MAKERERE UNIVERSITY

PREVALENCE AND RISK FACTORS OF *ECHINOCOCCUS GRANULOSUS* INFECTION IN DOGS IN MOROTO AND BUKEDEA DISTRICTS IN UGANDA

BY

OBA PETER

2012/HD17/2584U

BVM (Mak), IDAF (*PTC+, Barneveld*)

A DISSERTATION SUBMITTED TO THE DIRECTORATE OF RESEARCH AND GRADUATE TRAINING FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN INTERNATIONAL INFECTIOUS DISEASES MANAGEMENT OF MAKERERE UNIVERSITY

FEBRUARY 2015

Declaration

This study is original and has not been submitted for any other degree award to any other University before

Signature..... Date.....July 16th 2016

This dissertation has been submitted with the approval of the following supervisors:

a) **Professor Michael Ocaido, PhD**

Chair, Department of Wildlife and Aquatic Resources Management

School of Veterinary Medicine and Animal Resources

College of Veterinary Medicine, Animal Resources and Biosecurity

Makerere University

Signature..... Date.....17th/08/2016

b) **Associate Professor Francis Ejobi, PhD**

Chair, Department of Biosecurity, Ecosystems and Veterinary Public Health

School of Biosecurity, Biotechnical and Laboratory Sciences

College of Veterinary Medicine, Animal Resources and Biosecurity

Makerere University

Signature..... Date.....July 18, 2016

Dedication

To my wife, Agnes Acom and children, Diana Precious Akengo, Leticia Amongin, Celina Agadi Oba and Benjamin Frank Ebaat.

Acknowledgements

I wish to acknowledge the technical assistance received from my supervisors, Professor Michael Ocaido and Associate Professor Francis Ejobi. Financial support from DFG Project (Germany) through the Cystic Echinococcosis in Sub Saharan Africa Research Initiative (CESSARi) is highly appreciated. I wish to thank Professor Dr Peter Kern of University of Ulm, Germany, Dr Thomas Romig and Ms Marion Wassermann of University of Hoheinheim, Germany for all the support we received from the CESSARi Project especially training in R statistical data analysis at the University of Ulm, Germany. I also extend my sincere gratitude to Associate Professor Andrew Livex Okwi and Dr Ludwig Siefert of Makerere University for your important input into my research.

I wish to thank my colleagues, Dr Leonard Omadang and Mr. Martin Chamai for your support and cooperation during the study. I also thank Mr. Kenneth Okanga for your technical guidance in screening of samples; Mr. Alex Boobo, Mr. Sylvester Ochwo for providing assistance in molecular work and Mr. Mulinge Erasmus for providing a positive control sample from Kenya. Finally, I thank the following District Veterinary Officers namely Drs F.O. Inangolet, Dr Sam Elanyu and Dr. Francis Ongelec and farmers for your cooperation and participation in this study.

Finally, I wish to appreciate the support and patience of my family, especially my wife Agnes and children during my absence from home.

TABLE OF CONTENTS

List of tables

List of figures

List of abbreviations

CI	–	Confidence Interval
DALYs	–	Disability-Adjusted Life Years
DNA	–	Deoxyribonucleic Acid
DFID	–	Department for International Development
DRGT	–	Directorate of Research and Graduate Training
DSIP	–	Development Strategy and Investment Plan
DVO	–	District Veterinary Officer
ELISA	–	Enzyme-Linked Immunosorbent Assay
FAO	–	Food and Agriculture Organization of the United Nations
GOU	–	Government of Uganda
MAAIF	–	Ministry of Agriculture, Animal Industry and Fisheries
$MgCl_2$	–	Magnesium chloride
MOH	–	Ministry of Health
NCHE	–	National Council of Higher Education
NTDs	–	Neglected Tropical Diseases
OIE	–	World Organization for Animal Health
PBS	–	Phosphate Buffered Saline
PCR	–	Polymerase Chain Reaction
$TddH_2O$	–	Triple distilled de-ionized water
UBOS	–	Uganda Bureau of Statistics
UNDP	–	United Nations Development Programme
WHO	–	World Health Organization

Abstract

Cystic echinococcosis (CE) is a worldwide disease affecting domestic, wild animals and man. The definitive hosts are the members of the dog family, in which the adult tapeworm resides in the dog's intestines. Due to its zoonotic nature, CE is of public health and economic importance owing to medical costs of treatment in humans and losses in form of tissue condemnations in animals. A study was conducted in Moroto and Bukedea districts of Uganda from March to November, 2013 to determine the prevalence and risk factors for persistence of *Echinococcus granulosus* infection in dogs. Fresh dog faecal samples were collected, preserved in 70% ethanol and later screened for presence of taeniid eggs using a floatation test described. Positive samples were confirmed by a copro-PCR for *E. granulosus*. Data was collected using focus group discussions and structured questionnaires. Study sub-counties were selected by simple random sampling. Overall apparent prevalence of taeniid infection in dogs of 14.9% (39/261, CI 10.6-19.2) in both districts was recorded using the faecal floatation test. Copro-PCR results revealed a true prevalence of 14.4% (9.91- 19.0, 95% CI) in dogs in Moroto district and 7.4% (2.14-12.60, 95% CI) in Bukedea district. The overall true prevalence of CE infection was 12.2% (8.70-15.76, 95% CI) in both districts. The major risk factors identified using logistic regression were: (1) uncontrolled access of dogs to animal slaughter facilities; (2) higher cattle herd sizes and (3) lack of knowledge about the epidemiology of the disease. This study also revealed that meat inspection was important in preventing infection of dogs. From a public health perspective, enforcement of dog control and public health education were recommended to control the spread of this parasite and minimize risks of human infection. Dogs should be restricted from gaining access to condemned animal tissues and should be regularly dewormed.

CHAPTER ONE
INTRODUCTION

1.1 Background of study

Cystic echinococcosis / hydatidosis is a zoonotic parasitic disease affecting domestic, wild animals and man. It is caused by the larval stages (metacestodes) of the dog tape worm *Echinococcus* species. The disease has a worldwide distribution occurring in America, Europe, Middle East, Central and sub Saharan Africa. Several studies conducted have shown that hydatidosis is becoming an increasing public health and socioeconomic concern in many countries (Deplazes and Eckert, 2001; Kebede *et al.*, 2009). It is considered one of the re-emerging neglected zoonotic diseases in many developing countries (Budke *et al.*, 2006b; Eckert *et al.*, 2000).

The disease presents a serious animal health concern in many rural areas of the world, causing significant economic losses from reduced productivity and viscera condemnation in various livestock species (Budke *et al.*, 2006b; Cardena and Carmena, 2012; French *et al.*, 1982). Only recently, the World Health Organization (WHO) included echinococcosis and cysticercosis as part of a neglected zoonoses subgroup for its 2008–2015 strategic plans for the control of neglected tropical diseases (NTDs). Both echinococcosis and cysticercosis are to be included in a review of the Global Burden of Disease Study (Gemmell *et al.*, 2001; Torgerson *et al.*, 1995). Human cystic echinococcosis caused by *Echinococcus granulosus* and alveolar echinococcosis caused by *Echinococcus multilocularis*, are important public health threats in many parts of the world (FAO, 2011a; WHO/OIE, 2001).

The fact that echinococcosis contributes significant economic losses in livestock means appropriate control measures should be instituted to contain its spread. This is expected to lead to improvement of livestock productivity, which is the focus of the Agricultural Sector Development Strategy and Investment Plan (DSIP) of the Ministry of Agriculture, Animal Industry and Fisheries (MAAIF, 2010).

Three forms of echinococcosis occur: (1) cystic echinococcosis, caused by *Echinococcus granulosus;* (2) alveolar echinococcosis, caused by *Echinococcus multilocularis* and (3)

polycystic echinococcosis, caused by *Echinococcus oligarthrus* and *Echinococcus vogeli.* These are of special importance because of their wide geographical distribution, public health and economic impact to the livestock industry (Eckert and Deplazes, 2004a). Alveolar echinococcosis is associated with a high fatality rate and poor prognosis, especially if managed poorly. Polycystic echinococcosis, is less frequent and known to occur in Central and Southern America.

The current global burden of Cystic Echinococcosis (CE) is estimated at over 140,000 DALYs (disability-adjusted life years) lost (Hotez *et al.,* 2014). DALYs are used to assess the global burden of disease, which are important for disease prioritization and control. The Global Burden of Disease (GBD) study revealed the expected number of echinococcosis cases were 1,100,000 (95% CI 600,000-2,100,000), which is more than that of dengue fever (179,000 (109,000-299,000), visceral leishmaniasis 76,000 (61,000-93,500) and African trypanosomiasis 37,000 (9,000-106,000) (Hotez *et al,* 2014). This study was therefore conducted in Moroto and Bukedea districts so as to generate information on the magnitude of this disease in dogs as a basis for formulation of appropriate intervention measures.

1.2 Statement of the study problem

Globally, echinococcosis causes losses estimated to be between 1.6 to 3 billion disability-adjusted life years (DALYs) per annum (Torgerson and Craig, 2011). It has also been estimated that the annual costs of treating clinical cases and losses to the livestock industry could amount to US $ 2 billion (Torgerson and Craig, 2011). Echinococcosis disproportionately affects the socio-economically disadvantaged segments of society and causes serious human morbidity due to the effect of the parasite larvae in the liver, lung or brain of the host. Current evidence suggests that echinococcosis is spreading due to lack of meat control, dog management and appropriate legislation (Eckert and Deplazes, 2004b; Otero-Abad and Torgerson, 2013).

A study in south Western Uganda showed that 20 surgical cases were reported in hospitals per year (Macpherson *et al.,* 2004). Also a study conducted in Moroto district in Moroto found a prevalence of 66.3% of cystic echinococcosis in dogs (Inangolet *et al.,* 2010). However, this study was based on post mortem examination of dogs in Moroto district, which was quite costly, biohazardous and raised ethical issues. Otherwise, no previous studies have been conducted to

establish the key risk factors for the persistence of *Echinococcus granulosus* infection in dogs in Teso and Karamoja sub regions. This study was therefore formulated to establish the prevalence of infection in dogs and associated risk factors, as a basis for design of appropriate disease control strategies.

1.3 Significance and justification of the study

Echinococcosis surveillance in dogs, livestock and humans provides information for establishing pre-intervention baseline, assessing efficacy of control programmes and to guide control agencies for planning and resource allocation. Hydatid disease is a chronic debilitating disease, often common in remote settings where medical treatment is difficult to access (Torgerson, 2003), as in pastoral and agro-pastoral areas in Karamoja and Teso areas respectively in Uganda. Infection with hydatid disease in humans is often asymptomatic during an individual's life time, with many cysts only discovered at autopsy, or during surgery or radiography, related to other causes (Kern, 2010). Understanding the disease situation in the dog population is useful in assessment of disease transmission potential, zoonotic risks and optimization of intervention programmes (Butler and Bingham, 2000). In Uganda, little is known about the prevalence and factors promoting transmission of *Echinococcus granulosus* in dogs. Consequently, the motivation and design of appropriate methods for control of the disease cannot be attained. An improved understanding of the disease burden in dogs constitutes the basis for designing effective control measures. This would ultimately lead to reduction of the risk of echinococcosis infection among humans and livestock hence leading to improved livelihoods.

1.4 General objective

To determine the prevalence and risk factors for *Echinococcus granulosus* infection in dogs in Moroto and Bukedea districts in Uganda.

1.4.1 Specific objectives

i. Determine the prevalence of *Echinococcus granulosus* infection in dogs in Moroto and Bukedea districts in Uganda.

ii. Establish the major risk factors for *Echinococcus granulosus* infection in dogs in Moroto and Bukedea districts in Uganda.

1.5 Research questions

1. What is the current prevalence of *Echinococcus granulosus* infection in dogs in Moroto and Bukedea districts in Uganda?
2. What are the key risk factors responsible for persistence of *Echinococcus granulosus* infection in dogs in Moroto and Bukedea districts in Uganda?

1.5 Conceptual framework of risk factors for acquiring Echinococcus infection in dogs

The conceptual framework of risk factors for acquiring and maintenance of *Echinococcus* infection in dogs was as shown in Figure 1.

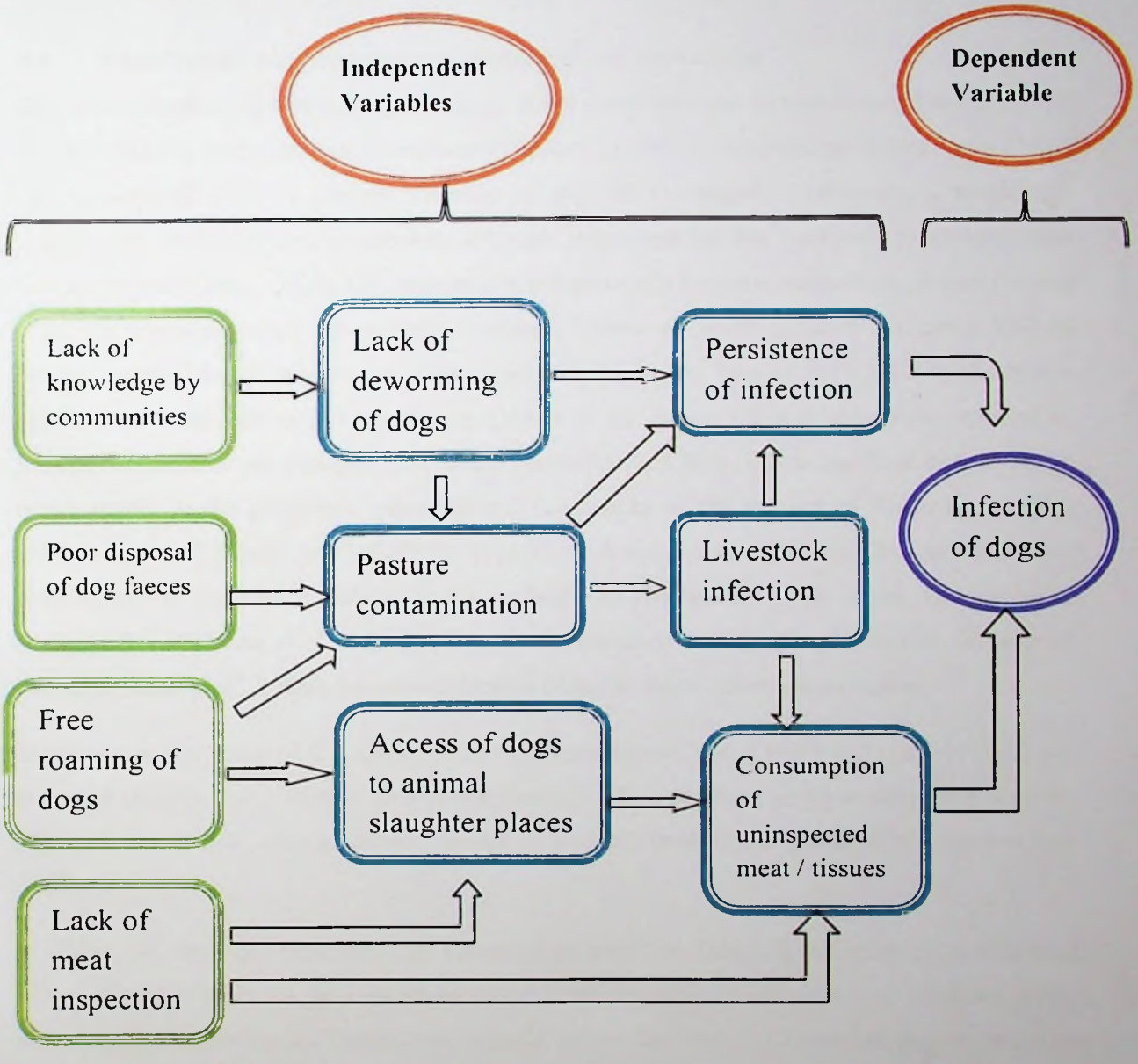


Figure 1: Conceptual model of factors responsible for persistence of CE infection of dogs

