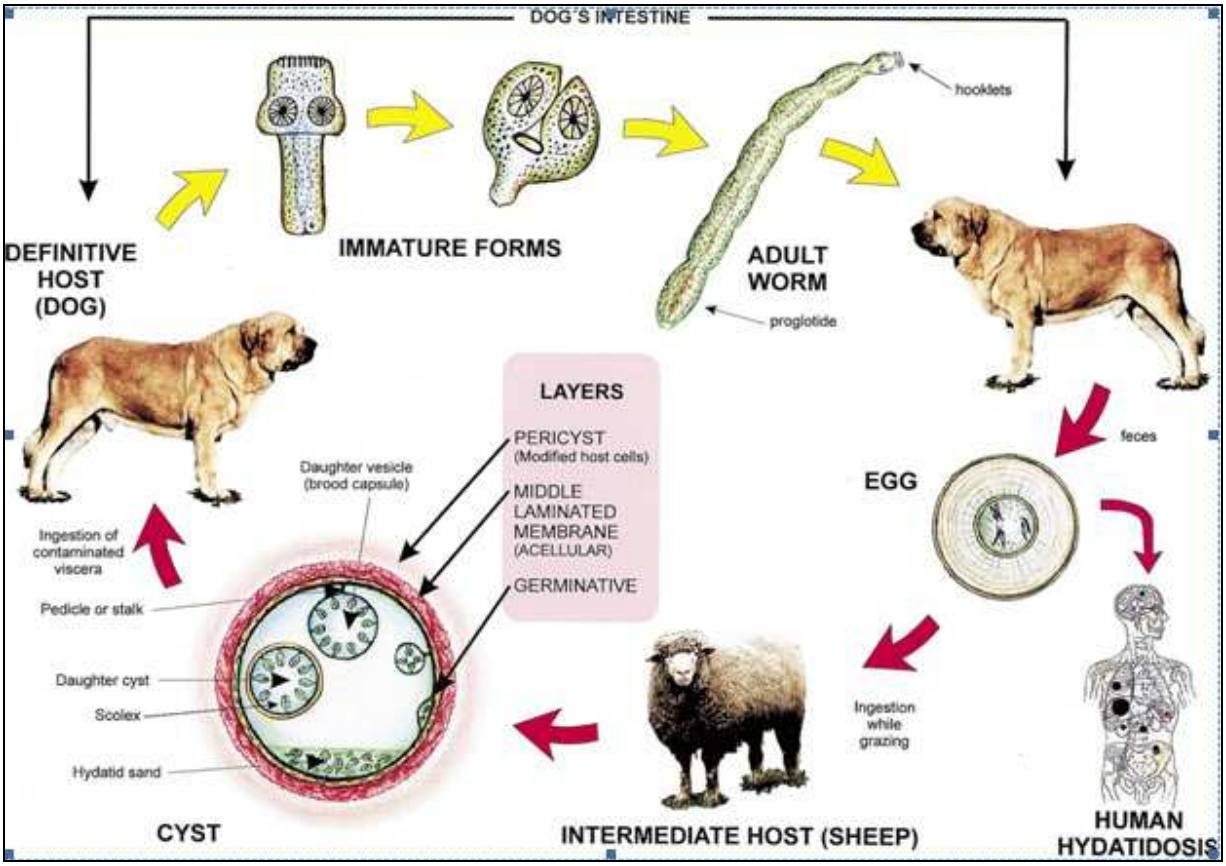

Figure 1. Life cycle of *Echinococcus granulosus* (adopted from Khanfar, 2004)

1.1.3 Biology of *Echinococcus* species

Echinococcus species is a small tapeworm (2 to 6 mm in length), it has three segments and other morphological characteristic which are important in species diagnosis (Thompson, 1995). The parasite has no digestive system nor circulatory or respiratory systems and all metabolic interchange take place across the tegument. They have excretory cells called flame cells (protonephridia). The parasite has reproductive organs of both sexes (hermaphroditic) with a common opening called genital pore, mature proglottids are full of embryonated eggs which detach from the strobila and shed outside the host with its faeces (Moro and Schantz, 2009).

1.2 Geographical distribution of cystic echinococcosis in Kenya

Cystic echinococcosis is globally distributed and particularly economically and medically important in rural pastoralist societies (Eckert, 2001). The disease poses important public health challenges in most parts of the world (WHO, 2001). The ability of the parasite to adapt to a wide variety of hosts contributes to the global distribution of the disease (Schantz and Schwabe, 1969). Although the disease is globally distributed, it is most prevalent where livestock farming is practiced. Kenya is an agricultural country and due to its landmass being largely marginal to semi-arid, pastrolism is a major economic activity in areas mainly occupied by pastoralist communities such as the Maasai, Turkana and Borana. These pastoralists' communities also keep dogs to help in herding of their livestock and guard at night thus increasing the incidence of the disease in such communities. (Eugster, 1978) (Fig. 2).

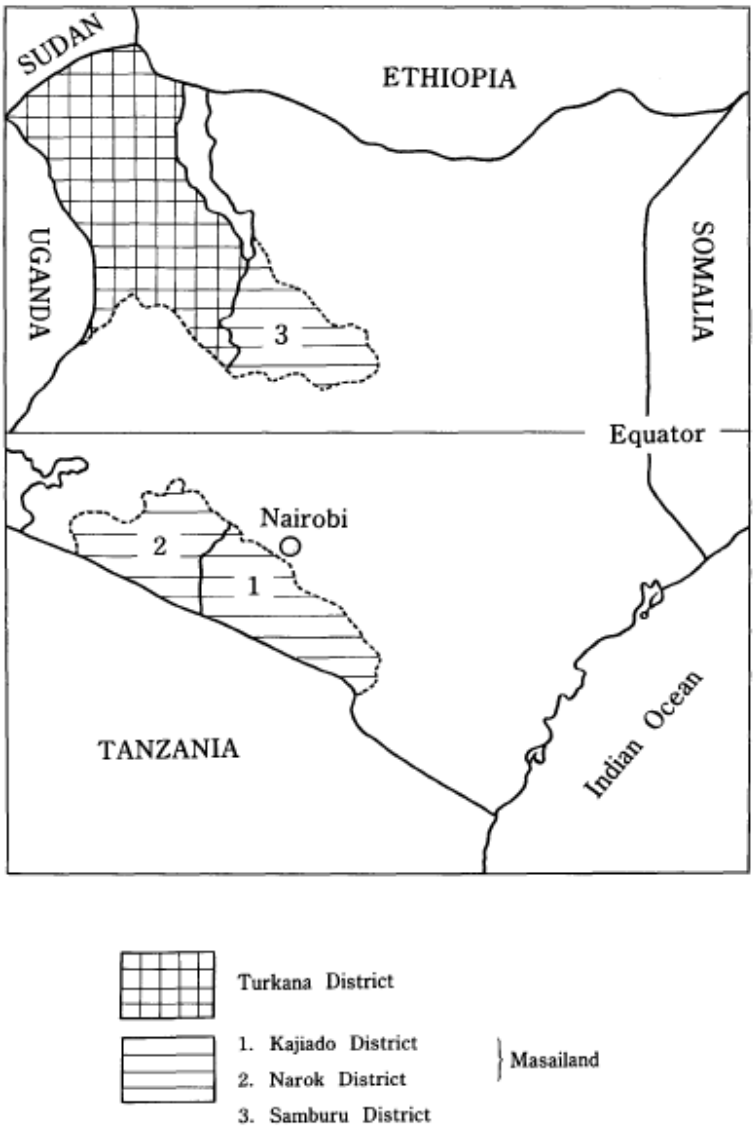

Figure 2: Map of Kenya showing areas of cystic echinococcosis endemicity

(Ministry of livestock development, 1990)

1.3. Transmission and control of cystic echinococcosis.

Echinococcus species is mainly maintained in a dog-sheep-dog cycle. In its domestic transmission cycle, dogs being definitive hosts of the adult tapeworm, while livestock such as sheep, goats, pigs and cattle are the intermediate hosts. The sheep strain (G1) of *E. granulosus* sensu stricto is the most epidemiologically important genotype in public health importance and geographic range (Jenkins *et al*., 2005). The infection is transmitted to dogs when they feed on infected viscera of intermediate host during slaughter, dogs also get infected through scavenging. The disease is transmitted to humans through direct contact with dogs and consuming vegetables and water contaminated with dogs' faeces. Humans are accidental intermediate host and are unable to transmit the disease (Budke *et al.,* 2006). (Fig.3). Intermediate hosts such as cattle, pigs, sheep and goats become infected following ingestion of eggs from faeces of infected definitive hosts (Wahlers *et al.,* 2012). The larval stage that emerges from the eggs gives rise to hydatid cysts which are found mostly in the liver and lungs, although other organs may also be affected.