CHAPTER TWO

LITERATURE REVIEW

2.1 Distribution and prevalence of *Echinococcus granulosus*

On a global basis, *Echinococcus granulosus* is the most common and widespread species, with endemic foci in every inhabited continent (Dakkak, 2010). In Sub Saharan Africa, only Cystic Echinococcosis (CE) is present (Romig *et al.*, 2011). Figure 2 presents a worldwide distribution of Echinococcus species, although some species are restricted to certain areas (Torgerson and Craig, 2011). CE represents a public health concern, particularly in low income countries where resources for control are limited. Present estimates indicate that cystic hydatid disease, caused by *Echinococcus granulosus*, results in the loss of 0.14 million disability-adjusted life years (DALYs) per annum (Hotez *et al,* 2014). CE has also been reported in Europe, Mediterranean regions, Russia and Australia. In China, CE is regarded as one of the major public health problems, where annual incidences of the disease in Xinjiang province were as high as 8.7 cases per 100,000 in 1990 (Eckert and Deplazes, 2004a). In Latin America, echinococcosis remains a public health problem in Argentina, Chile, Peru, Uruguay and southern Brazil (Moro *et al.*, 2000). CE is one of the most widely spread parasitic diseases in Sardinia (Scala *et al.*, 2006), the second largest island in the Mediterranean region.

In Europe, a few cases of CE are reported in Western and Central parts and most of these are imported (Budke *et al.*, 2006a). In Eastern Europe, CE is reported to be an emerging disease, with endemic foci in most countries, except in Iceland, Ireland and Denmark (Torgerson and Budke, 2003).

In Africa, CE has been reported in all countries south of the Sahara (see Figure 2) (Romig *et al.*, 2011). High prevalences or incidences have been reported in countries of northern Africa, including Sudan, Morocco, Tunisia and Algeria, where the camel is known to play an important role in the maintenance of the parasite (Dakkak, 2010). In Eastern Africa, there is a conspicuous presence of human cases of CE in areas of northwestern Kenya, northeastern Uganda, southeastern Ethiopia and the extreme southeast of Sudan (Romig *et al.*, 2011) (Figure 3).

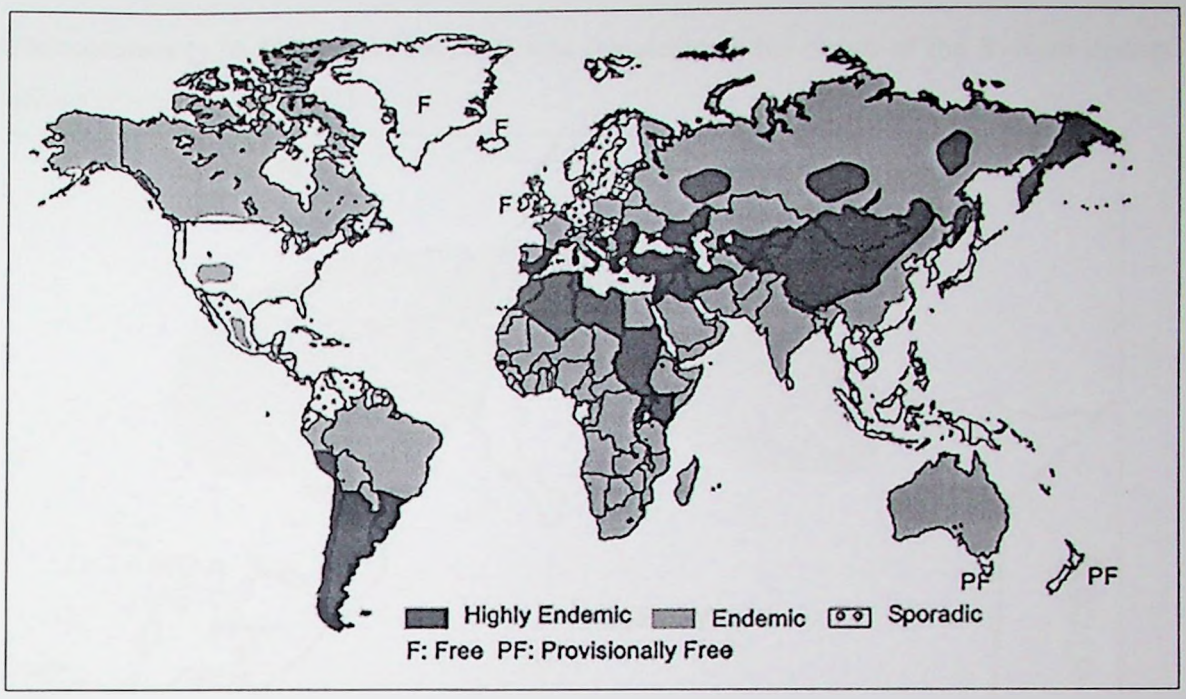


Figure 2: Global distribution of zoonotic strains of *Echinococcus granulosus* (Eckert and Deplazes, 2004).

Human CE caused by *E. granulosus* is prevalent in many developing countries, especially in poor marginal communities (Macpherson *et al.*, 1983). In dogs, previous studies on the prevalence of CE have reported widely varying results. In Karamoja sub region in Uganda with similar nomadic lifestyle as the Turkana region of Kenya, a prevalence of *E. granulosus* in dogs was found to be as high as 60% (Inangolet *et al.*, 2010). In Turkana district of Kenya, the prevalence in dogs was found to be 39.4% (Macpherson, 1985), while it was found to be 12.4% in Serengeti in Tanzania (Macpherson, 1985). In Ethiopia, a study revealed that 20.5% of cattle and 22% of dogs were infected (Wachira *et al.*, 1993). These differences could be associated with host distribution, dog population, cultural practices as well as climatic conditions in the different regions References.

In humans, the incidence of surgical cases of CE ranges from 0.1 to 45 cases per 100,000 and the real prevalence ranges between 0.22 and 24% in endemic areas (WHO, 2010). The prevalence of *Echinococcus granulosus* infection in dogs has been used as a key indicator of CE infection in humans (Walters and Clarkson, 1980). One of the highest annual surgical incidences of cystic

echinococcosis (176-220 cases /100,000) was registered in the North of the Kenyan district of Turkana (French *et al.*, 1982).

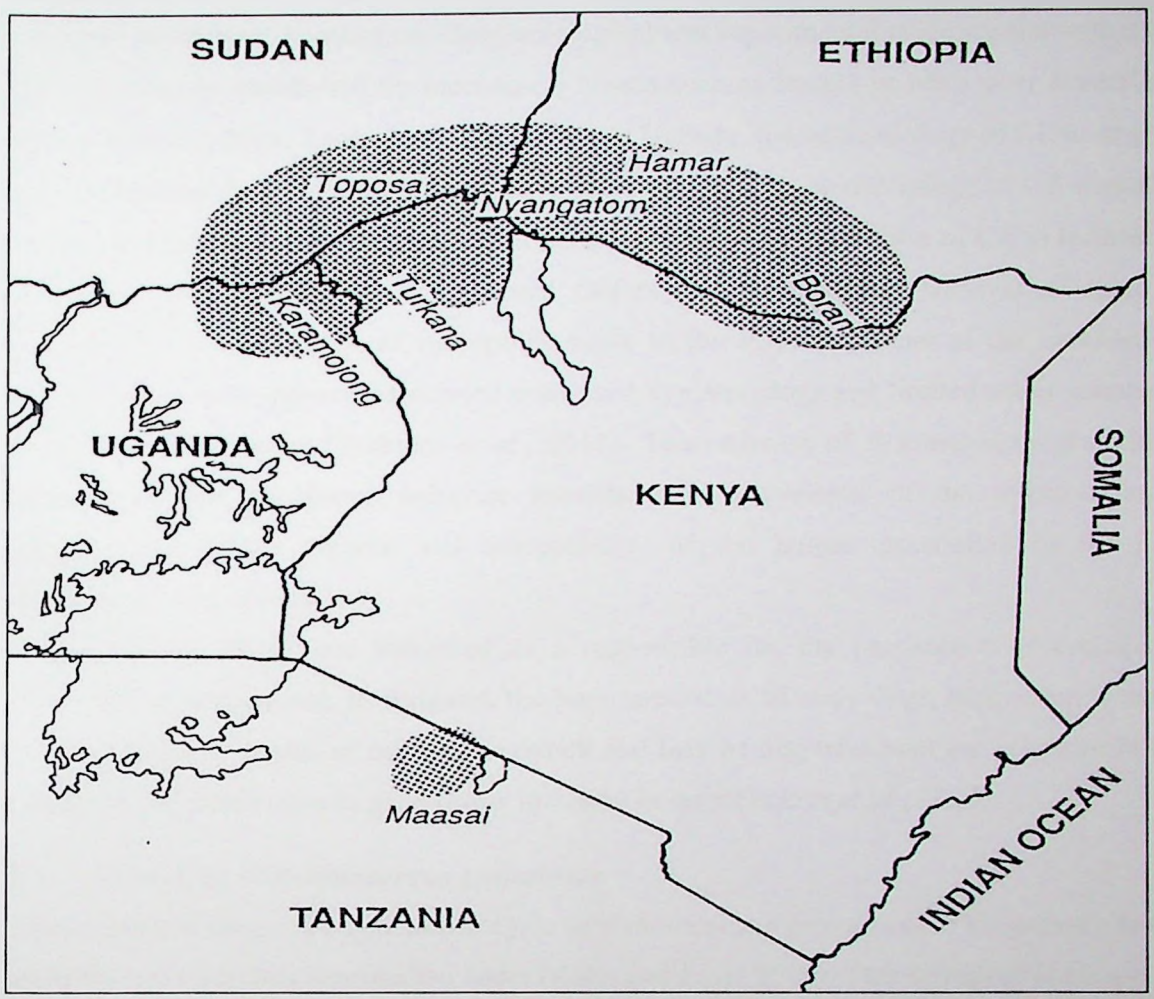


Figure 3: Approximate regions and ethnic groups with exceptionally high prevalence of Cystic Echinococcosis in Eastern Africa (Macpherson *et al.*, 1989 and Magambo *et al.*, 2006)

2.2 Epidemiology of cystic echinococcosis

CE is maintained in a domestic (synanthropic) and sylvatic cycle by dogs as definitive hosts. The domestic cycle is more common and poses the greatest threat to human health (Torgerson and Budke, 2003). In its natural transmission cycle, dogs and other canids are the definitive hosts, while the domestic ungulates (including cattle, goats, sheep, camels, etc.) are the intermediate hosts (Kahn and Line, 2010). Definitive hosts are infected by ingestion of tissues of intermediate

hosts containing viable protoscolices (Eckert *et al.*, 2010). Exposure to the eggs of Echinococcus species may be affected by a number of occupational and behavioral factors, which ultimately influence the disease distribution. Epidemiological and experimental evidence shows that taeniid eggs can also be transferred by mechanical means such as insects or birds over several metres (Gemmell *et al.*, 2001; Torgerson *et al.*, 1995). In Uganda, the epidemiology of CE in dogs is not well understood due limited information. In other regions, the epidemiology of CE was affected by the distribution of definitive and intermediate hosts. The distribution of CE is influenced by livestock production system, environment factors that influence the survival of eggs, strain pathogenicity and exposure of susceptible hosts to the infective forms of the parasite. CE is maintained in environments associated with poor hygiene, dogs and limited water sources as in pastoralist communities (Wahlers *et al.*, 2012). Transmission of *Echinococcus granulosus* to humans depends on human behavior towards dogs, prevalence of the infection in dogs, pathogenicity of the parasite and susceptibility of the human population to the parasite (Magambo, *et al.*, 2006).

Several factors have been identified as a responsible for the persistence or emergence of *Echinococcus granulosus*. In Bulgaria, the high population of stray dogs, lack of tap water, easy access of dogs to organs of infected livestock and lack of dog treatment are responsible for the persistence of *Echinococcus granulosus* infection in dogs (Eckert *et al.*, 2000).

2.3 Life cycle of *Echinococcus granulosus*

Figure 4 below shows the typical life cycle of *Echinococcus granulosus*. This parasite has a life cycle that involves two mammalian hosts (Kahn and Line, 2010). The definitive hosts are canids (especially dogs), in which the adult (strobilocercus form) is developed while the intermediate hosts are various herbivores, including cattle, sheep, goats, pigs, camels and donkeys where larval form (hydatid cyst) is developed. Humans can also become accidental hosts when they ingest eggs of the tapeworm from the definitive host, usually from contaminated water, hands or food (Deplazes and Eckert, 2001). Usually, humans are the 'dead-end' hosts, since in most communities dogs do not generally get access to human tissues.