An effective vaccine that would reduce hydatid infection in livestock would also have a substantial impact on the rate of transmission of the disease to humans (Lightowlers, 2006). An oncosphere antigen vaccine EG95 has been shown to be capable of inducing high level of protection against infection with *E. granulosus* eggs in sheep, the vaccine prevent development of oncosphere to hydatid cysts in sheep and thus stops development of adult gravid tapeworms in dogs (Lightowlers, 1996). Treatment of cystic echinococcosis is complicated because most lesions develop in the liver, lungs and other organs (Ci-Peng, *et al*, 2005) requiring surgical removal of the cysts. Anti-helminthic drugs including albendazole, mebendazole and praziquantel drugs have cure rate of up to 30% and approximately 10-20% of patients show

substantial regression of cyst size and symptom alleviation (Khuroo, 2002) Low priority has been given by respective governments to arid pastoral areas where cystic echinococcosis is most endemic. Sub-Saharan Africa is the world's poorest area with more than 46% of the population surviving on less than $1 a day. In Turkana District of northern Kenya for example a hydatid control program managed by African Medical Research Foundation (AMREF) was established in 1983. The control program has three main components; human treatment (chemotherapy, surgery and Puncture aspiration injection and reaspiration (PAIR), control of dog population (deworming of owned dogs, sterilization of female dogs and killing of stray dogs) and community education (settled, semi-settled and nomadic and in schools). Through this program a total of 1015 people had undergone surgery/PAIR by 2004 and 2500 treated by chemotherapy. This has reduced the prevalence of cystic echinococcosis in humans from 7% to 2.5%, and the prevalence of *E. granulosus* in dogs from 65% to 28% (Zeyhle *et al*., 2004).

Figure 3: Human-dog relationship contributes to cystic echinococcosis transmission

(Courtesy of Eberhard, 2012)

1.4. Economic impact of cystic echinococcosis

Cystic echinococcosis is of great economic importance both in humans and livestock. In terms of monetary losses and disability adjusted life years (DALYS) is about US$ 763,980,979 annually when underreported cases are considered (Budke *et al.,* 2006). The disease is of major public health and veterinary importance. In livestock it results in death, decreased meat, milk and fleece production. The disease also results in condemnation of infected organs in slaughter houses resulting in great economic losses (Jenkins *et al*., 2005; Wahlers *et al.,* 2012). Human hydatidosis has many important economic effects: reduced or complete loss of income during illness, cost of treatment and convalescent period. Economic and social losses associated with undiagnosed and therefore untreated cases should also be considered. Most reports have shown that between 1% and 2% cases of hydatidosis are fatal (Budke *et al.*, 2006). In Africa, the disease is widespread posing great challenges in most countries that practice large scale livestock economy (Omer *et al.,* 2010; Huttner *et al.,* 2009; Romig *et al.,* 2011). Apart from western and central Africa where only sporadic cases are known, hydatidosis is endemic in human and livestock in all the other countries of Africa (Romig *et al*., 2011). In Kenya, according to a slaughter house survey conducted in the whole country between 1977-1988, 4.1% and 4.8% of all cattle livers and lungs, respectively, were condemned due to hydatid cysts infection. In small stocks such as sheep and goats, 2.6% and 3.0% of livers and lungs, respectively, were condemned (Gathura and Gathuma, 1989). In humans, high incidence of the disease in Kenya is mainly confined to Turkana district and estimated at 220 cases per 100,000 populations (French and Nelson, 1982).

1.5. Literature review

1.5.1 Previous studies of cystic echinococcosis in Kenya

Field survey studies have been previously conducted on prevalence and diversity of cystic echinococcosis in Kenya. Although it is one of the most important helminth infections, cystic echinococcosis studies have proved difficult to come up with an accurate prevalence status in intermediate hosts in any part of the world. This is due to poor reliability of the available diagnostic tests and high costs of performing these tests under field conditions. Most prevalence studies have relied on slaughter data (Macpherson, 1981).

A study by Froyd, (1960) in Kenya showed a prevalence of 30% in cattle, 13% in sheep and 15% in goats. Etiology of the disease was first confirmed by Nelson and Rausch, (1963) who first identified *E. granulosus* as the causative agent of the disease in Kenya. Macpherson, (1985) conducted a survey in Maasailand and reported that the region was a hyperendemic focus having high level of prevalence in livestock and frequent occurrence of human cases. Similarly Njoroge *et al*., (2002) conducted a research study on prevalence of cystic echinococcosis in slaughter animals in three divisions of northern Turkana and showed that the prevalence was 19.4% in cattle, 3.6% in sheep and 61.4% in camels. Wachira *et al.,* (1993) and Dinkel *et al.,* (2004) genotyped cysts collected from southern Kenya. However the relative contribution of the species identified to the total burden of hydatidosis was not established because sample's collection was not done in a systematic manner. A study by Casulli *et al.,* (2010) showed that *E. canadensis* (G6) primarily use camels as their intermediate host. A similar study by Mulinge *et al.,* (2011) reported the presence of *E. ortleppi* in North-central Kenya.

A surveillance study conducted by Addy *et al.*, (2012) on prevalence and diversity of hydatidosis in Maasailand showed that the prevalence was 25.8% in cattle, 16.5% in sheep and 10.8% in goats. The average number of cysts per surveyed animal was 1.0, 0.4 and 0.3 for cattle, sheep and goats respectively. The number of cysts per infected animal varied between 1 to 16. They reported that about half of the infected animals harbored more than one cyst, intensity of infection was correlated with the age of the animal. In all the animals surveyed, the liver was found to be the most frequently affected organ, but liver cyst were less frequently fertile compared to lungs cysts (2.9 against 11.3%) in cattle (22.6 against 34.8%) in sheep and (0 against 50%) in goats. They also found that although multiple organ involvement is common in all animals, liver and lungs were the most frequently affected organs. In the study, they identified three *Echinococcus* species; *E. granulosus* G1, *E. ortleppi* G5 and *E. canadensis* G6, *E. granulosus* was the most dominant (279 of 285 specimens) which often reached its fertility in sheep.

1.5.2. Diagnosis of cystic echinococcosis infection

1.5.2.1. Serological diagnosis of *Echinococcus* species

Application of serodiagnostic techniques for study of hydatidosis provide more accurate results on prevalence of the infection as the technique can detect asymptomatic cyst carriers. Jenkins and Richard, (1984) reported that serodiagnosis can detect the infection as early as 4 to 6 weeks post infection. A serological test in sheep-dogs in Alava province in Spain conducted by Benito *et al*., (2006) revealed that the overall prevalence of *E. granulosus* infection is 8.0%, a rate that represents a public health threat. Similarly Tijjan *et al.*, (2010) conducted a study on prevalence in sheep in Yobe state, north eastern Nigeria, and reported a prevalence of 0.01%. Luka *et al.*, (2009) conducted a survey study on prevalence in sheep and reported a prevalence of 36.2% in

Kano Nigeria. Comparatively Dada and Belino, (1979) in Yobe state, north eastern Nigeria, conducted a survey on hydatidosis in sheep and reported a prevalence of 18.9%. A similar study by Magaji, (2011) on dogs in Sokoto, Nigeria, showed a prevalence of 26.69%. Raeghi *et al.*, (2007) in Kerman, Iran reported a seroprevalence of 9.2%, 6.8% and 7.4% in sheep, goats and stray dogs respectively. They also demonstrated an infection of 0.22 per 100,000 population. Another study in Tripoli, Libya by Buishi *et al*., (2005) revealed that 25.8% of stray dogs and 21% of owned dogs tested by coproantigens ELISA were positive for *Echinococcus* species parasite. Serodiagnosis has shortcomings in that cross reactivity with antigens from other parasites mainly other taeniid cestodes is a major problem. It has also been found that intermediate host animals produce very poor antibody responses to infection (Lightowlers and Gottstein, 1995). According to a study conducted by Jenkins and Richard, (1986) sheep were found to be the main intermediate host of *E. granulosus* in most endemic areas worldwide and antibodies to various antigens were detectable in sera of some but not all infected sheep (nonresponders).

1.5.2.2 Molecular characterization of *Echinococcus* species

Genetic characterization of *Echinococcus* species is important in understanding transmission patterns of the parasite (s) between definitive hosts, humans and intermediate hosts, this will assist in diagnosis and control of the disease (Thompson *et al.*, 1995; Thompson and MacManus, 2002). Various DNA-based techniques have been applied to genetic classification of *Echinococcus* species and genotypes (Abushhewa *et al.*, 2010). To date 10 distinct genotypes (G1-G10) of *E. granulosus* sensu lato and *Echinococcus fidelis* have been defined based on mitochondrial DNA studies (Lavikainen *et al.*, 2003). However, due to lack of molecular data on *E. granulosus* in many countries of Africa, South America and Asia, the full extent of genetic

variation within *E. granulosus* might not be known and new genotypes could be discovered in new studies.

Wachira *et al*., (1993) examined 208 isolates of *E. granulosus* from intermediate hosts and 40 adult worms and showed the presence of two *E. granulosus* strains; the sheep strain (G1) with known pathogenicity in humans was identified in sheep, goats, cattle, camels and humans and the camel strain with apparently low infectivity to humans was found only in camels and goats. Similarly Mwambete *et al*. (2004) Carried out molecular analysis in Spain and identify three strains, G1 infecting sheep, cattle, goats, pigs, wild boars and humans, G7 infecting pigs, goats and wild boars and *E.equinus* infecting horses. A molecular analysis by Lahmar *et al*., (2004) in Tunisia demonstrated the presence of the G1 sheep strain and the G6 camel strain. In the same country Farjallah *et al*., (2007) characterized 38 isolates of *E. granulosus* collected from different regions and hosts and indicated the circulation of the common sheep strain (G1) in all species. Varcasia *et al*., (2008) detected G1, G2, G3, G4 and G7 genotypes in different hosts in central-southern Italy. A similar study by Rinaldi *et al*., (2008) detected G2 genotype in water buffaloes from Campania region (Southern Italy).

A study conducted by Varcasia *et al*., (2007) in Peloponnesus, Greece on the prevalence and genotypes of *E. granulosus* in sheep and goats showed that the prevalence was 30.4% in sheep and 14.7% in goats, they also revealed that 18 of 20 sheep examined harboured the G1 (sheep) strain while all the goats examined harboured the G7 (pig) strain. Another study conducted in Brazil by Haag *et al.,* (1999) for the first time showed the presence of the sheep strain (G1) and the cattle strain (G5) of *E. granulosus* in sheep and cattle respectively. Similarly Dela Rue *et al.,* (2006) in southern Brazil showed that the G1 genotype was the most common taxon both in sheep and cattle, while few cysts in cattle were identified as *E. ortleppi*. Gudewar *et al.,* (2009)

demonstrated that four genotypes, namely the sheep strain (G1), Tasmanian sheep strain (G2), Indian buffalo (G3) and cattle strain (G5) of *E. granulosus* are present in livestock in India. In Argentina, Kamenetzky *et al.,* (2002) isolated G1 and G6 from human, G2 from sheep and human and G7 from pigs. In eastern Libya Tashani *et al*., (2002) analyzed protoscolices from humans, cattle, camels and sheep and showed the occurrence of the sheep strain of *E. granulosus* G1. Another molecular study conducted by Manterola *et al.,* (2008) in Chile on human beings revealed the circulation of G1 and G6 genotypes. In Peru, Santivanez *et al.,* (2008) characterized G1 and G6 variants in humans. G1 genotype was also isolated from sheep, cattle and goats, G6 from goats and G7 from pigs in the same country. A study by Moro *et al*., (2009) in Turkey showed that the most prevalent strain was G1 in both human and livestock while G3 and G7 were detected in a few isolates.

A molecular analysis conducted by Pednekar *et al.,* (2010) in north India revealed that G3 genotype was the predominant genotype 29/46 in all species of livestock followed by cattle strain (G5) 9/46, G1 or the common sheep strain 6/46 and the G2 genotype or Tasmanian sheep strain 2/46. Similar genotypes with slightly high prevalence were recorded by Latif *et al.,* (2010) in Pakistan. In Maasailand of southern Kenya Addy *et al.,* (2012) identified *E. granulosus* G1, *E. ortleppi* and *E. canadensis* G6 in livestock. In the neighboring Sudan Dinkel *et al.,* 2004 and Omer *et al.,* (2010) reported that the camel strain of *E. canadensis* is the dominant genotype. Similarly Mailard *et al.,* (2007) reported a frequent presence of G1 from the north and northeast of Africa. In another study by Wachira *et al.,* (1993) in Maasailand Kenya, G6 was isolated from a cow and a goat. Mulinge *et al.,* (2011) reported the presence of *E. ortleppi* from cattle in the north central part of Kenya. According to the data currently available *E. ortleppi* is widespread in other parts of the world but rare in eastern Africa. Huttner *et al.,* (2009) recorded the presence of

E. fidelis from lions in the queen Elizabeth Park Uganda. A similar study by Jenkins *et al.*, (2005) reported the presence of *E. equines* in South Africa.

1.6. Justification

In Kenya, agriculture is the major economic activity, of the total Kenya land only about 7% is suitable for crop production yearly and about 5% which can only support crops during years of high rains, the remaining land is mainly arid and semi-arid suitable for livestock rearing (Oniang'o, 2001). In Olokurto division Narok County, Kenya, the main economic activity is rearing of cattle and sheep. The community also keep dogs that help in herding of their livestock and guard at night. During herding, livestock interact with wild canids and herbivores which in the process lead to transmission of the disease. Most areas within the division where herding is practiced are mostly forested providing suitable habitat for wild animals.

Residents of the division obtain water for domestic use from the numerous streams originating from forested areas which are also grazing grounds for livestock. The water is usually contaminated especially during rainy seasons when wild animals' feaces and livestock dung are washed into water bodies. These feaces may contain infective eggs which are transmitted into water bodies, infective eggs are also transmitted to children through dogs handling.

This study is justified from the economic point of view. In humans the annual incidence in endemic areas is 220 cases per 100,000 populations (French and Nelson, 1982). In livestock it results in death, decreased meat, milk and fleece production. Information obtained from the study will be used to come-up with recommendation for control of the disease. Current information on status of the disease in the area is also not available, the study will also fill the knowledge gap.

1.7. Objectives

The general objective of this study was to determine the level of infection of cystic echinococcosis in sheep in selected locations in Olokurto division, Narok County, Kenya.

The specific objectives were:

1. To determine the prevalence and distribution of hydatidosis in sheep within Olokurto division.
2. To determine the intensity of infection of hydatidosis in sheep in Olokurto division.
3. To characterize and identify the different molecular genotypes of *Echinococcus* species in the region

CHAPTER 2

MATERIALS AND METHODS

2.1. Study site

The project was conducted in Olokurto division of Narok County, Kenya. Olokurto division is situated about 220km west of Nairobi. The study area was divided into five locations to ensure that the study covers the entire area, the five locations are Ololong'oi, Olorropil, Olposimoru, Kisiriri and Eneng'etia. The area has a high altitude characterized by high and reliable rainfall, fertile agricultural land and dispersed population.

The means of transport in the area is earth roads which are usually impassable during rainy seasons. The area has many rivers and streams originating from the Mau forest complex and most are permanent. The area is generally characterized by low temperatures throughout the year. Rainfall is bimodal with long rain from March to August and low rainfall between September and February. The area experiences less wind and low evapo-transpiration rate, the area also experiences high humidity, especially between May and July.

Olokurto division is considered as one of the major sheep rearing areas in Narok county, majority of the population live in rural areas and are involved in livestock keeping mainly cattle and sheep as the area is cold hence does not favour goat keeping. Sheep are the principal animals slaughtered for human consumption on social and religious occasions, slaughtering in Olokurto division occurs in the main abattoir located at the Likia centre along the Narok-Nakuru road and most sheep from the division are transported there for slaughter. A larger percentage of the meat is then transported to bigger towns like Nakuru and Nairobi.

Most locations within the division lack abattoirs and slaughtering takes place in streets, traditional slaughter houses and markets, this enhances poor disposal of infected offals and carcasses which are in turn consumed by stray dogs roaming around and other carnivores such as jackals, enhancing spread of the disease. The area therefore favors *Echinococcus* species due to interaction between the wild carnivore, wild herbivore, livestock and dogs.

Figure 4: The map of Olokurto division showing sampling site.

(Narok survey office, 2013)

2.2. Study design and Sample size

A cross section study was carried out to determine the level of infection of hydatidosis in sheep in Olokurto division. The sample size was calculated by use of a formula described by Thrusfield, (2005)

$$N=Z^2 p (1-p) /d^2$$

Where **N** is the minimum sample size, **Z** is the confidence interval which is the range of values in which the population mean is likely to be maintained with a given level of probability, defined by the standard errors of the sampling distribution, **p** is the expected frequency of the condition of interest and $\mathbf{d^2}$ is the inverse of 95% allowable error which is the desired precision, expected frequency is 15.6%.

2.3. Field sample collection

The abattoirs which are key facilities for slaughter were visited on slaughter days. A total of 180 sheep above one year old selected randomly from each location were inspected for hydatid cysts in all organs of the pleural and abdominal cavities following slaughter. Sheep above one year old were selected because hydatid cyst establishment is correlated to the age of the sheep in contrast to sheep below one year old which although may be infected are unlikely do present with established cysts in the visceral organs. The age of the sheep was determined by dentition, sheep whose all teeth are present, strong and intact were considered as age two years, sheep that have loss some teeth and with loose teeth were considered as three years while sheep that have loss most of the teeth were considered as four years, the sex of the sheep was also recorded. Visceral organs identified with hydatid cysts were preserved in 70% ethanol in clean plastic containers and transported to the laboratory for analysis.

2.4. Examination of hydatid cysts

In the laboratory cysts of infected organs were physically counted and their sizes measured using a 15 cm ruler and the cysts size in square centimeter calculated and data recorded. Individual cysts were cut-opened using a surgical blade and the cyst's fluid volume collected and measured using a measuring cylinder. Each individual cyst was then examined at X410 magnification under a dissecting microscope for the presence of protoscolices. The fertility of the cysts was determined and recorded based on the criteria established by EI-Ibrahim, (2009). Cysts with protoscolices were considered as fertile cysts, while fluid filled cysts without protoscolices were considered as sterile cysts while solid and sand contained cysts were considered as calcified cysts. The protoscolices from fertile cysts and germinal layer which is tissue obtained from sterile/calcified cysts were fixed and preserved in 70% ethanol to be used in DNA extraction.