The disease is maintained in two cycles: - the sylvatic (wildlife) and the domestic cycle involving domestic ungulates, usually cattle, sheep, goats, pigs, camels and donkeys (Dinkel *et*

al., 2004; Wachira *et al.*, 1993). From Figure 4, the adult *Echinococcus granulosus* designated as (1) (3 to 6 mm long) resides in the dog's intestines. Adult female worm gravid proglotids release eggs designated as (2) that are passed out with faeces (WHO/FAO/OIE, 2011). The intermediate host, which is usually cattle, goats, sheep, camels and other wild ungulates, is infected by ingestion of eggs in contaminated pastures or water. The egg hatches in the small intestines into an oncosphere designated as (3) penetrates the intestinal wall and migrates through the circulatory system into other organs, especially the liver, lungs or other viscera (Hendrix, 2010). In these organs, the oncosphere develops into a hydatid cyst designated as (4), which gradually enlarges in size, producing daughter cysts. The definitive host becomes infected by ingesting cyst-containing tissues of the intermediate host (WHO/OIE, 2001a). Upon ingestion, the protoscolices designated as (5) evaginate and attach onto the intestinal mucosa and develop into adult stages (1). The whole life cycle is completed between 32 to 80 days.

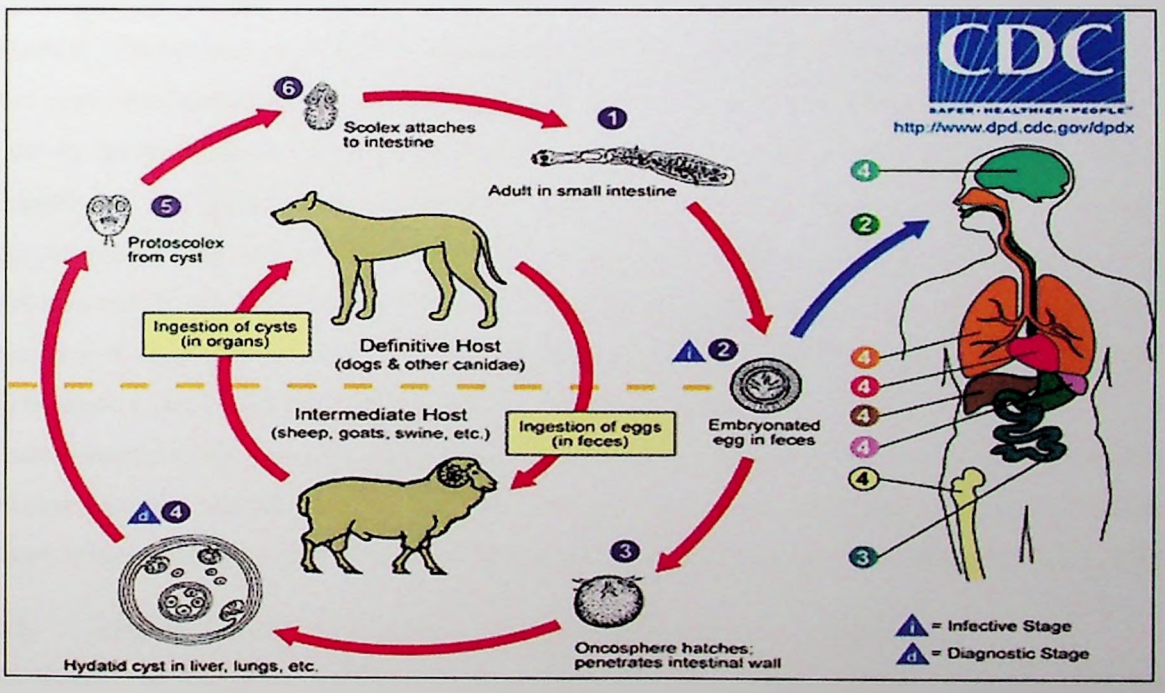


Figure 4: Schematic life cycle of *Echinococcus granulosus* (Adopted from CDC, 2012)

2.4. Survival of *Echinococcus granulosus* eggs in the environment

Various ecological and environmental factors play a role in determining the transmission patterns of *Echinococcus* species. In studies to determine the transmission dynamics of CE it was found that eggs of *E. granulosus* could survive only a few hours under the high ambient temperatures in Turkana land (Njoroge *et al.*, 2002; Wachira *et al.*, 1991). When exposed to sunlight and high temperatures, they become desiccated and did not hatch even when consumed by intermediate hosts (Eckert *et al.*, 2001; Wachira *et al.*, 1991). However, under favorable conditions, *Echinococcus granulosus* eggs can survive and remain viable in the environment (pastures) for several weeks or months. However *Echinococcus* species eggs can be very resistant to environmental conditions. If deposited inside the soil, eggs have been found to be viable for up to one year (WHO/OIE, 2001b). Echinococcus eggs have been found to survive in water or damp soil for 3 weeks at 30 °C (Wachira *et al.*, 1991). Previous studies have demonstrated survival of *Echinococcus granulosus* eggs inside canine faeces in inferior arid environment for up to 41 months (Thevenet *et al.*, 2005). This characteristic facilitates the transmission of this parasite to the next host, particularly in areas where the population of susceptible hosts is high enough to sustain the transmission cycle. This has important epidemiological implications since one dog is capable of infecting several susceptible hosts when they ingest pastures or water which is contaminated with dog faeces. The number of eggs ingested by the intermediate host is therefore determined by the infectivity of the eggs and the level of pasture contamination. Furthermore, number of eggs that develop into hydatid cysts is determined by the immune status of the host (Thompson and McManus, 2002). Thus various climatic factors can play a significant role in the transmission of the parasite. The potential for domestic transmission of *E. granulosus* may be highest in poor countries where the level of education is low, veterinary services inadequate and there is the widespread practice of home slaughtering of animals (Torgerson and Budke, 2003)

2.5. Risk factors for transmission of *Echinococcus granulosus* in dogs

Abundance of definitive and intermediate hosts play a key role in influencing the transmission patterns of CE (Magambo *et al.*, 2006). A study in northwestern Turkana by Macpherson *et al.* (1985) found that the prevalence of CE in dogs was higher in areas with high stocking densities of livestock. Nomadic people use dogs for various socio-economic functions. The combination of poor hygiene, sanitation conditions and scarce water resources, provide ideal conditions for

the spread of *Echinococcus* species (Wahlers *et al.*, 2012). Hygiene and sanitary conditions play a key role in the transmission of CE since dogs can contaminate the environment and water ways with their faeces (Macpherson, 2005). Previous studies have revealed that dogs fed on condemned viscera or raw offal were more likely to be copro-antigen positive for *E. granulosus* (Otero-Abad and Torgerson, 2013). Likewise, dogs with more possibilities of contact with domestic livestock were likely to become infected (Otero-Abad and Torgerson, 2013). Previous studies revealed that the key risk factors for infection of dogs with *Echinococcus* species is age of the dog of less than 5 years; free roaming of dogs, access to raw offal (giving a 12-fold increase in likelihood of infection), frequency of home slaughters and species of animal slaughtered (Buishi *et al.*, 2006; Moro *et al.*, 2005).

In a recent study done by Nyakarahuka *et al.* (2013) in Kasese district, Uganda found potential risk factors for CE in humans to be: dog ownership, presence of stray dogs, absence of dog de-worming, home slaughtering of animals, lack of hand washing; use of un-boiled drinking water and feeding uncooked infected organs to dogs.

Prevention strategies used against CE include improved slaughter hygiene, regular anthelmintic treatment of dogs and public health educational programmes. Vaccination of lambs is being evaluated as an additional strategy (Lightowlers, 2006a). The World Health Organization is working towards validation of effective CE control strategies by 2018 (Kern, 2013; WHO, 2010).

2.6. Diagnosis of cystic echinococcosis

The diagnosis of echinococcosis in dogs or other carnivores requires the demonstration of the adult cestodes of *Echinococcus* species or immature segments in their faeces or the small intestine or the detection of specific coproantigens or copro-DNA. In the intermediate host, diagnosis depends on the detection of the larval cyst form, which can occur in almost any organ, but particularly in the liver and lungs. Necropsy is the best diagnostic test for CE (Eckert *et al.*, 2001) and is used as a reference test to estimate the sensitivity and specificity of immunodiagnostic tests (Gatti *et al.*, 2007). However, this method is not 100% accurate in intermediate hosts because of possible wrong diagnosis and the bias of slaughtering older animals more often than younger animals (Gatti *et al.*, 2007).

In definitive hosts, diagnostic assays for detection of parasite antigens (coproantigens) using antigen capture ELISA in dog faecal samples have been developed (Allan *et al.*, 1992) but this procedure is primarily genus-specific and not species-specific and its sensitivity appears to be affected by parasite burdens (Shehabi *et al.*, 2000). In addition, although coproantigen procedure is reported to have a high sensitivity (Craig, 1997) and specificity (approximately 85%), cross reactions between antigens of *E. granulosus* and *E. oligarthrus* or with other *Taenia* species do occur (Torgerson and Deplazes, 2009; Allan, *et al.,* 1992; Christofi *et al.,* 2002). Besides, coproantigens are not detectable in faeces treated with organic solvents and are not preserved well in ethanol (Allan JC, *personal observation*).

The faecal flotation technique is used as a screening test to concentrate *E. granulosus* eggs using Zinc chloride (specific gravity 1.45 g/cm^3), as described by Eckert *et al.* (2001), Mathis *et al.* (1996) and Huttner *et al.*, (2009). The advantage of this method is that it is relatively cheaper and is less invasive. However to differentiate *E. granulosus* eggs from other *taeniid* eggs or from other Echinococcus species, a Polymerase Chain Reaction (PCR) should be performed. The copro-PCR is reported to have a high sensitivity (Eckert and Deplazes, 2004b), and the specificity is close to 100% (Mathis and Deplazes, unpublished data). This is used as a confirmatory test for *Echinococcus granulosus* (Abbasi, *et al.* (in press); Mathis and Deplazes, unpublished; Thompson and McManus, 2002).

2.7. Treatment and control of *Echinococcus granulosus* infection in dogs

Supervised treatment of dogs four to eight times a year using praziquantel orally is the single most important intervention (WHO/FAO/OIE, 2011). However, the effectiveness of this intervention depends on the proportion of the dog population treated and community participation. In humans, treatment is usually effected by surgical excision of cysts. However, this depends on the degree of tissue involvement, location and size of the cysts. Control of this parasite is by restricting access of dogs to infected offal, regular deworming of dogs, meat inspection at slaughter places / abattoirs and public health education on the mode of spread of the parasite (WHO/OIE, 2001b). Prevention methods that have been used for *Echinococcus* infections in man include avoiding contact with dog faeces, periodic deworming of dogs (WHO, 2014) hand washing, improved sanitation and reducing dog populations (CDC, 2012; WHO/OIE,

2001a). In sheep, trials using the EG95 vaccine have demonstrated a 95% protection (Lightowlers, 2006b). Effective control requires strong collaboration between medical, public health and the veterinary professionals. In many developing countries, collaboration between these sectors is often weak, and this may perpetuate further spread and persistence of the parasite.

From what has been cited in literature it becomes apparent that the prevalence and risk factors for CE differ from place to place hence requiring area-specific studies to understand the epidemiology of CE so that appropriate control measures can be designed and implemented.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area description

This survey was conducted in Moroto and Bukedea districts of Uganda. Moroto district is located in north eastern region (Karamoja sub region), with coordinates 2° 30' 0''North and 34° 15' 0'' East and Bukedea district, located in the central part of the eastern region of Uganda (with coordinates 1° 22' 0'' North and 34° 8' 0'' East). Details of the locations of Moroto and Bukedea districts are as shown in Figure 5. These two districts were chosen for the study because Moroto district lies in an arid belt (purely pastoral livestock production system), close to Turkana of North-western Kenya where the prevalence of cystic echinococcosis had been reported to be high (French *et al.*, 1982; Macpherson *et al.*, 1983). Bukedea district was chosen to represent a mixed crop-livestock agro-pastoral area for comparison.

Bukedea district, occupied by mainly the Iteso ethnic group, is composed of five sub counties and one town council. The district is generally a flat land that covers an estimated area of 1,051.7 km^2, with a mean elevation of 1,071 metres above sea level (UBOS, 2012). The projected population by 2012 is 186,400 persons with average density of 177.2 persons per km^2 (UBOS, 2002). Over 84% of the population are mainly subsistence farmers, engaged in crop cultivation and livestock (cattle, goats, sheep, pigs and poultry) rearing (UBOS, 2012). The district has annual temperatures ranging from 16 to 32 °C with a mean of 26 °C (Dept. of Meteorology Entebbe, 2012) while annual rainfall (bimodal pattern) ranges from 800 – 1500 mm (FAOSTATS, 2010). The dog population in Bukedea district is estimated to be 1,320 dogs (DVO Bukedea, *personal communication*).

Moroto district is inhabited by the Bokora and the Matheniko ethnic groups. The 2014 mid-year projected population was 151,900 persons, with a density of 23 persons per square km (UBOS, 2014). The district has a mean altitude of 1,327 metres above sea level and a monomodal rainfall pattern, ranging between 500 – 800 mm annually, with annual temperatures from 18 to 32.5^0 C (GOU, 2009). The district lies in a semi-arid belt, with most inhabitants living a pastoral or agro-pastoral lifestyle. Over 80% of the population lives below the poverty line (UNDP, 2007).

Livestock rearing forms a major source of livelihoods for a majority of the population, in addition to subsistence crop cultivation. The dog population in Moroto district is estimated to be 2,250 (DVO, Moroto).

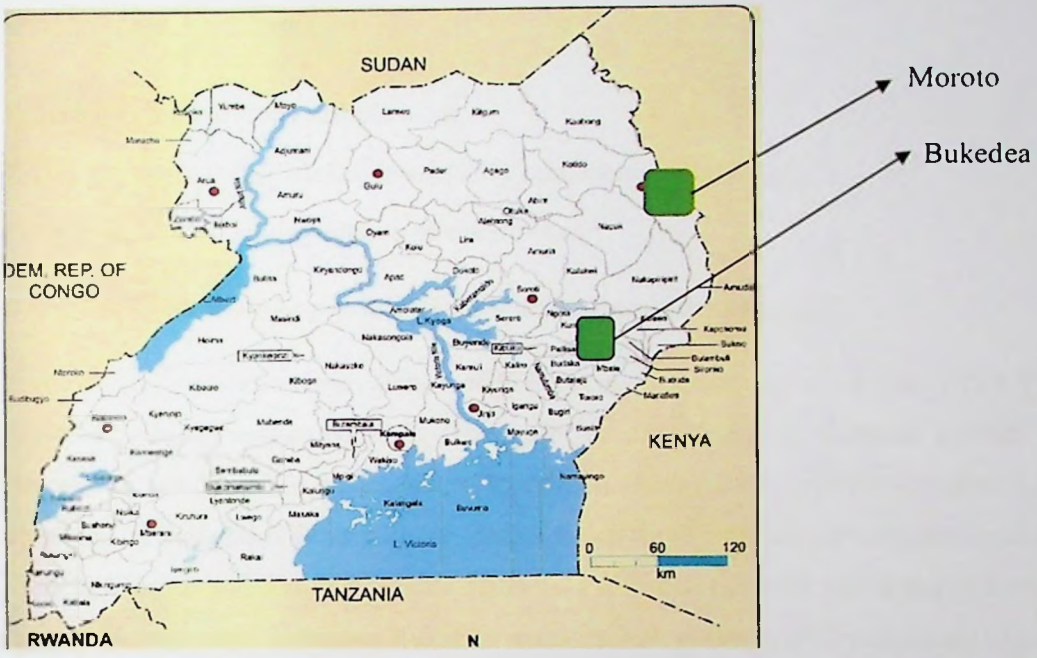


Figure 5: Map of Uganda showing locations of study areas: Moroto and Bukedea districts

3.2 Study design and sample size determination

A cross sectional study was conducted from March to November 2013. In each of the study districts, sub counties were selected for the study by simple random sampling. In Moroto district, three (3) sub counties were selected and these were Moroto municipality, Rupa and Nadunget. In Bukedea district, the four (4) sub counties randomly selected were Kachumbala, Kolir, Bukedea town council and Malera.

The total population of domesticated dogs was obtained from the District Veterinary Office. The sampling units were the households owning dogs in the study areas. Households keeping dogs were sampled from the sub counties using a systematic random sampling method, with every

third household being considered. A representative sample size of the domesticated dogs in the study area was obtained by a simple random sampling procedure.

The estimated sample size was computed from the formula below (Thrusfield, 2005):

$$n = \frac{Z\alpha^2 \, P_{exp} \left(1 - P_{exp}\right)}{d^2}$$

Where **n** is the required sample size;

$\mathbf{Z\alpha^2}$ is the desired confidence level (95% confidence interval);

Pexp is the expected prevalence of the disease

d is the desired absolute precision (allowable error of the estimate).

Previous studies found a prevalence of *Echinococcus granulosus* infection of 24.5% (95% CI 20-29.5) in semi-domesticated dogs (Inangolet *et al.,* 2010). In Turkana district of Kenya, the prevalence was found to be 39.4% (Macpherson *et al.*, 1985), while it was found to be 12.4% in Serengeti in Tanzania (Ernest, *et al.,* 2013). From these figures, the estimated mean prevalence is 15 % in Moroto and 5% in Bukedea districts. From this formula, the required sample size of 196 dogs in Moroto and 72 dogs in Bukedea was derived, giving a total sample size of 268 dogs.

3.3 Sampling design and sampling of study dogs

A list of households keeping dogs in the respective sub counties was obtained from the area veterinarian. The households were then selected by systematic random sampling, with a sampling interval of 3 households for Bukedea and 2 households for Moroto district. The selected households were then visited for collection of faecal samples from dogs and administration of questionnaires. In households that kept 1-2 dogs, both dogs were considered in the study. Where there were 3 dogs or more, 30% (3/10) of the number of dogs was considered. The domesticated dogs were the target of this study, due to anticipated difficulties of trapping stray dogs. Routine visits were made to these sites and field assistants were recruited and trained to help in data collection.

3.4 Collection of faecal samples and administration of questionnaires

With the assistance from the owners, dogs were restrained with a mouth gag and faecal samples were taken directly from the rectum with lubricated gloved hands. Faecal samples (approx. 5 g)

collected from dogs were kept in 10 ml plastic containers containing 70% ethanol till analyzed. Information on prevalence of *E. granulosus* infection was obtained by screening faecal samples for the presence or absence of taeniid eggs using the faecal floatation method, as described by Huttner *et al.*(2009) and Eckert *et al.* (2001) and subsequently identified using a PCR test.

In each household where dog faecal samples were collected, a structured questionnaire (Appendix 2) was administered to the head of the household or any available adult. Questions were translated from English to a local language by myself or trained assistants. The questionnaire captured data mainly on demographic characteristics, dog keeping practices, attitudes and knowledge level in regard to cystic echinococcosis. A total of 74 and 65 questionnaires were administered in Bukedea and Moroto districts respectively.

3.5 Laboratory analysis of faecal samples

Laboratory analysis of faecal samples was done at the College of Veterinary Medicine, Animal Resources and Biosecurity, Makerere University. The eggs were recovered using a floatation technique described by Huttner *et al.*, (2009) (Appendix 7).

Briefly, the procedure was carried out as follows: Untreated faecal sample was diluted in proportions of 1:4 in phosphate buffered saline (PBS) containing 0.3% Tween 20. The suspension was centrifuged for 10 min at 1600 g and supernatant discarded. The pellet (about 2 ml) was re-suspended in 8 ml zinc chloride solution (density 1.45 g/cm^3) and centrifuged for 30 min at 400 g – the supernatant solution contains the taeniid eggs.

The supernatant was then passed through the sieve with mesh size 45-50µm and rinsed thoroughly with water – taeniid eggs were in the flow-through fraction. The flow-through fraction was passed through the sieve with mesh size 20-25 µm and rinsed thoroughly with water – taeniid eggs were retained by the sieve. The sieve was placed upside down in a funnel standing in a collection tube to collect the taeniid eggs by washing the sieve thoroughly with water.

The water was then centrifuged for 10 min at 400 g to concentrate the eggs and the supernatant carefully removed by using a pipette until approximately 1 ml was left in the tube. A small volume (2 ml) of the supernatant was then placed on a glass slide using a 3 ml pipette and observed using Olympus Cx21 microscope at 10X magnification for the presence of taeniid eggs.

The pellet / taeniid eggs with the remaining water was transferred to a 1.5 ml tube for safe storage. A positive sample was considered to have a typical taeniid egg that is ovoid, brown striated appearance with characteristic hooklets (WHO/OIE, 2001) (Appendix 7).

Because eggs of *Echinococcus granulosus* are not distinguishable morphologically from those of *Echinococcus multilocularis* or from other *Taenia* species (WHO/OIE, 2001b), a copro-DNA was used for identification of *E. granulosus*. However, although necropsy is considered a gold standard for CE diagnosis, it was not used due to ethical issues. Besides, the dog owners were less likely to accept to consent to the study involving killing of their dogs. The Polymerase chain Reaction (PCR) was thus used to distinguish between *Echinococcus granulosus* from other *Taenia* species. Samples that were found positive by the faecal floatation method were further subjected to a copro-DNA test.

3.6 DNA (PCR) procedure

The PCR protocol for identification and confirmation of *Echinococcus granulosus* eggs was done as described by Huttner *et al.*, 2008 and Huttner *et al.*, 2009. Using a MicroCL 17 Thermo^{TM} centrifuge, taeniid eggs were concentrated from the supernatant fluid by centrifugation at 12000 rpm for 5 minutes and placed in 200µl PCR tubes. Ten (10) µl of 0.02M Sodium Hydroxide was added then heated at 90° for 30 minutes. This was then used as the template. Genotypic identification of the parasite was achieved by amplifying the target gene nad1 which amplifies a segment of 1073 base pairs (Huttner *et al*, 2008).

Amplification of the target gene nad1 was done as follows: The reaction mixture (25µl by volume) used for gene amplification used a PCR kit obtained from Qiagen (Qiagen^{TM}). The PCR procedure was conducted using a 2720 Thermal Cycler (Applied Biosystems^{TM}) at the College of Veterinary Medicine, Animal Resources and Biosecurity Molecular Biology laboratory. 5 µl template DNA prepared as above (section 3.6) was mixed with PCR-Buffer (at conc. 10 mM Tris-HCl, pH 8.3; 50 mM KCl), 4 µl of 2 mM $MgCl_2$, one µl of 200 µM of each dNTP, 1.25 µl of 12.5 pmol of each of the forward and reverse primers and 0.25 µl of 1.25 U of Taq polymerase. Triple distilled de-ionized water (TdH_2O) was added to make a 25 µl reaction.

Initial denaturation was done at 94 °C for 5 minutes followed by denaturation (for 35 cycles) at 94 °C for 30 seconds. Annealing was then performed at 55°C for 30 seconds, followed by elongation at 72°C for 1 minute and final elongation done at 72°C for 5 minutes. A known positive control from Isiolo National Game Park in Kenya courtesy of Mr. Mulinge Erasmus was included in the assay and distilled water (Qiagen™) was also included as a negative control.

This PCR procedure was designed to produce a banding pattern of 1075 base pairs when viewed at 2% ethidium-stained agarose electrophoretic gel under ultraviolet light.

The following primer sequences were used for gene amplification (Huttner *et al.*, 2008):

Table..... showing primer sequences were used for gene amplification

Forward Primer "nadA"	5' TGT TTT TGA GAT CAG TTC GGT GTG 3'
Reverse Primer "nadC"	5' CAT AAT CAA ACG GAG TAC GAT TAG 3'
Nested PCR	
Forward Primer "nadB"	5' CAG TTC GGT GTG CTT TTG GGT CTG 3'
Reverse Primer "nadD"	5' GAG TAC GAT TAG TCT CAC ACA GCA 3'

3.7 Data management and analysis

Raw questionnaire data was coded, entered into Excel software (Version 16.0); cleaned and exported to R Statistical Software program, *Version 3.0.3* (The R Core Team, 2014) for analysis and presentation.

Descriptive statistics was conducted on study variables. The relationship between the different independent and dependent (response) variables was examined at 95% confidence interval. Univariate and multivariate logistic regression analysis was used to identify possible risk factors for infection of dogs with *Echinococcus granulosus*. Logistic regression analysis for risk factor identification was done by use of R statistical analysis software program, *Version 3.0.3* (The R Core Team, 2014).

CHAPTER FOUR

RESULTS

4.1 Household demographic characteristics

Of the total number of respondents interviewed, males constituted 85% (n = 63) and 80% (n = 52), while females were 15% (n = 11) and 20% (n = 13) in Bukedea and Moroto districts respectively. No significant difference was observed in the sex distribution of respondents between Moroto and Bukedea district (p = 0.036)

The median age group of respondents was 43 years and the mean was 44.4 (95% CI, 41.7-47) years across the two districts. The modal age group was 35 to 54 years.

Most respondents (80.6%, n = 112) were peasant farmers, 6.5% (n = 9) had formal or informal jobs, 7.2% (n = 10) were involved in business, while others constituted 5.7% (n = 8). There was no significant difference (p>0.05) in occupation type by district of origin.

However, Moroto district had a higher proportion of respondents (86.5%) who were engaged in one only form of livelihood (i.e. livestock pastoralists) as opposed to Bukedea district where (62.4%) were engaged in mixed (crop/livestock) production.

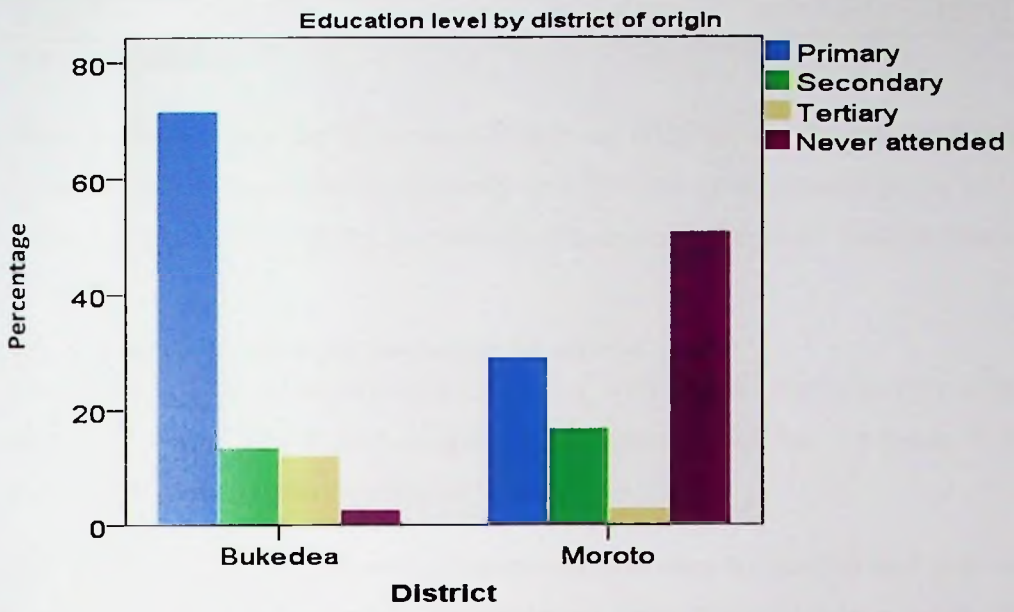


Figure 6: Percent distribution of education level of respondent by district of origin

Figure 6 shows the percent distribution of education level of respondents, which showed that Moroto district had a significantly higher proportion of respondents (50.7%, n = 33) who had never attended school compared to Bukedea district (2.7%, n = 2) at $p<0.001$. Education level was highly associated with the district of origin of respondent. Bukedea had a significantly higher education level of respondents than Moroto district at $p<0.05$. Education level was however, not found to be significantly associated with infection status of dogs ($p= 0.083$).

4.2. Number of dogs kept per household

The mean number of dogs per household were found to be 1.815 ± 1.22 and 2.419 ± 1.70 in Moroto and Bukedea district respectively. No significant association was found between the mean number of dogs per household and *E. granulosus* prevalence in dogs ($p>0.05$).