2.5. DNA extraction from samples

DNA was extracted from protoscolices obtained from fertile cysts and germinal layer tissue materials of sterile cysts as described by Nakao *et al.,* (2003), lysing in 0.02 M NaOH and heated at 95°c for 10 minutes. An alternative DNA extraction process described by Dinkel *et al*., (2004) was used for tissues where there was sub-optimal yield of DNA following alkaline lysis to allow for PCR amplification. Briefly, approximately 0.5 g of the germinal layer was cut into small pieces using a surgical blade and digested using 2 mg/ml proteinase K in 500 µl of 10 mM Tris-HCl (pH 7.5), 10 mM EDTA, 50 mM NaCl, 2% sodium dodecyl sulphate and 20 mM dithiothreitol. The DNA was then extracted using phenol-chloroform-isoamyl alcohol (25:24:1) and precipated using cold absolute ethanol, and the pellet washed in 70% ethanol. The DNA pellet was then air dried and dissolved in 100 µl nuclease free water.

2.6. Oligonucleotide primers

The primers for use in a nested PCR amplification assay were originally designed by Huttner *et al*., (2008), to amplify the Nicotinamide adenine dinucleotide hydride (NADH) dehydrogenase subunit 1 (nad-1) gene. An aliquot of reconstituted primers were kindly provided by Erastus Mulinge of the Centre for Microbiology Research located at the Kenya Medical Research Institute (KEMRI). The primer sequence used is indicated in Table 1 below.

Table 1: Sequence of primers

Primer		Sequence(5'-3')	Bases
Outer primers	NAD A	TGT TTT TGA GAT CAG TTC GGT GTG	24
	NAD C	CAT AAT CAA AGG GAG TAC GAT TAG	24
Inner primers	NAD B	CAG TTC GGT GTG CTT TTG GGT CTG	24
	NAD D	GAG TAC GAT TAG TCT CAC ACA GCA	24

2.7. Polymerase chain reaction

All PCR amplifications were done as described by Huttner *et al*., (2008) in a total reaction volume of 25 µl containing 18.625 µl of DNase/RNase free water, 2.5 µl 10X PCR buffer, 0.5 µl 10 mM dNTPs, 0.625 µl of each primer (0.25 units/µl), 0.125 µl of Taq DNA polymerase (5 units/ µl) and 2 µl of the DNA from the test sample whose concentration cannot be quantify because it is not pure DNA, it also contain sodium hydroxide used during lysis. A set of purified genomic DNA from *E. granulosus* sensu stricto (G1, G2 and G3), *E. ortleppi* G5 and *E. canadensis* G6 were included as positive controls during all PCR assays and distilled water as a negative control. In the first round of the reaction the outer primers (NAD A and NAD C) were used under the following reaction conditions: 1 cycle of 95°c for 5 minutes followed by 35

cycles of denaturing at 94°c for 30 seconds, annealing at 55°c for 30 seconds, elongation at 70°c for 1 minute and final elongation at 72°c for 5 minutes. In the second round of reaction, 1 µl of the primary PCR product was used as the template DNA and the outer primers substituted with the inner primers (NAD B and NAD D) and all the other reaction conditions were as described for the primary PCR. Following amplification, the PCR products were resolved at 100 volts for 30 minutes on 1.5 % agarose gel (1x) (prepared by dissolving 1.5 g of agarose in 100 ml of Tris-acetate-EDTA (TAE) buffer). The gels were stained with Ethidium bromide (0.2 units/µl) and visualized under UV.

2.8. Restriction fragment length polymorphism of NAD-1 amplicons

Digestion of NAD-1 amplicons was performed as described by Huttner *et al.,* (2009) using Hph 1 restriction enzyme (10 units/ µl) synthesized by Thermo Fisher Scientific in a total reaction volume of 20 µl containing 10 µl of the secondary PCR product, 7.5 µl of DNase/RNase free water, 2 µl 10x buffer and 0.5 µl Hph 1 enzyme. The reaction mixture was incubated at 37°c overnight and the banding patterns were detected on 2% agarose gel (prepared by dissolving 2 g of agarose in 100 ml of Tris-acetate-EDTA (TAE) buffer) and the gels visualized following Ethidium bromide staining as described in section 2.7 above. The sample's genotypes were determined by comparing them to defined patterns of *E. granulosus* sensu stricto (G1, G2 and G3), *E. ortleppi* G5 and *E. canadensis* G6.

2.9. Data analysis

The data collected which included the number of cysts, cysts size, nature of cysts, age and sex of the sheep were analyzed using SPSS version 16.0 software at 95% confidence interval for analysis. Variables which included age of the sheep and number of cysts, age of the sheep and size of the cyst, age of the sheep and location of the cyst, infection and age of the sheep, infection and sex of the sheep and infection and sampling site were compared using MANOVA (Multivariate analysis of variance). Overall infection rate of cystic echinococcosis in the area was calculated in percentage by taking the total number of infected sheep divided by the total number of surveyed sheep and multiplied by 100.

Infection rate in each sampling site was calculated in percentage by taking the total number of sheep infected in each site divided by the total number of surveyed sheep in each sampling site multiplied by 100. A correlation analysis was also performed to establish whether there was a relationship between the number of cysts and age of the sheep, cyst size and age of the sheep. Intensity of infection was determined by calculating the average number of hydatid cysts per surveyed sheep and also calculating the average number of hydatid cysts per infected sheep.

CHAPTER 3

REULTS

3.1. Prevalence and distribution of cystic echinococcosis

The overall prevalence of hydatidosis in sheep in Olokurto division was 16.0% (144/900). (Fig. 5, Appendix 1) shows the prevalence and distribution of hydatidosis in sheep in the area. Ololong'oi site had the highest infection prevalence of 21.1% followed closely by Olorropil at 19.4%, Olposimoru, Kisiriri and Eneng'etia had the prevalence at 16.7%, 10.6% and 12.2%, respectively. There was a significant difference in infection rates in the five sampling sites ($P<0.05$). Least significance difference, a post hoc test for multiple comparisons showed that the prevalence were only statistically different ($P<0.05$) between Ololong'oi and Eneng'etia, Olorropil and Kisiriri and between Ololongoi and Kisiriri. A post hoc test did not show a significance difference in prevalence between Ololong'oi, Olorropil and Olposimoru because $P>0.05$. Although the study survey showed that more males than females were slaughtered (55.8% males against 42.2% females), the number of infected males and females were found to be equal (50% males against 50% females).

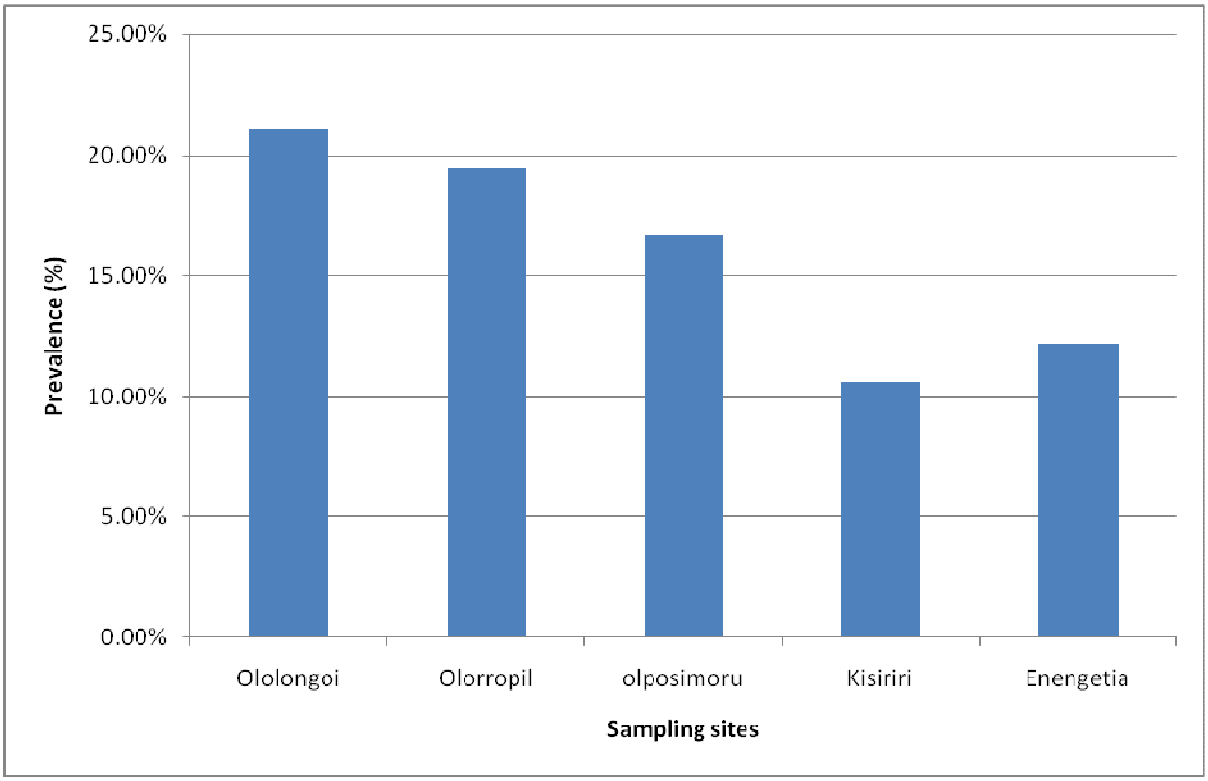

Figure 5: Prevalence of infection within the five locations

3.2. Load of *Echinococcus* species infection in sheep

The liver was the most commonly infected organ in all the sheep surveyed although infections were also detected in the lungs. Mixed infection involving both liver and lung infection were also observed. The load of infection differed within different sampling sites and organ's infection also differed in all the sheep surveyed, the liver being the most affected organ (50.7%), lung (36.8%) infection while 12.5% had infection both in the liver and lungs (Table 2). No hydatid cysts were observed in the spleen, kidneys or other internal visceral organs. A total of 343 hydatid cysts were collected during the survey and the average number of cysts per surveyed sheep was 0.38 (343/900) while the average number of cysts per infected sheep was 0.4 (144/343).