Table 1: Frequency distribution (percent) of sex of dog in households by district of origin

District	NA	Males	Females	Both male & female	Total
Bukedea	1 (1.35%)	26 (35.1%)	8 (10.8%)	39 (52.7%)	74 (52.7%)
Moroto	2 (3.0%)	31 (47.7%)	17 (26.15%)	15 (23.1%)	65 (47.3%)

Total	2 (2.2%)	40 (41.0%)	16 (18.0%)	46 (38.8%)	139 (100%)

NA – data missing

Table 1 above shows that there was a significant difference in the sex distribution of dogs in the 2 districts. Male dogs were significantly ($p<0.05$) more predominant (41%, n = 40) than females (18%, n = 16). Exactly 38.8% (n = 46) of all households kept both male and female dogs.

4.3 Cattle herd size per household by district

Cattle herd size varied significantly by district, with Moroto district having a significantly higher mean herd size of 17.6 ± 3.84 compared to Bukedea, which had a mean herd size of 4.9 ± 1.62 cattle per household interviewed ($p = 0.021$).

4.4 Prevalence of *Echinococcus granulosus* in dogs by district and sub county

A total of 180 faecal samples were collected from Moroto district, while 81 samples were collected from Bukedea district, making an overall total of 261 samples from both districts. The table below presents results of the faecal floatation procedure.

Table 2: Prevalence of taeniid infection in dogs by faecal flotation method

District	Sub county	Faecal samples collected	Total positive	% positivity	95% Confidence Interval
Moroto	Municipality	112	24	21.4	13.8 – 29.0
	Rupa	48	6	12.5	3.1 – 21.8
	Nadunget	20	2	10	3.1 – 23.1
Bukedea	Kachumbala	20	1	5	4.5 – 14.5
	Kolir	21	2	9.5	3.4 – 22.4
	Bukedea T/C	20	2	10	3.1 – 23.1
	Malera	20	2	10	3.1 – 23.1
Total		**261**	**39**	**14.9**	**10.6 – 19.2**

Table 2 above presents the overall prevalence of taeniid infection of 14.9% (39/261, CI 10.6-19.2) in both districts. In Moroto district, the prevalence was 17.8% (32/180, 95% CI 12.2-23.4), while it was 8.6% (7/81, 95% CI 2.5-14.7) in Bukedea district.

Of the studied sub counties, the highest prevalence of 21.4% (95% CI 13.8-29.0) was recorded in Moroto Municipality in Moroto district, while the lowest prevalence of 5% (95% CI 4.5-14.5) was recorded in Kachumbala Sub County in Bukedea district.

4.5 PCR Genotyping Results

The figure 7 below presents the results of a PCR genotyping procedure.

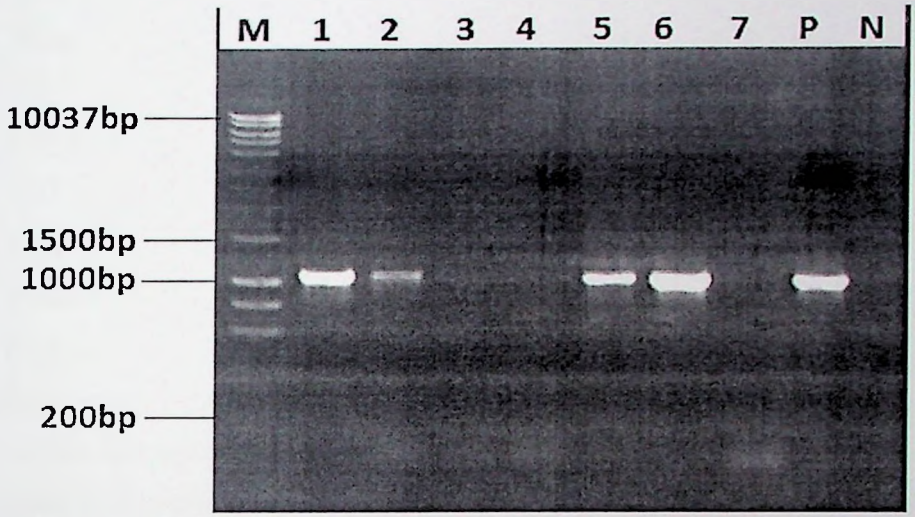


Figure 7: A 2% agarose gel showing amplification products and banding patterns of the NADH dehydrogenase subunit 1 (nad1) gene. M is the 1 Kb DNA ladder (HyperLadder^{TM}) marker; Lanes 1, 2, 5 and 6 show samples identified as *Echinococcus granulosus* (1075bp); Lanes 3, 4 and 7 were negative. P is a positive control, N is the negative control.

From the PCR genotyping results, 81.25% of the positive samples by faecal flotation from Moroto district (n = 26) and 85.7% of the samples from Bukedea district (n = 6) were identified as *Echinococcus granulosus* infection.

In Moroto district, the true prevalence of CE infection in dogs was found to be 14.4% (26/180; 9.91 - 19.0%, 95% CI) while it was 7.4% (6/81; 2.14 - 12.6%, 95% CI) in Bukedea district. Of the 39 samples that were identified as positive by the faecal floatation method in both districts, 82% (n = 32) samples were confirmed to be *Echinococcus granulosus*. Thus, overall true prevalence of *Echinococcus granulosus* in dogs in both Moroto and Bukedea districts was found to be 12.2% (n = 32; 8.6-16.7 95% CI).

Results showed Moroto had a significantly higher prevalence of CE than Bukedea district (p = 0.01137).

4.6 Risk factors for *Echinococcus granulosus* infection in dogs

4.6.1 Knowledge about cystic echinococcosis infection in dogs

When asked in a local language about knowledge of CE infection, most respondents had never heard about CE in dogs. In Bukedea district, 74.3% (n = 55) while 65% (n = 42) of the respondents in Moroto district had never heard of CE infection in dogs.

4.6.2 Dog ownership and methods used to keep dogs

Results showed that 98.6% (n = 73) and 96.9% (n = 63) of the respondents interviewed owned dogs in Bukedea and Moroto districts, respectively. As for the reasons advanced for keeping dogs, security was a more pronounced reason (82.4%, n = 61) in Bukedea and 52.3% (n= 34) in Moroto district), followed by hunting (14.8%, n = 11) in Bukedea, and 44.6% (n = 29) in Moroto district. However, the proportion of dogs used for hunting in Moroto (44.6%; n= 29) was significantly higher ($p< 0.05$) than that in Bukedea district (14.8%; n = 11).

Results showed that 85% (n = 53) and 96.9% (n = 70) of domesticated dogs were free to roam in Moroto and Bukedea districts respectively. Only 15% (n = 10) of domesticated dogs in Moroto district and 3.1% (n = 3) of domesticated dogs in Bukedea district were confined at home. In all the districts, 90% of the dogs were free to roam. Bukedea district had a significantly higher proportion of dogs that were free to roam ($p<0.05$) compared to Moroto district.

4.6.3 Removal of dog faecal matter from compounds

In Moroto district, 96.9% (n = 63) of the respondents never remove dog faeces from their compounds as a routine practice, while only 21.3% (n = 16) of respondents in Bukedea district said they never remove dog faeces from their compounds. There was a significant difference (χ^2 = 19.9, p = 0.029) in the proportion of respondents who routinely remove dog faecal material from their compounds between the 2 districts. More respondents cleaned their compounds of

faecal material in Bukedea than in Moroto district. In Bukedea district, 77% (n = 50) of the respondents said dog faeces is taken to the pit latrine or placed in a dug out pit on a nearby ground and covered with soil.

4.6.4 Treatment of dogs against worm infections

Eighty two percent (82%, n = 60) and 80% (n = 52) of the respondents in Moroto and Bukedea districts respectively, never deworm their dogs. No significant difference was observed in the proportions of dogs that were never dewormed in both districts ($p>0.05$). This had no effect on prevalence of CE in dogs.

4.6.5 Post-mortem observations of cysts in animal tissues

In Bukedea district, only 32.4% (n = 24) of the respondents had ever seen cysts in livestock carcasses, while 63% (n = 41) of respondents in Moroto district had ever seen cysts in animal tissues. Cysts were significantly ($p<0.05$) more commonly observed in Moroto than in Bukedea district. Since Moroto district had a higher prevalence of the infection, observations of cysts in livestock carcasses was significantly ($p<0.05$) associated with infection of dogs with *E. granulosus*.

4.6.6 Community knowledge and practices about tissue cysts in slaughtered animals

In Moroto district, only 40% (n = 26), while 24% (n = 18) of respondents in Bukedea district had prior information about cysts. In Karimojong language (Moroto district), cysts were called "amilil", while in Ateso language (Bukedea district), they were called "adeka na icida". However, a majority were unaware of the potential dangers of presence of cysts in animal tissues. In Moroto, 92.3% (n = 60) of the respondents and 83.8% (n = 62) in Bukedea district could either throw cysts away or feed them directly to the dogs.

4.7 Results of univariate regression analysis of risk factors for CE infection

The summary of univariate analysis of prevalence of *Echinococcus granulosus* in dogs are as shown in Table 3.

Table 3: Summary of univariate regression analysis of potential risk factors for infection of dogs with Echinococcus granulosus in Moroto and Bukedea districts

Potential risk factor	Variable category	Factor present	Factor absent	Chi-square statistic	Fishers Exact test OR (95% CI)	p-value
Origin	Moroto	14/65	51/65	6.407	3.75 (1.181-14.19)	0.01137*
	Bukedea	5/74	69/74			
Observations of cysts in animal tissues (yes)	Moroto	41/65	24/65	-	3.525 (1.671-7.62)	0.00030**
	Bukedea	24/74	50/74			
Access to slaughter facilities	Yes	15/57	42/57	-	6.865 (2.018-30.25)	0.00067**
	No	4/82	78/82			
Herd size (high		10/112	18/27	15.76	-	0.00127*
County of origin		5/75	42/52	7.5806	-	0.0226*
Meat inspection (No)		9/19	63/120	-	0.9948 (0.3316-2.94)	1.000
Method used to keep dogs (Free to roam)		1/13	105/123	0.9656	-	0.6171
Type of feed given to dogs		18/120	110/128	05187	-	0.9148

**p<0.05 statistically significant; ** p< 0.01 statistically significant*

Dogs from Moroto counties were more likely to be infected with *E. granulosus* than those in Bukedea county ($p<0.05$). This is reflected by the higher prevalence of the infection in Moroto than in Bukedea district. Dogs kept in sub counties located in the urban settings (19.6%, n=26) were significantly ($\chi^2 = 4.7499$, $p = 0.029$) likely to be positive to *E. granulosus* infection than those in the rural areas (10%, n = 13).

The difference in prevalence *E. granulosus* in dogs according to different variables (district, access to slaughter facility and herd size is as shown in Table 5.

Table 4: Prevalence of *E. granulosus* in dogs according to different variables

Variable		Infection status		χ^2	df; P-value
		Infected	Not infected		
District	Moroto	32	148	6.407	df=1; 0.01137*
	Bukedea	7	74		
Access to slaughter facility	Yes	15	42	13.09	df=1; 0.0007**
	No	4	78		
Herd size	1-16	10	102	15.76	df=3;
	17-32	5	9		0.00127**
	33-49	4	0		
	Beyond 50	0	4		

** Statistically significant at $p<0.05$; ** statistically significant at $p< 0.01$*

4.8 Multivariate regression analysis

Using univariate regression, the following potential risk factors for CE infection in dogs were identified: (1) district of origin, (2) observations of cysts in slaughtered livestock tissues, (3) access to livestock slaughter facilities, (4) herd size, (5) Sub County and (6) the county of origin of the dog. A backward stepwise approach was used in model building and the following logistic regression models were arrived at in identifying potential risk factors:

Table 5: Results of multivariate regression analysis of risk factors for CE infection in dogs

The results of multivariate logistic regression analysis were as shown in table 5 below

Reg Model 1: glm (formula = Infection.Status ~ Herd.Size + Cysts.seen + Access2slauterfac, family = binomial (logit), data = Dataset)

Coefficients	Log of Odds Ratio	Std Error	Z value	Pr (>|z|)
Intercept	2.0891	0.5117	4.08	4.5e-05***
Herd size	-0.0501	0.0175	-2.86	0.0042**
Cysts seen (yes)	-0.6951	0.5628	-1.23	0.2169
Access to slaughter facilities	2.0940	0.6465	3.24	0.0012**

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
(Dispersion parameter for binomial family taken to be 1)
AIC: 95.32
Number of Fisher Scoring iterations: 6

In this model, herd size and access of dogs to livestock slaughter facilities (Figures 9, 10 & 11) were identified as key risk factors for infection of dogs with *E. granulosus*. Observations of cysts in animal carcasses, though not found to be statistically significant in this model, are associated with possible infection.

Table 6: Results of multivariate regression analysis of risk factors for CE infection in dogs

Reg Model 2: glm (formula = Infection.Status ~ Herd.Size + Access2slauterfac + Meat.Inspection, family = binomial (logit), data = Dataset)

Coefficients	Log of Odds Ratio	Std Error	Z value	Pr (>|z|)
Intercept	2.2165	0.5191	4.27	2e-05***
Herd size	0.0598	0.0182	-3.28	0.00102**
Access to slaughter facilities	2.4018	0.6940	3.46	0.00054***
Meat inspection (yes)	-1.0193	0.6008	-1.70	0.08975

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
(Dispersion parameter for binomial family taken to be 1)

AIC: 93.91

Number of Fisher Scoring iterations: 6

In this model, herd size (OR = 1.06) and access of dogs to livestock slaughter facilities (OR = 11) were significantly ($p<0.001$) associated with infection of dogs and were therefore identified as key risk factors for infection of dogs with *E. granulosus*. The estimated parameter for meat inspection (OR = 0.36), found to be marginally significant in this model, is associated with reduced infection of dogs (protective factor) and based on biological plausibility, is therefore forced into the model.

Table 7: Results of multivariate regression analysis of risk factors for CE infection in dogs

Reg Model 3: glm(formula = Infection.Status ~ Herd.Size + Access2slauterfac + Educ, family = binomial(logit), data = Dataset)

Coefficients	Log of Odds Ratio	Std Error	Z value	Pr (>|z|)
Intercept	-2.767	1.245	-2.22	0.026 *
Cysts.seen[T.Yes]	-0.134	1.146	-0.12	0.907
Decision	0.320	0.449	0.71	0.476
Educ[Secondary]	0.175	0.787	0.22	0.825
Educ[Tertiary]	-15.056	1155.751	-0.01	0.990
Educ[Never attended]	1.227	0.608	2.02	0.044 *
Freq.purchases	-0.872	0.587	-1.49	0.137
Heard	1.149	0.724	1.59	0.113
Information about cysts	0.540	0.620	0.87	0.384

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for binomial family taken to be 1)

Null deviance: 110.897 on 138 degrees of freedom

Residual deviance: 84.685 on 133 degrees of freedom

AIC: 96.685

Number of Fisher Scoring iterations: 17

This model revealed that failure to attend any formal education was significantly associated with increased likelihood of infection of dogs ($p<0.05$). Although not found to be statistically significant ($p>0.05$), the estimated model parameter for tertiary education (OR = 2.89e-7), as opposed to those who never attended any education (OR = 3.41) revealed that tertiary education was important in preventing infection of dogs. Further analysis showed that location of the dog by district, county of origin and Sub County were confounding variables and were therefore left out of the model. Based on the Akaike information criterion (AIC), regression model 2 was found to be the best fit for risk factor identification.

CHAPTER FIVE

DISCUSSION

From the results shown above, Moroto had a significantly higher prevalence of *Echinococcus granulosus* infection (14.4%, 9.91-19, 95% CI) than Bukedea district (7.4%, 2.14-12.6, 95% CI) ($p = 0.01137$). Overall prevalence of cystic echinococcosis infection in dogs in both districts was found to be 12.2% (8.7-15.76% 95% CI). This shows that the threat of infection (pressure) for humans was relatively higher in Moroto compared to Bukedea district.

These findings on prevalence of CE infection in dogs are lower than those of Inangolet *et al.*, (2010), who found a prevalence of 24.6 % (20.1-29.5 95% CI) in partly domesticated and 32.4 % (27.5-37.7, 95% CI) in domesticated dogs in Moroto district. Due to the high cost of a Copro-PCR, I was able to test 39 samples that were found positive by the faecal floatation method. Although the specificity of the faecal floatation test was found to be relatively high (93%), the likelihood of false negatives could have altered (increased) test sensitivity and hence the final prevalence. We believe these figures could be lower than the actual situation, considering the lower sensitivity of the faecal floatation method (78%). Copro-PCR is reported to have a high sensitivity (Eckert and Deplazes, 2004) and specificity is close to 100% (Mathis and Deplazes, unpublished). The high specificity of the Copro-PCR test implied that 7 out of the 39 samples tested were likely to be true negatives.

In categorizing sub counties by location, Moroto municipality (urban setting) was found to have the highest prevalence (16.9%, 10.9 – 22.9, 95% CI), than Bukedea Town council (10%, 3.1-23.1, 95% CI). Kachumbala Sub County (rural setting) had the lowest prevalence (4.28%, 3.85 - 12.42%, 95% CI). This is probably explained by increased access of dogs to slaughter facilities in Moroto municipality, as the only slaughter slab available was always open, allowing dogs free access. These variations could also be related to the differences in farming systems and climatic conditions between Moroto and Bukedea district. Whereas a significant proportion of dogs in Moroto were always moving with the livestock herds, this was not a common feature in Bukedea district. Mean cattle herd size per household was significantly ($p<0.05$) lower in Bukedea

compared to Moroto district and that a majority of animals (cattle, sheep and goats) were tethered in Bukedea district. As a result, the rate of slaughters was likely to be lower in Bukedea district compared to Moroto and this could probably explain the lower prevalence of the infection in Bukedea compared to Moroto district.

Overall prevalence of *Echinococcus granulosus* in dogs in Moroto and Bukedea districts is comparable to that found in Ngorongoro district of Tanzania, where it was found to be 10% (Ernest *et al.,* 2004 and Magambo *et al,* 2006). In eastern Ethiopia, Mersie (1993) found that 20.5% of cattle and 22% of dogs were infected with CE. In another study by postmortem examination of adult stray dogs, a prevalence of 16.7% in Tigray region, Northern Ethiopia was recorded (Kebede *et al.*, 2009b).

In Turkana district of Kenya, Macpherson found that more than 50% of dogs were positive for *Echinococcus granulosus* when investigated by postmortem (Macpherson *et al.,* 1985). This agreed with the findings of Inangolet *et al.* (2010). Similar studies reported varying prevalence of infection in dogs from 13 % to 70 % in different regions of Turkana, Kenya (Kamiya and Gathura, 1990).

Because of higher demand, the number of animals slaughtered is generally higher in urban areas compared to rural areas. Exposure of dogs to condemned animal tissues increases with the slaughter rate and this could account for the high prevalence of the infection in urban dogs relative to rural dogs in both districts.

Most respondents in Bukedea district, 74.3% (n = 55) and 65% (n = 42) in Moroto district had never heard about cystic echinococcosis infection in dogs. Cattle herd size ($p = 0.00102$) and lack of knowledge about tissue cysts was significantly associated with increased infection of dogs. This finding agreed with an earlier study in northwestern Turkana, which found that the prevalence of cystic echinococcosis in dogs was higher in areas with high stocking densities of livestock (Macpherson *et al.*, 1985). In addition, in a recent study in Turkana district of Kenya, dogs with more possibilities of contact with domestic livestock were likely to become infected (Otero-Abad and Torgerson, 2013). This finding could be explained by increased probability of access of dogs to condemned animal tissues, increasing their chances of infection. Magambo *et*

al. (2006) also reported that an owner of a dog who lacked knowledge about cystic echinococcosis in livestock and its transmission increased the risk of coproantigen positivity in dogs.

The study revealed that Bukedea district had a significantly ($p = 0.027$) higher mean number of dogs kept per household (2.42 ± 1.7) than Moroto district (1.81 ± 1.22). As for the reasons advanced for keeping dogs, security was a more pronounced reason by 82.4% ($n = 61$) of the respondents in Bukedea than 52.3% ($n= 34$) in Moroto district. Dogs were kept to give alarms by barking whenever there was an attack by livestock raiders. The second important reason for keeping dogs was hunting, as stated by 14.8% ($n = 11$) of respondents in Bukedea, and 44.6% ($n = 29$) in Moroto district. Because hunting of wild animals was significantly ($p<0.05$) more pronounced in Moroto district than Bukedea, it could explain the increased prevalence of CE in dogs observed in Moroto district in view of the sylvatic cycle.

The high number of potential intermediate hosts (cattle, goats and sheep) in Moroto district than Bukedea district increases the likelihood of access of dogs to condemned animal tissues, leading to possible infection.

Access of dogs to livestock slaughter facilities was significantly associated ($p<0.001$) with infection of dogs (OR = 11). Dogs which had access to livestock slaughter facilities were more likely to be infected with *E. granulosus* than those which did not ($p<0.05$). In both districts, the higher proportion of roaming dogs (about 90%) that have access to slaughter facilities could explain this finding. This finding agreed with previous study by Otero-Abad and Torgerson (2013) which found that dogs fed on condemned viscera or raw offal were more likely to be coproantigen positive for *E. granulosus*. This also agrees with previous studies by Buishi *et al.*, 2006, which revealed that dogs that had access to condemned offal were 12 times more likely to be infected with *E. granulosus*. A previous study in Moroto district by Inangolet *et al.* (2010) also revealed a higher prevalence of CE infection in stray dogs than in domesticated dogs. This was attributed to indiscriminate disposal of offal that is unfit for human consumption, to which stray dogs could easily get access. In this study, evidence of lack of control of dogs to available slaughter facilities was obtained (See appendices 8-10), as observed in Moroto municipality. Because the gates were always open, dogs were able to gain unrestricted entry to the slaughter

slab, from where they would likely consume condemned animal tissues. This was therefore a major risk factor for infection of dogs with *E. granulosus*.

As regards hygiene practices in relation to CE, 98.6% (n = 73) of the respondents in Moroto district and only 23% (n = 15) of the respondents in Bukedea never remove dog faecal matter from their compounds as a routine practice. This practice could increase the likelihood of pasture contamination with the tapeworm eggs increasing the risk of infection for domestic livestock species and humans.

Observations of cysts in animal tissues by respondents were highly associated with the district. Hydatid cysts were significantly more likely to be seen in livestock slaughtered in Moroto than in Bukedea district (at $p<0.05$). This correlates well with the fact that in Bukedea district, 74.3% (n = 55) and 65% (n = 42) of the respondents Moroto district had never heard of CE in dogs. As to what respondents did with the cysts or condemned tissues / organs, most respondents in Moroto (92.3%, n = 60) and 83.8% (n = 62) in Bukedea district threw them away or fed them directly to dogs. Since Moroto district had a higher prevalence of the infection, observations of cysts in animal carcasses was significantly ($p<0.05$) associated with infection of dogs with *E. granulosus*. In Moroto district, the fact that 92.3% (n = 60) of the respondents and 83.8% (n = 62) in Bukedea district either threw cysts or condemned tissues away or fed them directly to the dogs could perpetuate further transmission of the parasite. This reveals a knowledge gap in regard to the transmission of echinococcosis to dogs.

Although not found to be significant ($p>0.05$), keeping of dogs as domestic pets appeared to protect dogs from possible infection. As opposed to keeping dogs for security and hunting purposes, households that kept dogs as pets tended to pay more attention to preventive healthcare practices such as routine deworming and vaccination. They are also more likely to confine their dogs at home rather than leave them to roam in the neighborhoods, which exposes them to scavenging on leftover animal tissues.

Meat inspection was found to be marginally associated with reduced infection of dogs ($p<0.05$) in both districts. However, in the final regression model, the estimated model parameter (OR = 0.36) showed that meat inspection was important in preventing infection dogs, which agrees with

previous studies (Kamiya and Gathura, 1990), which showed meat inspection significantly reduced the risk of dog infection with CE. This finding is similar to a regression model (not shown here), in which estimated parameters for meat inspection and frequency of inspection (OR = 0.66) and 0.72 respectively) showed protective effects against possible infection of dogs. This conforms to internationally established methods of containing the spread of the disease (WHO / DFID / FAO, 2005).

In a third regression model, education level (never attended school) was found to be significantly associated with infection of dogs ($p<0.05$). The estimated parameter for respondents who had never attended school (OR = 3.41) was found to be a risk factor for infection of dogs, whereas the estimated parameter for tertiary education (OR = 2.89e-07) showed a protective effect on infection status. Thus, dogs owned by respondents who had never attended any formal education were 3.4 times more likely to be infected than those who had attended school. Households that acquired tertiary education were probably more aware of the risks of zoonotic disease from animals and were likely to practice certain preventive measures such as deworming. This model revealed that tertiary education was important in preventing or reducing the risk of possible infection of dogs.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

i. Moroto district had a significantly higher prevalence of infection (14.4%; 26/180) of dogs with *Echinococcus granulosus* than Bukedea (7.4%; 6/81) district. The traditional system of livestock rearing (nomadic pastoralism) coupled with the practice of allowing dogs to freely roam with the livestock herds increases the risks of pasture contamination, increasing the risks of infection for the domestic animal species. The large livestock numbers in Moroto district may probably help to maintain the high endemicity of the parasite.

ii. The major risk factors for transmission of *E. granulosus* in dogs include easy access of dogs to livestock slaughter facilities; higher cattle herd sizes and lack of or limited knowledge about the disease and its transmission dynamics. This study revealed that meat inspection is important in preventing possible infection of dogs with the parasite.

6.2 Recommendations

i) In order to contain the spread of this disease, livestock slaughtering facilities should be strictly controlled to prevent dogs from gaining access to them. Meat inspection regulations should be enforced to limit access of dogs to condemned animal tissues, which may perpetuate the parasite.

ii) Public health education is crucial to improve understanding of the parasite's transmission dynamics. Efforts to create public awareness should be made on the high risk practices, such as allowing dogs to feed on waste tissues that may help to spread the disease in animals as well as in humans. This could help to reduce the prevalence of the infection.

iii) A program to ensure regular deworming of dogs in communities should be designed and implemented to minimize infection rate.

iv) Further studies are justified on the epidemiology and economic impact of cystic echinococcosis in the region, which would be important in the formulation of appropriate control measures.

REFERENCES

Allan, J., Craig, P., Garcia, N., Mencos, F., Liu, D. and Wang, Y. (1992). Coproantigen detection for immunodiagnosis of echinococcosis and taeniasis in dogs and humans. *Parasitology,* 104: 347-355.

Budke, C. M., Deplazes, P. and Torgerson, P.R. (2006). Global socio-economic impact of cystic echinococcosis. *Emerging Infectious Diseases. Pubmed* 12: 296-303.

Budke, C. M., White, Jr. A. C. and Garcia, H. H. (2009). Zoonotic Larval Cestode Infections: Neglected, Neglected Tropical Diseases? *PLos Neglected Tropical Diseases* 3: e319. doi: 10.1371/journal.pntd.0000319

Buishi, I., Njoroge, E., Zeyhle, E., Rogan, M.T. and Craig P.S. (2006). Canine echinococcosis in Turkana (north-western Kenya): a copro-antigen survey in the previous hydatid-control area and an analysis of risk factors. *Ann. Trop. Med. Parasitol.* 100: 601-610.

Butler, J. R. A. and Bingham, J. (2000). Demography and dog-human relationships of the dog population in Zimbabwean communal lands. *Veterinary Record,* 147: 442–446. [HYPERLINK "http://europepmc.org/abstract/MED/11079440" \t "pmc_ext" PubMed]

Cardena, G. A. and Carmena, D. (2013). A review of the global prevalence, molecular epidemiology and economics of cystic echinococcosis in production animals. *Veterinary Parasitology,* 192: 10-32. doi: http://dx.doi.org/10.1016/j.vetpar.2012.09.027

CDC (2010). Neglected Tropical Diseases. Centers for Disease Prevention and Control, Atlanta, Georgia, USA. http://www.dpd.cdc.gov/dpdx/HTML/Echinococcosis.htm Accessed on 16th January 2015.

CDC (2011). Neglected Tropical Diseases. Centers for Disease Prevention and Control, Atlanta, Georgia, 1600 Clifton Rd. Atlanta, GA 30329-4027, USA. http://www.dpd.cdc.gov/dpdx/HTML/Echinococcosis.htm Accessed on 16th January 2015.