Table 2: Intensity of *Echinococcus* species infection in organs within the five divisions

Study site	**Number of sheep with infection in different organs**			**Total**
	Liver	Lung	Mixed infection (Liver & Lung)	
Ololong'oi	19	13	6	38
Olorropil	21	13	1	35
Olposimoru	14	13	3	30
Kisiriri	8	6	5	19
Eneng'etia	11	8	3	22
Total	73	53	18	**144**

Sheep of all age groups showed infection in the liver, lungs and mixed infection (liver and lung). The number of hydatid cysts in organs per infected sheep showed a general increase as the age of the sheep advanced, liver and lung infection cases (mixed infection) in all the age groups was higher compared to single organ infection because they had high number of hydatid cysts when compared with the low number of sheep with mixed infection cases in each age group. (Fig.6, Appendix 2).

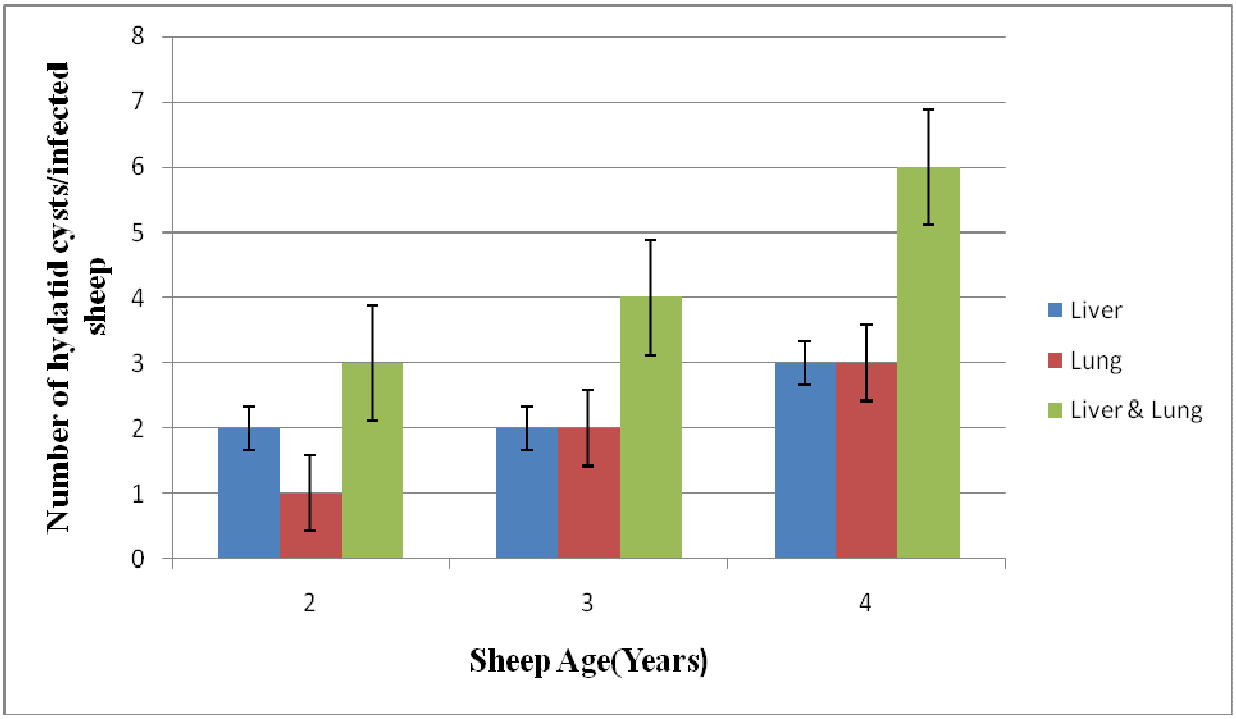

Figure 6: Comparison of distribution of infection in organs in age groups

There was a significant correlation between the age of the sheep and size of the hydatid cysts in square centimetres with cysts sizes showing a general increase with advanced age. (Fig.7) The volume of the hydatid fluid in milliliters was also correlated with the age of the sheep and it showed that the volume of the hydatid fluid increased with the age of the sheep. (Appendix 3)

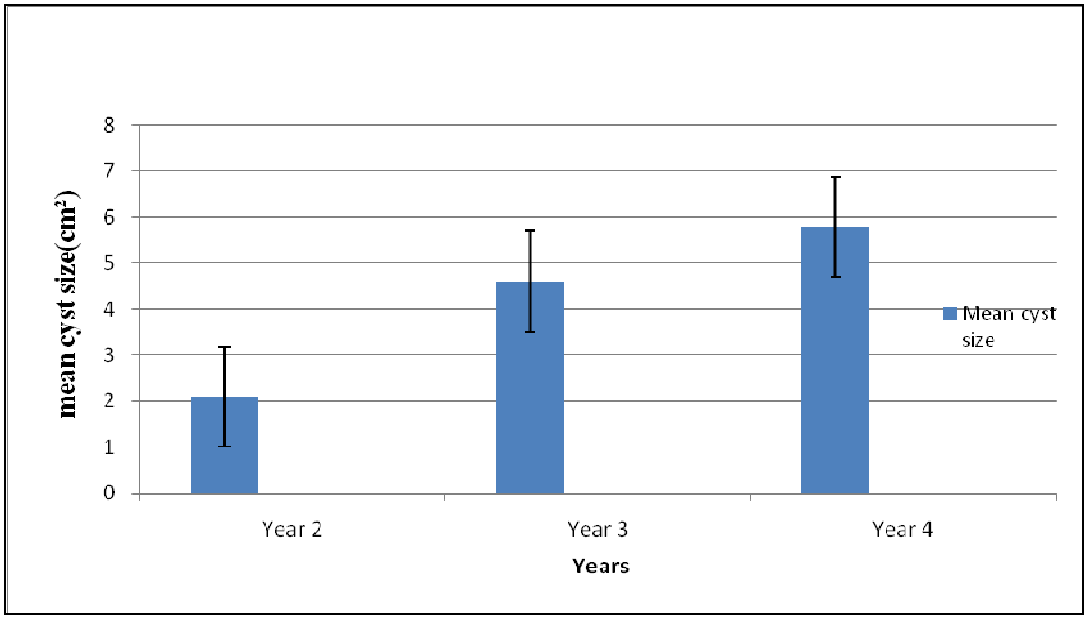

Figure 7: Mean cysts size in square centimeters (cm^2) with 5% probability Error bar

Fig.8 shows representative fertile and sterile cysts that were detected in infected organs. Microscopic examination of hydatid fluid revealed that out of 343 hydatid cysts collected during the survey, 62.1 % were fertile and contained protoscolices, while 35.2 % were sterile and 2.7 % were calcified. Fertile cysts were more localized in the lung and accounted for 73.02 % of the cysts this compared to 53.4 % fertile cysts that were observed in the liver.

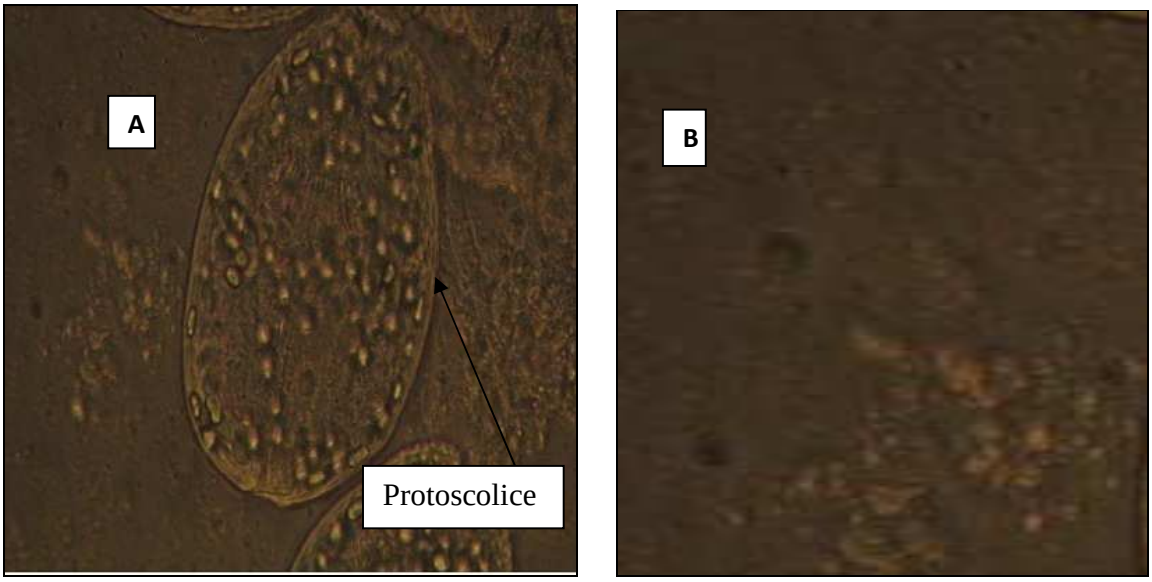

Figure 8: A fertile cyst (A) with protoscolice and sterile cyst (B) of *E. granulosus*

3.3. Characterization of different *Echinococcus* species in the region

All the 343 cysts isolates recovered during the survey were characterized to the species/genotype level by PCR/RFLP. Analysis of PCR amplified products revealed DNA fragments of between 1073-1078bp. (Fig.9).