CDC (2012). Neglected Tropical Diseases. Centers for Disease Prevention and Control, Atlanta, Georgia, 1600 Clifton Rd. Atlanta, GA 30329-4027, CDC website: www.cdc.gov/parasites/echinococcosis; http://www.dpd.cdc.gov/dpdx/HTML/Echinococcosis.htm Accessed on 8th July 2014

Craig, P. (1997). Immunodiagnosis of *Echinococcus granulosus* and a comparison of techniques for diagnosis of canine echinococcosis. In F. Andersen, H. Ouhelli & M. Kachani (Eds.), *Compendium on cystic echinococcosis in Africa and in the Middle Eastern countries with special reference to Morocco* (pp. 85-118). Provo, Utah: Bringham Young University.

Christofi, G., Deplazes, P., Christofi, N., Tanner, I., Economides, P., Eckert J., (2002). Screening of dogs for *Echinococcus granulosus* coproantigen in a low endemic situation in Cyprus. *Vet Parasitol.* 104: 299-306.

Dakkak, A. (2010). Echinococcosis / hydatidosis: A severe threat in the Mediterranean Countries. *Veterinary Parasitology*, 174: 2-11

Deplazes, P. and Eckert, J. (2001). Veterinary aspects of alveolar echinococcosis — a zoonosis of public health significance. *Veterinary Parasitology,* 98: 65-87. doi: http://dx.doi.org/10.1016/S0304-4017(01)00424-1

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 the *Echinococcus granulosus*-complex, with reference to the epidemiological situation in eastern Africa. *Int J Parasitol, 34*(5), 645-653. doi: 10.1016/j.ijpara.2003.12.013

Eckert, J. and Deplazes, P. (2004). Biological, Epidemiological, and Clinical Aspects of Echinococcosis, a Zoonosis of Increasing Concern. *Clinical Microbiology Reviews,* 17: 107-135.

Eckert, J., Conraths, F. J., and Tackmann, K. (2000). Echinococcosis: an emerging or re-emerging zoonosis? *Int J Parasitol,* 30: 1283-1294.

Eckert, J., Gemmell, M.A., Meslin F. X., Pawlowski Z.S (2010). *WHO/OIE Manual on Echinococcosis in Humans and Animals: A Public Health Problem of Global Concern.* Paris, France: World Organization of Animal Health. pp. 267.

Eckert, J., Gottstein, B., Heath, D. and Liu, F.-J. (2001). Prevention of echinococcosis in humans and safety precautions. *in* J. Eckert, M. Gemmell, F. Meslin & Z. Pawlowski (Eds.), *WHO/OIE manual on echinococcosis in humans and animals: a public health problem of global concern* (pp. 238-247). Paris, France: World Organization for Animal Health.

Ernest, E., Nonga, H., Kassuku, A. and Kazwala, R. (2009). Hydatidosis of slaughtered animals in Ngorongoro district of Arusha region, Tanzania. *Trop Anim Health Prod,* 41 (7): 1179-1185.

Ernest, E., Kassuku, A. and Kazwala, R. (2004). Studies on the epidemiology of echinococcosis / hydatidosis in Ngorongoro district, Arusha, Tanzania. *Int Arch Hydatid;* 35:43

FAO (2011a). *Echinococcosis.* FAO Statistical Databases. Available from http://www.faostat.fao.org/ Accessed on 16th November, 2014.

FAO (2011b). Food and Agriculture Organization of the United Nations. *Manual for Recognition of Exotic Diseases of Livestock.* Rome. Available from http://www.spc.int/rabs/

FAOSTATS (2010). FAO Statistical Databases. Available from http://faostats.fao.org.

French, C., Nelson, G. and Wood, M. (1982). Hydatid disease in the Turkana District of Kenya. I. The background to the problem with hypotheses to account for the remarkably high prevalence of the disease in man. *Ann Trop Med Parasitol,* 76: 425-437.

Gathura, P.B. and Kamiya, M. (1990). Echinococcosis in Kenya: Transmission, characteristics, incidence and control measures. *Japanese Journal of Veterinary Research,* 38: 107-116.

Gatti, A. (2007). Ovine echinococcosis. Immunological diagnosis by enzyme immunoassay. *Veterinary Parasitology,* 12: 112-121.

Gemmell, M. A., Roberts, M. G., Beard, T. C., Diaz, S. C., Lawson, J. R. and Nonnemaker, J. M. (2001). *WHO/OIE manual on echinococcosis in humans and animals: a public health problem of global concern.* Paris, France: WHO/OIE. *pp.* 267.

Hotez P. J, Alvarado M, Basáñez M. G, Bolliger I, Bourne R, Boussinesq M, *et al.* (2014). The Global Burden of Disease Study 2010: Interpretation and Implications for the Neglected Tropical Diseases. *PLoS Negl Trop Dis* 8(7): e2865. doi:10.1371/journal.pntd.0002865

Huttner, M., Nakao, M., Wassermann, T., Siefert, L., Boomker, J. D. F., Dinkel, A., Sako, Y., Mackenstedt, U., Romig, T., Ito, A., (2008). Genetic characterization and phylogenetic position of *Echinococcus felidis* (Cestoda: Taeniidae) from the African lion. *International Journal of Parasitology* 38: 861-868. doi: 10.1016/j.ijpara.2007.10.013

Huttner, M., Siefert, L., Mackenstedt, U. and Romig, T. (2009). A survey of *Echinococcus* species in wild carnivores and livestock in East Africa. *International Journal of Parasitology*. 39: 1269-1276 doi: 10.1016/j.ijpara.2009.02.015.

Huttner, M. and Romig, T. (2009). *Echinococcus species* in African wildlife. *Parasitology* 10: (1): 1089-1095.

Inangolet, F. O., Biffa, D., Opuda-Asibo, J., Oloya, J. and Skjerve, E. (2010). Distribution and intensity of *Echinococcus granulosus* infections in dogs in Moroto district, Uganda. *Tropical Animal Health and Production,* 42: 1451-1457.

Kahn, C. M.. and Line, S. (2010). *The Merck Veterinary Manual.* Whitehouse Station, New Jersey, USA: Merck and Company. Tenth ed., pp 2945.

Kebede, W. (2009). Echinococccosis / hydatidosis: its prevalence, economic and public health significance in Tigray region, North Ethiopia. *Tropical Animal Health and Production* 6: 865-71.

Kern, P. (2013). Current status of cystic echinococcosis: a global perspective. *A paper presented at the Cystic echinococcosis in Sub-Saharan Africa Research Initiative (CESSARi) Symposium held at Imperial Royale Hotel in Kampala Uganda on 6th May, 2013.*

Lightowlers, M.W. (2006a). Vaccines against cysticercosis and hydatidosis: Foundations in taeniid cestode immunology. *Parasitology International,* **55:** S39-S43

Lightowlers, M.W. (2006b). Cestode vaccines: origins, current status and future prospects. *Parasitology*, 133: S27-S42. Doi 10.1017/S003118200600179X.

MAAIF (2010) Agriculture Sector Development Strategy and Investment Plan, *Ministry of Agriculture, Animal Industry and Fisheries, Entebbe, Uganda.* pp. 160.

Macpherson CNL, Siefert L, Francis B, Kabuusu R, Butler B, Bakamanume B. (2004). Human cystic echinococcosis in Uganda. *Int Arch Hydatid*, **35:** 42

Macpherson, C., Karstad, L., Stevenson, P. and Arundel, J. (1983). Hydatid disease in Turkana district of Kenya III. The significance of wild animals in the transmission of *Echinococcus granulosus*, with particular reference to Turkana and Masailand in Kenya. *Ann Trop Med Parasitol,* 77:66-68.

Macpherson, C., French, C., Stevenson, P., Karstad, L. and Arundel, J. (1985). Hydatid disease in the Turkana district of Kenya IV. The prevalence of *Echinococcus granulosus* infection in dogs and observations on the role of the dog in the life style of the Turkana. *Ann Trop Med Parasitol,* 79: 51-61.

Macpherson, C. N. L. (1985). Epidemiology of hydatid disease in Kenya: a study of the domesticated intermediate hosts in Masailand. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 79: 209-217.

Macpherson, C. N. L. (2005). Human behavior and the epidemiology of parasitic zoonoses. *International Journal of Parasitology,* 35: 1319-1331.

Macpherson, C.N.L., Craig, P.S., Romig T., Zeyhle, E. and Watschinger, H. (1989). Observations on human echinococcosis (hydatidosis) and evaluation of transmission factors in the Maasai of northern Tanzania. *Ann. Trop. Med. Parasitol.* **83:** 489-497

Macpherson, C.N.L. and Wachira, T. (1997). Cystic echinococcosis in Africa South of the Sahara. *In: F. Andersen, H. Ouhelli, & M. Kachani (Eds.), Compendium of Cystic Echinococcosis in Africa and in Middle Eastern Countries with Special Reference to Morocco.* 245-277. Brigham Young University, Provo.

Magambo, J., Njoroge, E. and Zeyhle, E. (2006). Epidemiology and control of echinococcosis in sub-Saharan Africa. *Parasitology International,* 55:S193-S195 doi:10.1016/j.parint.2005.11. 029

Mathis, A., Deplazes, P. and Eckert, J. (1996). An improved test system for PCR-based specific detection of *Echinococcus multilocularis* eggs. *J. Helminthol.* 70:219-222.

Mersie, A. (1993). Survey of echinococcosis in eastern Ethiopia. *Vet Parasitol.* 47:161-163.

Moro, P. L., Gonzalez, A. and Gilman, R. H. (2000). *Hunter's Tropical Medicine* (8 ed.): WB Saunders Company.

Moro, P. L., Lopera, L., Bonifacio, N., Gonzales, A., Gilman, R. H. and Moro, M. H. (2005). Risk factors for canine echinococcosis in an endemic area of Peru. *Veterinary Parasitology,* 130: 99-104. doi: 10.1016/j.vetpar.2005.03.015

Murray, C. J. L. and Lopez, A. D. (1997). Alternative projections of mortality and disability by cause 1990–2020: Global Burden of Disease Study. *The Lancet,* 349: 1498-1504. doi: http://dx.doi.org/10.1016/S0140-6736(96)07492-2

Njoroge, E. M., Mbithi, P. M. F., Gathuma, J. M., Wachira, T. M., Gathura, P. B., Magambo, J. K., E., Zeyhle (2002). A study of cystic echinococcosis in slaughter animals in three selected areas of northern Turkana, Kenya. *Veterinary Parasitology,* 104: 85-91. doi: http://dx.doi.org/10.1016/S0304-4017(01)00614-8

Nyakarahuka, L., Ejobi, F., Lalobo, M.O., Kankya C., Siefert, L. and Ocaido, M. (2013). A survey of potential risk factors associated with cystic echinococcosis in pastoral communities in Kasese district, Uganda. *Advance Tropical Medicine and Public Health International*, 3: 10-24.

OIE (2008). OIE Manual of Diagnostic Tests and Vaccines for Terrestial Animals (online). Paris OIE, 2008. Echinococcosis/hydatidosis. Available at http://www.oie.int/eng/normes/mmanual/2008/pdf/2.01.04_ECHINOCOCCOSIS.pdf. *Accessed 15 Sept 2009.*

Otero-Abad, B. and Torgerson, P.R. (2013). A Systematic Review of the Epidemiology of Echinococcosis in Domestic and Wild Animals. *PLoS Negl Trop Dis* 7: e2249. doi:10.1371/journal.pntd.0002249

Romig, T., Omer, R. A., Zeyhle, E., Huttner, M., Dinkel, A., Siefert, L., Elmahdi, I. E., Magambo, J., Ocaido, M., Menezes, C.N., Ahmed, M.E., Mbae, C., Grobusch, M.P. and Kern, P. (2011). Echinococcosis in sub-Saharan Africa: emerging complexity. *Veterinary Parasitology*, 181: 43-47.

Romig, T., Dinkel, A., and Mackenstedt, U. (2006). The present situation of echinococcosis in Europe. *Parasitol. Int.* 55 (Suppl): S187-S191.

R Core Team (2014). R: A language and environment for statistical computing. *R Foundation for Statistical Computing, Vienna, Austria.* ISBN 3-900051-07-0 URL http://www.R-project.org/.

Scala, A., Garippa, G., Varcasia, A., Tranquillo, V. and Genchi, C. (2006). Cystic echinococcosis in slaughtered sheep in Sardinia (Italy). *Vet. Parasitol., 15*(1), 33-38.

Schwabe, C. (1986). Current status of hydatid disease: a zoonosis of increasing importance. London: Allen and Unwin.

Shehabi, F. E., Kamhawi, S., Schantz, P., Craig, P. and Hafez, S. A. (2000). Diagnosis of canine echinococcosis: comparison of coproantigen detection with necropsy in stray dogs and red foxes from Northern Jordan. *Parasitology,* 7:83-90.

Thevenet, P. S., Jensen, O., Drut, R., Cerrone, G. E., Grenovero, M. S., Alvarez, H. M., Targovnik, H. M., and Basualdo, J. A (2005). Viability and infectiousness of eggs of *Echinococcus granulosus* aged under natural conditions of inferior arid climate. *Veterinary Parasitology,* 133: 71-77. doi: 10.1016/j.vetpar.2005.05.048

Thompson, R. and McManus, D. (2002). Towards a taxonomic revision of the genus Echinococcus. *Trends in Parasitology,* 18:542-547.

Thrusfield, M. V., (2005). Veterinary Epidemiology, *3rd ed. Blackwell Publishing.* ISBN 13:978-1-405-15627-1

Torgerson, P., Pilkington, J., Gulland, F. and Gemmell, M. (1995). Further evidence for the long dispersal of taeniid eggs. *Int. J. Parasitol.,* 25:265-267.

Torgerson P.R., (2003). Economic effects of echinococcosis. *Acta Tropica,* 85:113-118.

Torgerson, P.R. and Budke, C.M. (2003). Echinococcosis- an international public health challenge. *Research in Veterinary Science,* 74:191-202. doi: 10.1016/S0034-5288(03)00006-7.

Torgerson, P. and Craig, P. (2011). Updated global burden of cystic and alveolar echinococcosis. *Report of the WHO Informal Working Group on cystic and alveolar echinococcosis surveillance, prevention and control, with the participation of the Food and Agriculture Organization of the United Nations and the World Organisation for Animal Health*, 22-23.

Torgerson, P.R., and Deplazes, P., 2009. Echinococcosis: diagnosis and diagnostic interpretation in population studies. *Trends in Parasitology,* 25:164-170.