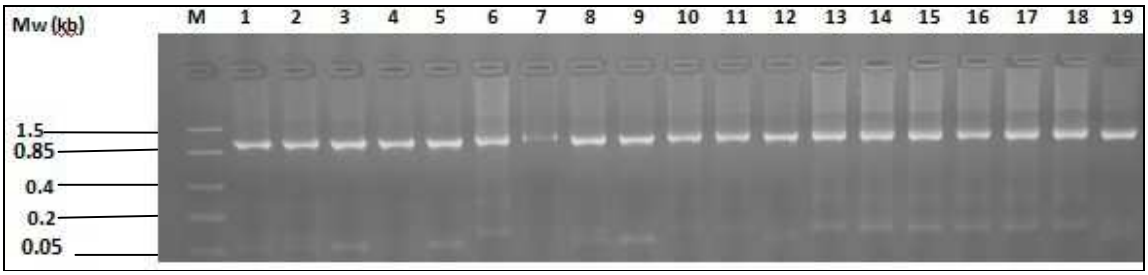

Figure 9: Representative image of an agarose gel showing PCR amplified DNA - fragments using NAD nested PCR primers. M is 100bp molecular marker and 1-19 are the tested sheep samples.

Analysis of the PCR amplified DNA fragments showed that all the samples were *E. granulosus* sensu stricto (G1-G3). The sheep digested samples produced a three band product of 485bp, 320bp and 202bp which is characteristic of *E. granulosus* G1 sheep strain. This confirmed that all cysts collected during the survey were G1 genotype. (Fig.10)

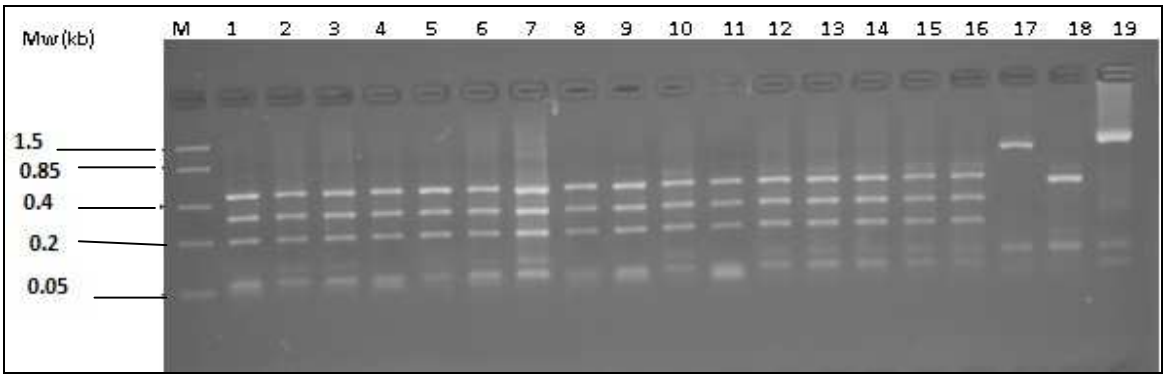

Figure 10: Representative gel image of electrophoresis following restriction digest of DNA. M is 100bp molecular marker, 1-15 are digested sheep samples, 16-19 are positive controls representing *E. granulosus* sensu stricto, *E. ortleppi*, *E. canadensis* and sheep undigested PCR product respectively.

CHAPTER 4: DISCUSSION, CONCLUSION AND RECOMMENDATIONS

4.0. Discussion

4.1. The prevalence and distribution of cystic echinococcosis in Olokurto division

These results demonstrate the presence of cystic echinococcosis in Narok area in Kenya. The overall prevalence was 16.0 % (144/900) infected sheep which is similar to a previous study conducted in Maasailand Kenya where the reported prevalence was 16.5 % (Addy et al., 2012).

The findings reflect a higher prevalence when compared to other studies done in other pastoralist areas in Kenya, such as a study by Froyd, (1960) which showed a prevalence of 13 %, another study conducted by Njoroge *et al*., (2002) in northern Turkana showed a prevalence of 3.6 %. The findings reflect the current status of the disease in the area because important factors that can influence the outcome of the study have been considered, for instance only sheep aged two years and above were used in the study and the area of study divided into five locations to ensure that the study covers the entire area. Presence of the disease in the area could be attributed to home slaughter where dogs can easily access infected offals which results in perpetuation of the disease (Macpherson, 1985) as well as high numbers of stray dogs, interaction of livestock with wild animals during grazing and poorly maintained slaughter facilities in the area.

The difference in prevalence rate in the five sampling sites could be attributed to the large number of livestock kept in Ololong'oi, Olorropil and Olposimoru sites, these three sites also border forest reserves and majority of the area residents graze their livestock in the forest reserves where livestock interact with wild animals such as jackals, warthogs and hyenas and are likely to be infected while grazing. These pastoralists also keep dogs to help in herding and

guarding the bomas at night. Since the dogs are the definitive hosts of *Echinococcus* species they may also increase transmission of the disease. In Kisiriri and Enengetia sites, there are small number of sheep and cattle and interaction of livestock with wild animals is limited since the two sites are neither forested nor border any forest reserve hence less transmission of the disease. Proportion of infected male and female sheep was the same suggesting that all sheep were at risk of acquiring infection.

4.2. Intensity of infection

The liver was the most affected organ with 50.7% of infected sheep having liver infection, 36.8% lung infection and 12.5% had both liver and lung infections. The liver is the most affected organ perhaps because it is the first organ into which the blood flows after leaving the intestines which result in most of the oncospheres being filtered into it, the ones that are not filtered in the liver move to lungs and other organs (A-Khalidi, 1998).

The study showed that older sheep had higher rate of infection, with more number of cysts, compared to younger sheep. This is attributed to the following factors; higher age reflect a much longer period of risk of infection, it is also easy to detect cysts at meat inspection in older animals due to their bigger sizes and also because older sheep cysts have more time to enlarge and pass on cysts to other organs (EI-Ibrahim, 2009).

The study results also showed that multiple organ involvement increase with increase in age of the sheep, sheep of age group 2 years had the lowest number of multiple organ involvement cases, while sheep of age group 4 years had the highest number of cases. Distribution of the parasite in various organs results in condemnation of more organs in slaughter houses which is of great economic loss (Omer *et al.*, 2010). Microscopic examination of hydatid cysts revealed that

62.1% of the hydatid cysts were fertile which is an indication that sheep is the most important intermediate host for *E. granulosus* sensu stricto.

4.3. Strains/Genotypes of *Echinococcus* species

The study results showed that all the hydatid cysts analyzed for species/genotypes identification were G1. The PCR amplified fragments were between 1073-1078bp since *E. granulosus* sensu stricto comprise of G1, G2 and G3, all the species being G1 imply that sheep is its dominant intermediate host in Olokurto division.

The presence of G1 in the area could also be a likely hood of the high prevalence of human hydatidosis among the Maasai people as this taxon has been shown to be the most pathogenic form of hydatidosis in humans, Wachira *et al*., (1993) and perhaps this should be investigated.

4.4. Conclusion

The study results showed the presence of cystic echinococcosis in Olokurto division, Kenya. A prevalence of 16.0 % which is higher compared to results of previous studies conducted in other pastoralist areas in Kenya. This is an indication that the disease will continue to be of medical and veterinary importance both in humans and livestock. All the cysts collected being G1 genotype imply that sheep is its dominant intermediate host in Olokurto division. A higher proportion of hydatid cysts collected being fertile (62.1 %) imply that the nature of the cyst is an important factor that can affect stability of *E. granulosus* parasite. Cyst's size, number and location in the body of infected sheep are directly correlated to the age of the sheep. It can therefore be concluded that the findings reflect the current status of the disease in the area.

4.5. Recommendations

1. Control programs should be established to minimize and ensure effective protection of contracting the disease in both human and livestock.

2. Vaccines which have already been tested in other areas in the world should be imported and tested for instance EG95 vaccine.

3. Treatment of dogs with anti-helminthic drugs such as albendazole, mebendazole and praziquantel.

4. Proper maintenance of slaughter facilities in the area and proper disposal of infected offals to prevent spread of the disease.

5. Killing of stray dogs in the area.

References

Abushhewa, M. H., Nolan, M. J., Jex, A.R. and Campbell, B.E. (2010). Genetic classification of *Echinococcus granulosus* cysts from humans, cattle and camels in Libya using mutation scanning based analysis of mitochondrial loci. *Mol. Cell. Probes* 2010, 24, 346-351.

Addy, F., Alakonya, A., Wamae, N., Mbae, C., Mulinge, E., Zeyhle, E., Wassermann, M., Kern, P. and Romig, T. (2012). Prevalence and diversity of cystic echinococcosis in Livestock in maasailand, Kenya. *Parasitol Res*.111 (6) 2289-2294

Al-Khalidi, N.W. (1998). Cystic echinococcus in sheep, goats and camels in shahat abattoir. Al-jabal, Libya. Proceedings of the third annual meeting for animal production under arid condition, 1, 143-149.

Benito, A., Carmena, D., Joseph, L., Martinez, J. and Guisantes, J. A. (2006). Dogs' echinococcosis in northern Spain: Comparison of coproantigens and serum antibody assays with coprological exam. *Vet. Parasitol*. 142, 102-111

Budke, C.M., Deplazes, P. and Torgeson, P.R. (2006). Global socioeconomic impact of cystic echinococcosis. *Emerg. Infect. Dis* 12, 296-303.

Buishi, L., Njoroge, E.M., Bouamra, O., Craig, P.S., (2005). Canine echinococcosis in northwest Libya: Assessment of coproantigen ELISA and a survey of infection with analysis of risk factors. *Vet. Parasitol*.130, 223-232

Casulli, A., Zeyhle, E., Brunetti, E., Pozio, E., Meroni, V., Genco, F., Filice, C., (2010). Molecular evidence of the camel strain (G6) of *Echinococcus granulosus* in human from Turkana, Kenya. *Trans. R. Soc. Trop. Med. Hyg* 104 (1): 29-32.

Ci-Peng, J., McManus, P.D. and Malcom, J. (2005). Liver alveolar echinococcosis in china: clinical aspects with relative basic research. *World. J. Gastroenterol*, 11, 4611-4617.

Dada, B.J.O. and Belino, E.D., (1979). Prevalence of hydatidosis and cysticercosis in slaughtered livestock in sokoto state, Nigeria. *Int. J. of Zoonoses*, 6, 115-117.

De la Rue, M.L., Dinkel, A., Mackenstedt, U., Romig, T., (2006). New data on *Echinococcus* species in southern Brazil. *Rev. Inst. Med. Trop. S. Paulo* 48, 103-104.

Dinkel, A., Njoroge, E.M., Zimmermann, A. Walz, M., Zeyhle, E., Elmahdi, I.E., Mackenstedt, U. and Romig, T. (2004). A PCR system for detection of species and genotypes of *Echinococcus granulosus* complex, with reference to the epidemiology situation in eastern Africa. *Int .J. Parasitol*. 34 (5) 645-653.

Eckert, J., Gemmell, M.A., Meslin, F. X. and Pawlowski, Z.S., World Health Organization. (2001). Manual on echinococcosis in human and animals: A public health problem of global concern. Paris-France.72-99.

EI-Ibrahim, J.H. (2009). Prevalence of sheep hydatidosis in North west Bank-Palestine. Msc thesis, An-Najah National University.

Eugster, R.O. (1978). A contribution to the epidemiology of echinococcosis/hydatidosis in Kenya with special reference to Kajiado district. DVM thesis, Zurich University.

Farjallah, S., Busi, M., Mahjoub, M.O., Slimane, B.B., Said, K., D'Amelio, S., (2007). Molecular characterization of *E. granulosus* in Tunisia and Mauritania by mitochondrial rrnS gene sequencing. *Parasitologia* 4, 239-246.

French, C.M. and Nelson, G.S., (1982). Hydatid disease in Turkana District of Kenya. 11. A study in geography. *Ann. Trop. Med. Parasitol*. 76, 439-457.

Froyd, G. (1990). Cysticercosis and hydatid disease of cattle in Kenya. *J. Parasitol*. 46, 491-496.

Gathura, P.B. and Kamiya, M. (1990). *Echinococcus* in Kenya: Transmission characteristics, incidence and control. *Jpn. J. Vet .Res*, 38: 107-116.

Githura, P.B. and Gathuma, J.M. (1989). Recent trends in the incidence of livestock and human hydatid disease in Kenya. *Bull. Anim. Hlth. Prod. Afr* (special issue): 159-162.

Gudewar, J., Pan, D., Bera, K., Das, S.K., Konar, A., Rao, J.R., Tiwari, A.K. and Bhattacharya, D. (2009). Molecular characterization of *Echinococcus granulosus* of Indian animal isolates on the basis of nuclear and mitochondrial genotype. *Mol. Biol. Rep*. 36, 1381-1385.

Haag, K.L., Araujo, A.M., Gottstein, B., Siles-Lucas, M., Thompson, R.C.A. and Zaha, A. (1999). Breeding systems in *Echinococcus granulosus* (Cestoda; Taeniidae): selfing or outcrossing? *Parasitol* 118, 63-71.

Hüttner, M., Nakao, M., Wassermann, T., Siefert, L., Boomker, J.D.F., Dinkel, A., Sako, Y., Mackenstedt, U., Romig, T. and Ito, A. (2008). Genetic characterization and phylogenetic Position of *Echinococcus fidelis* from the African lion. *Int. J. Parasitol* 38 (7):861-868.

Hüttner, M., Siefert, L., Mackenstedt, U. and Romig, T. (2009) .A survey of *Echinococcus* Species in Wild carnivores and livestock in East Africa. *Int. J. Parasitol* 39 (11):1269–1276.

Jenkins, D.J. and Richard, M. D. (1984). Haematological and serological data from dogs raised worm free and monospecifically infected with helminthes. *Aust. Vet. J.* 61: 309-311.

Jenkins, D. J., Romig, T. and Thompson, R.C., (2005). Emergence/re-emergence of *Echinococcus* spp. A global update. *Int. J. Parasitol*. 35, 1205-1219.

Kamenetzky, L., Gutierrez, A.M., Canova, S.G., Haag, K.L., Guarnera, E.A., Parra, A., Carcacia, G.E. and Rosenzvit, M.C. (2002). Several strains of *Echinococcus granulosus* infect livestock and humans in Argentina. *Infect. Genet*. 2*:* 129-136.

Khanfar, N. (2004). Hydatid disease: a review and update. Current Anaesthesia and Critical Care. 15, 173-183

Khuroo, M.S. Hydatid disease: current status and recent advances. *Ann. Saudi. Med* (2002); 22, 56-64.

Lahmar, S., Debbek, H., Zhang, L.H., MacManus, D.P., Soussi, A., Chelly, S., Torgerson, P.R., (2004). Transmission dynamics of the *Echinococcus granulosus* sheep-dog strain (G1 genotype) in camels in Tunisia. *Vet. Parasitol*.121, 151-156.

Latif, A.A., Akhtar, T., Magbool, A., Siddiqi, N., Tanner, M.K.and Traub, R.S. (2010). Morphological and molecular characterization of *Echinococcus granulosus* in livestock and human in Punjab, Pakistan. *Vet. Parasitol.* 170 (1-2) 44-49.

Lavikainen, A., Lehtinen, M.J., Meri, T., Hirvela-Koski, V., Meri, S. (2003). Molecular genetic characterization of the fennoscandian cervid strain, a new genotypic group (G10) of *Echinococcus granulosus. Parasitol;* 127: 207-215.

Lightowlers, M.W. and Gottstein, B. (1995). *Echinococcus*/hydatidosis: antigens, immunological and molecular diagnosis, P. 355-410.

Lightowlers, M.W., Lawrence, S. B., Gauci, C. G., Young, J., Ralston, M., Maas, D. and Heath, D.D. (1996). Vaccination against hydatidosis using a defined recumbent antigen. *Int. J. Parasitol*. 18, 457-462.

Lightowlers, M.W. (2006). Cestode vaccines: origins, current status and future prospects. *Parasitol*, 133, 27-42

Luka, S.A., Ajogi, I., Nock, I., Kodi, C. and Umoh, J. (2009). Evaluation of Enzyme Linked Immunosorbent Assay (ELISA) and western blotting for the immunodiagnosis of hydatid disease in sheep and goats. *Int. J. Vet. Med*. 5 (2) 3-6.

Macpherson, C.N.L (1981). Epidemiology and strain differentation of *Echinococcus granulosus* in Kenya. PhD Thesis University of London.

Macpherson, C.N.L (1985). An active internediate host role for man in the life cycle of *Echinococcus granulosus* in Trukana, Kenya. *AM. J. TROP. Med. Hyg.* , 79, 209-217.

Macpherson, C.N.L. (1985). Epidemiology of hydatid disease in Kenya: a study of domestic intermediate hosts in Maasailand. *Trans. R. Soc. Trop. Med. Hyg*, 79 (2): 209-217.

MacPherson, C.N.L., Karstad, L., Stevenson, P. and Arundel, J.H. (1983). Hydatid disease in Trukana District of Kenya 111: The significance of wild animals in the transmission of *Echinococcus granulosus* with particular reference to Trukana and Masailand in Kenya. *Ann. Trop. Med. Parasitol* , 77: 61-73

Magaji, A.A., Oboegbulem, S.L., Daneji, A.L., Garba, H.S., Salihu, M.D., Junaidu, A.U., Mohammed, A.A., Lawal, M., Aminus, S., Yakubu, Y. and Mamuda, A., (2011).

Incidence of hydatid cyst disease in food animals slaughtered at sokoto central abattoir, sokoto state, Nigeria. *Vet. world*, 4 (5):197-200.

Maillard, S., Benchikh-Elfegoun, M.C., Knapp, J., Bart, J.M., Koskei, P., Gottsein, B. and Piarroux, R. (2007). Taxonomic position and geographical distribution of the common sheep G1 and camel G6 strains of *Echinococcus granulosus* in three African countries. *Parasitol Res* 100 (3): 495-503.

Manterola, C., Benavente, F., Melo, A., Vial, M. and Roa, J.C. (2008). Description of *Echinococcus granulosus* genotypes in human hydatidosis in a region of southern Chile. *Parasitol. Int* 157: 342-346.

McManus, D.P. and Thompson, R.C.A. (2003). Molecular epidemiology of cystic

Echinococcus. Parasitol, 127:537-551.

Moro, P.L., Nakao, M., Ito, A., Shantz, P.M., Cavcro, C. and Cabrera, L. (2009). Molecular identification of *Echinococcus granulosus* isolates from Peru. *Parasitol. Int* 58*:* 184-186.

Moro, P., and Schantz, P. M. (2009). Echinococcosis: a review. *Int. J. Dis*, *13*(2), 125-133.

Mulinge, E., Magambo, T., Mbae, C., Huttner, M., Zeyhle, E., Kern, P. and Romig, T. (2011). First report of *Echinococcus ortleppi* in Kenyan cattle. xxivth world congress of hydatilogy. *Urumqi China* 14-18 September 2011, pg 178.

Mwambete, K.D., Ponce-Gordo, F. and Cuesta-Bandera, C. (2004). Genetic identification and host range of the Spanish strains of *E. granulosus*. *Acta. Trop*. 91, 87-93

Nakao, M., McManus, D.P., Schantz, P.M., Craig, P.S. and Ito, A. (2007). A molecular Phylogeny of the genus *Echinococcus* inferred from complete mitochondrial genomes. *Parasitol* 134 (5): 713-722.

Nakao, M., Sako, Y. and Ito A. (2003). Isolation of polymorphic microsatellite loci from the tapeworm *Echinococcus multilocularis*. *Infect Genet* 3 (3): 159-163.

Nelson, G. and Rausch, R.L. (1963). Echinococcosis infection in man and animals in Kenya. *Ann. Top.Med. Parasitol.* , 57, 136-149.

Njoroge, E.M., Mbithi, P.M., Gathuma, J.M., Wachira, T.M., Magambo, J.K. and Zeyhle E. (2002). A study of cystic echinococcosis in slaughter animals in three selected areas of northern Turkana, Kenya. *Vet. Parasitol* 104 (1): 85-91.

Omer, R.A., Dinkel, A., Romig, T., Mackenstedt, U., Elnahas, A.A., Ahmed, M.E., Elmalik, K.H., Adam, A. and Aradaib, I.E (2010). A molecular survey of cystic echinococcosis in Sudan. *Vet. Parasitol* 169 (11): 340-346.

Oniang'o, R.K. (2001). Enhancing people's through revitilisation of Agriculture and related activities. *Afr. J. F and N. Sci*, 1 (1), 43-50.

Pednekar, P.R., Gatne, L.M., Thompson, R.C.A., Traub, R.J. (2009). Molecular and morphological characterization of *Echinococcus* from food producing animals in India. *Vet. Parasitol*. 165: 58-65.

Raeghi, S., Harandi, M.F., Zia-Ali, N. and Ardakani, R.F. (2007). Hospitals cases six year old with hydatid cyst: demographic characterization and blood parameters in Kerman University hospitals. Proceedings of 4th national congress on hydatid cysts. Iran. Armaghane-danesh12, P 42. Persian.

Rinaldi, L., Maurelli, M.P., Capuano, F., Perugini, A.G., Veneziano, V., Cringoli, S., (2008). Molecular update on cystic echinococcosis in cattle and water buffaloes of southern Italy. *Zoonoses. Pub. Hlth* 55, 119-123.

Romig, T., Ibrahim, K., Kern, P. and Omer, R.A. (2011). A molecular survey on cystic *Echinococcus* in Sinnar area, Blue Nile estate (Sudan). *Chin. Med. J.* 124 (18) 2829-2833.

Santivanez, S.J., Gutierrez, A.M., Rosenzvit, M.C., Muzulin, P.M., Rodriguez, M.L., Vasguez, J.C., Rodriguez, S., Gorizalez, A.E., Gilman, R.H. and Garcia, H.H. (2008). Cysticercosis working group in Peru. *Am. J. Trop. Med. Hyg*, 79: 89-92.

Schantz, P.M. and Schwabe, C. (1969). Worldwide status of hydatid disease control. *J. Am. Med*. 55:2104-2120.

Schwabe, C.W. (1969). Veterinary medicine and human Health Balliere, Tindell. London. 169.

Tashani, O.A., Zhang, L.H., Boufana, B., Jegi, A., McManus, D.P., (2002). Epidemiology and strain characterization of *Echinococcus granulosus* in the Benghazi area of eastern Libya. *Ann. Trop. Med. Parasitol*. 96, 369-381.

Thompson, R.C. and Lymbery, A.J., (1988). The nature, extent and significance of variation within the genus *Echinococcus. Adv. Parasitol*. 27, 209-258.

Thompson, R.C. and McManus, D.P., (2002). Towards a taxonomic revision of the genus *Echinococcus. Trends. Parasitol*.18, 454-457.

Thompson, R.C., Limberly, A. J. and Constantine, C.C. (1995). Variation in *Echinococcus:* towards a taxonomic revision of the genus. *Adv. Parasitol*. 35*:*145-176.

Thompson, R.C.A. (1995). Biology and systematic of *Echinococcus,* P 1-50. CAB international Wallingford, united kingdom.

Thrusfield, M. (2005). Veterinary Epidemiology. (3rd ed.). UK: Blackwell Science Ltd.

Tijjan, A.O., Musa, H.J., Atsanda, N. N. and Mamman, B. (2010). Prevalence of hydatidosis in sheep and goats slaughtered at Damatura abattoir, Yobe state, Nigeria. *Nigerian.* Vet. J. 31 (1) 71-75.

Varcasia, A., Tosciri, G., Pedes, T., Pipia, A.P., Scala, A., Garippa, G. and Seimenis, A. (2007). Molecular characterization of *E. granulosus* in sheep and goats of Peloponnesus (Greece). *Parasitol. Res*, 4, 1135-1139.

Varcasia, A., Garippa, G., Pippia, A. P., Scala, A., Brianti, E., Gianetto, S., Batteli, G., Poglayen, G, and Micagni, G., (2008). Cystic echinococcosis in equids in Italy. *Parasitol. Res*. 102, 815-818.

Wachira, T.M., Bowles, J., Zeyhle, E. and McManus, D. P. (1993). Molecular examination of the sympatry and distribution of sheep and camel strains of *Echinococcus granulosus* in Kenya. *Ann. J. Trop. Med. Hyg* 48 (4): 473-479.

Wahlers, K., Menezes, C.N., Wong, M. L., Zeyhle, E., Ahmed, M.E., Ocaido, M., Stijnis, C., Romig, T., Kern, P., Grobusc, M.P., (2012). Cystic echinococcosis in sub-Saharan A Africa. *Lancet. Infect. Dis.* 12, 871-880.

Zeyhle, E., Njoroge, E., Magambo, J., Wachira, T.W.M., Wachira, J. and Romig, T. (2004). Control of cystic echinococcosis - a viable option for nomadic communities in East Africa. *Int. Arch. Hydatid*; 35:75

Appendices

Appendix 1: Prevalence of infection rates in different sampling sites

Count	**Sampling sites**					**Total**
	Ololongoi	Olorropil	Olposimoru	Kisiriri	Enengetia	
Infected	38(21.1%)	35(19.44%)	30(16.7%)	19(10.6%)	22(12.22%)	144(16.0%)
Un-infected	142	145	150	161	158	756
Total	180	180	180	180	180	900

Appendix 2: Distribution of *E. granulosus* infection in organs

Sheep age (years)	**Number of hydatid cysts in organs per infected sheep**			**Average**
	Liver	Lung	Liver & Lung	
2	2	1	3	2
3	2	2	4	3
4	3	3	6	4
Average	2	2	5	**3**

Appendix 3: Mean cyst size in square centimeters (cm^2) and in millilitres (ml) (± SD)

Sheep age (years)	**Mean cyst size in:**	
	square centimeters(cm^2)	**milliliters(ml)**
2	2.12 ±1.89	2.01 ±1.80
3	4.57 ±4.32	4.39 ±3.93
4	5.80 ±4.64	4.40 ±3.58
Overall mean size	4.42 ±3.56	3.91 ±3.12