UBOS. (2002). The 2002 Uganda Population and Housing Census, Population Size and Distribution. October 2006, Kampala, Uganda (Statistics) *Analytical Report* (pp. 72). Kampala: Uganda Bureau of Statistics.

UNDP (2007). Human Development Report 2007/2008. Fighting Climate Change: Human solidarity in a divided world. United Nations Development Programme. pp. 162

Wachira, T., Macpherson, C. and Gathuma, J. (1991). Release and survival of *Echinococcus* eggs in different environments in Turkana, and their possible impact on the incidence of hydatidosis in man and livestock. *J. Helminthol.,* 65: 55-61.

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

Wahlers, K., Menezes, C. N., Wong, M.L., Zeyhle, E., Ahmed, M. E., Ocaido, M., Stijnis, C., Romig, T., Kern, P., Grobusch, M.P. (2012). Cystic echinococcosis in sub-Saharan Africa. *Lancet Infect Dis.* 12:871-880. doi: 10.1016/S1473-3099(12)70155-X

Walters, T. M. and Clarkson, M. J. (1980). The prevalence of *Echinococcus granulosus* in farm dogs in Mid-Wales. *Veterinary Parasitology,* 7: 185-199.

WHO / DFID / FAO (2005). The control of neglected zoonotic diseases. A route to poverty alleviation. *Report of a Joint WHO/DFID-AHP Meeting with the participation of FAO and OIE.* Geneva: World Health Organization. pp 65.

WHO (2014). Echinococcosis, Fact Sheets. Available from www.who.int/mediacentre/factsheets/.../e *Accessed 10th December 2014*

WHO (2010). Working to overcome the global impact of neglected tropical diseases. First WHO report on neglected tropical diseases. In: *D. W. T. Crompton & P. Peters (Eds.).* Geneva, Switzerland. Pp 184.

WHO/DFID/FAO. (2005). The Control of Emerging Neglected Diseases.WHO, Geneva, Switzerland. pp 65.

WHO/FAO/OIE (2011). Report of the WHO Informal Working Group on Cystic Echinococcosis and Alveolar Echinococcosis Surveillance, Prevention and Control, with the Participation of the Food and Agriculture Organization of the United Nations and the World Organization for Animal Health. Geneva, Switzerland: WHO. pp 26.

WHO/OIE (2001). WHO/OIE Manual on Echinococcosis in humans and animals. Geneva, Switzerland. pp 267.

APPENDICES

Appendix 1: LETTER OF INTRODUCTION

MAKERERE
P.O Box 7062, Kampala Uganda
TELEGRAM: Makunika

UNIVERSITY

Telephone: 256-414-534061 / 533402
Fax: 256-414-554685 / 533402
Email: warm@vetmed.mak.ac.ug

College of Veterinary Medicine, Animal Resources and Biosecurity

Dept. of Wildlife and Aquatic Animal Resources

Our ref: Your ref: Date: 1st March, 2013

TO WHOM IT MAY CONCERN

Dear Sir / Madam,

RE: Introduction of Dr. PETER OBA

This is to introduce to you Dr Peter Oba who is doing research on "Prevalence and risk factors of *Echinococcus granulosus* infection in dogs in pastoral and agro-pastoral areas in Uganda". He is doing this work on behalf of Cystic Echinococcosis project in Uganda.

Please accord him the necessary assistance.

Yours sincerely,

Professor Michael OCAIDO,
Ugandan Node Coordinator Echinococcosis Project
HEAD OF DEPARTMENT
Phone: +256-772-506104
Email: mocaido@vetmed.mak.ac.ug

Appendix 2: QUESTIONNAIRE FOR HOUSEHOLD SURVEY

RISK FACTORS FOR *ECHINOCOCCUS GRANULOSUS* INFECTION IN DOGS

Date.....

Dear respondent,

You have been selected to provide important information in order to identify risk factors for the transmission of the dog tape worm, *Echinococcus granulosus* among dogs. This tape worm infection is zoonotic in nature i.e. it can be transmitted to humans and also contributes significant economic losses to livestock farmers in terms of organ or tissue condemnations in animals.

The information you provide will therefore be used to design appropriate preventive and control measures for this disease. This information will be for entirely academic purposes and will be kept with utmost confidentiality. We shall examine your dogs to establish infection status and the procedure will not harm your dog(s). This survey is entirely voluntary.

Thanking you for participating in this survey.

Name of researcher: Peter Oba Sign.....

Instructions for filling the questionnaire

- Fill in the details as required. Where applicable, simply mark with a tick or circle accordingly

a) Household characteristics

i) District..... County..... Sub county.....
Parish..... Village..... GPS location.....

ii) Name of household head..... Sex: M/ F..... Age .....yrs

iii) Occupation: 1= Farmer..... 2= Formal job..... 3= Business.....
4= Others..... (specify)

iv) Education level: 1= Primary..... 2= Secondary .....3 =Tertiary 4 = Other.....(none)

v) Religion: 1= Catholic 2= Protestant 3= Pentecostal 4= Muslim 5= Other.....

b) Livestock production system used:

i) No of animals kept: cattle..... Goats..... sheep..... others.....

ii) Cattle: 1= Communal grazing 2= tethering 3 = zero grazing 4 = Others

iii) Goats: 1= Communal grazing 2= tethering 3 = Left to stray 4 = Others.....

Sheep: 1= Communal grazing 2= tethering 3 = Left to stray 4 = Others.....

c) Dog ownership:

vi) Do you own dogs 1 = yes 2 = no

i) No. of dogs owned: 1 = one 2= two 3= three 4= four 5 = >5

ii) Which breed of dog(s) do you keep?

1= Local breed..... How many..... Age(s).....

2= Exotic breed(s).....(specify breed) How many.....

Age(s).....

iii) Sex of dog kept: 1= male Number..... 2= female Number.....

iv) Reasons for keeping dogs: 1= for security 2= for hunting 3 = as pets

4= Others.....(specify)

v) How do you keep your dogs? 1= Confined or restrained at home.....

2= Left to stray.....

If confined at home, where is it housed? 1= kennel 2= other.....

vi) What do you feed your dog(s) on ? 1= kitchen leftovers..... 2= purchase food from market / town 3= feeds on its own..... 4= Other specify).....

vii) Sources of dog food: 1= given raw food 2= cooked food 3= other.....

viii) Where is the food placed before being fed to the dog? 1= plate 2= on ground 3= other.....

ix) How do you dispose off dog faeces? 1= Deep burial on ground..... 2= latrine ..... 3= Others .....(specify)

x) How often do you dispose off dog faeces? 1= daily 2= weekly 3= not at all

xi) How long have you kept dogs? 1= Less than 2 years 2= Between 3 to 5 years 3= More than 5 yrs

xii) Are your dogs usually treated? 1= yes 2= no

xiii) If yes, against what disease? .....

xiv) Name of drug(s) used.....(describe)

xv) Have dogs suffered any disease here before? 1=yes 2= no

xvi) If yes, what were the signs.....

xvii) How was it managed? .....

xviii) History of vaccination: Type/ name of vaccine..... When given.....

d) Marketing of livestock and livestock products

i) Do you sell your animals? Yes..... No.....

ii) If yes, where do you sell them? At home..... Market ..... Exchanged for other goods.....

iii) What is the distance to the nearest market / trading centre? Less than 2 km..... Between 3 to 5 km..... More than 5 km.....

iv) Have you seen cysts in meat? 1= yes 2= no **(show photo of cysts)**

v) If yes, what do you do with them? 1= cook and eat 2= thrown away 3= give to dogs

4= other ..... (specify)

vi) How do you call cysts in a local language? .....

vii) Do you believe cysts can cause disease in dogs? 1= yes 2=no

viii) Do you know if cysts are harmful?. 1 =yes 2 =no

e) How do you obtain animal products (meat)?

i) 1= Home slaughter .....2= Purchase from markets / town / trading centre.....

3= Others.....(specify)

ii) If by home slaughter, how often?: 1= Regularly.....(weekly 2= Rarely.....

iii) Type of meat purchased: 1= cattle..... 2= Goats..... 3=Sheep.....

4 =Poultry..... 5= Others..... (specify)

iv) If by purchase from towns/ markets, how often do you buy meat? 1= Regularly.....(weekly) 2= Rarely..... 3= Never.....

v) Is the meat inspected? 1= Yes..... 2= No.....

vi) If yes, how often is the meat inspected? 1=Daily.....2= Once or twice a week..... 3= Rarely..... 4 =Never.....

vii) If yes, by whom? 1= Veterinary officer.....2= Veterinary assistant / community animal health worker..... 3 = Other.....

f) Availability of abattoirs or slaughter slabs (Tick one or more where applicable)

i) Where are animals in your area slaughtered? 1=Abattoir..... 2= Slaughter slab..... 3= Under trees..... 4= At home.....

ii) Access to livestock slaughter facilities: 1= Yes, 2= No

g) Knowledge and attitudes towards dog tapeworms

i) Have you heard any information about dog tape worms? 1=Yes..... 2= No.....

ii) If yes, where did you get that information? 1= From community.....2=From an extension worker..... 3 = From school.....4= from media 5 = Others .....

iii) Do you know how dogs or other animals get infected with tape worms? 1= Yes..... 2= No.....

If yes, how? ..... (Explain)

iv) What is the effect of tape worms on dogs?

.....

v) Do you have any idea of how to prevent tape worms?

.....

.....

vi) Any comments / suggestions?.....

End and Thank you for your participation

Appendix 3: QUESTIONNAIRE FOR ABATTOIR AND SLAUGHTER SLAB WORKERS

RISK FACTORS FOR *ECHINOCOCCUS GRANULOSUS* INFECTION IN DOGS

Date.....

Dear respondent,

You have been selected to provide important information in order to identify risk factors for the transmission of the dog tape worm, *Echinococcus granulosus* among dogs. The information you provide will therefore be used to design appropriate preventive and control measures for this disease. This information will be used for entirely academic purposes and will be kept with utmost confidentiality.

Thanking you for participating in this survey.

Name of Researcher: Peter Oba Sign.....

Instructions for filling the questionnaire

Fill in the details as required. Where applicable, simply mark with a tick or circle accordingly

a) **Slaughter facilities:**

vii) Type of slaughter facility: 1= abattoir 2 = slaughter slab 3= under tree

viii) Name of facility: .....

b) **Location details**

District..... County..... Sub county.....

Parish..... Village..... GPS location.....

i) Ownership: 1 = Govt 2 = private

ii) If at abattoir or slaughter slab, is it fenced or in open places? 1 = fenced 2= Not fenced

iii) How many are they in the sub county? 1= Publically owned..... 2= Privately owned.....

iv) Is the meat inspected? 1= Yes 2 = No

v) If yes, who does the inspection? 1 = Vet officer 2= AHO / CAHW 3= Other.....

i) How often is the meat inspected? 1= daily 2= once or twice per week 3= rarely 4= never

ii) Do you have knowledge of cysts in meat? 1 = yes 2 = no

iii) If yes, have you come across tissue cysts in meat? 1=yes 2 = No

iv) What type of meat are cysts most common? 1 = cattle 2 = goats 3= sheep 4 = other.....

vi) What do you do with affected tissues? 1 = sold to public 2= bury on ground 3= throw away 4 = give to dogs 5 = other ..... (specify)

vii) How do you call cysts in a local language?.....

viii) Do you believe they are harmful? 1 = yes 2 = No

ix) Do you know that cysts can be transmitted to humans? 1 = Yes 2 = No

x) If meat inspection is not done, why? .....

.....

xi) Any relevant information or comments you would give.....

.....

.....

End and thanks for your time.

Appendix 4: QUESTIONNAIRE FOR VETS AND ANIMAL HEALTH WORKERS

RISK FACTORS FOR *ECHINOCOCCUS GRANULOSUS* INFECTION IN DOGS

Date.....

Dear respondent,

You have been selected to provide important information in order to identify risk factors for the transmission of the dog tape worm, *Echinococcus granulosus* among dogs. The information you provide will therefore be used to design appropriate preventive and control measures for this disease. This information will be for entirely academic purposes and will be kept with utmost confidentiality.

Thanking you for participating in this survey.

Name of Researcher: Peter Oba Sign.....

Instructions for filling the questionnaire

Fill in the details as required. Where applicable, simply mark with a tick or circle accordingly

h) Location details

ix) District..... County..... Sub county.....
Parish..... Village..... GPS location.....

x) Area / Sub county you are in charge.....

xi) Estimated dog population.....

xii) Importance attached to dogs in the area: Please rank in order of importance, 1 = most important , 4 = least important

- for hunting
- security
- as household pets
- Others..... (specify)

xiii) If for hunting, what animals are usually or commonly hunted? (list in order of importance)

.....

xiv) How do you rate cystic echinococcosis as a disease in this area? 1= very important 2= important 3= less important 4= not important

i) Disease control and prevention in dogs

v) What dog diseases do you most frequently encounter in this area?.....
.....
.....

vi) How do you manage them?.....
.....

vii) Vaccination history: have you done any vaccination of dogs before? 1= yes 2= No
viii) If yes, when was the last vaccination held?.....
ix) Type / name of the vaccine.....
x) Do you conduct treatment(s) of dogs? 1= yes 2= No
xi) How often? 1= monthly 2 = quarterly 3= annually 4 = never
xii) If yes, against what disease? .....
.....
xiii) What drug(s) are used? .....

j) Meat inspection

xii) Where are livestock mainly slaughtered in this area? 1= abattoir 2= slaughter slabs 3= under trees 4= other.....(specify)
xiii) If at abattoirs or slaughter slabs, are they fenced or in open places? 1 = fenced 2= Not fenced
xiv) How many are they? Public owned..... Private owned.....
xv) Is the meat inspected? 1= Yes 2 = No
xvi) If yes, who does the inspection? 1 = Vet officer 2= AHO / CAHW 3= Other.....
xiv) How often is the meat inspected? 1= daily 2= once or twice per week 3= rarely 4= never
xv) Do you have knowledge of cysts in domestic animals? 1 = yes 2 = no
xvi) If yes, have you come across tissue cysts in animals? 1=yes 2 =No
xvii) What do you do with affected tissues? 1 = bury on ground 2= throw away 3 = given to dogs 4 = other ..... (specify)

xviii) If meat inspection is not done, why? .....
.....
xix) Any relevant information or comments you would give.....
.....
.....

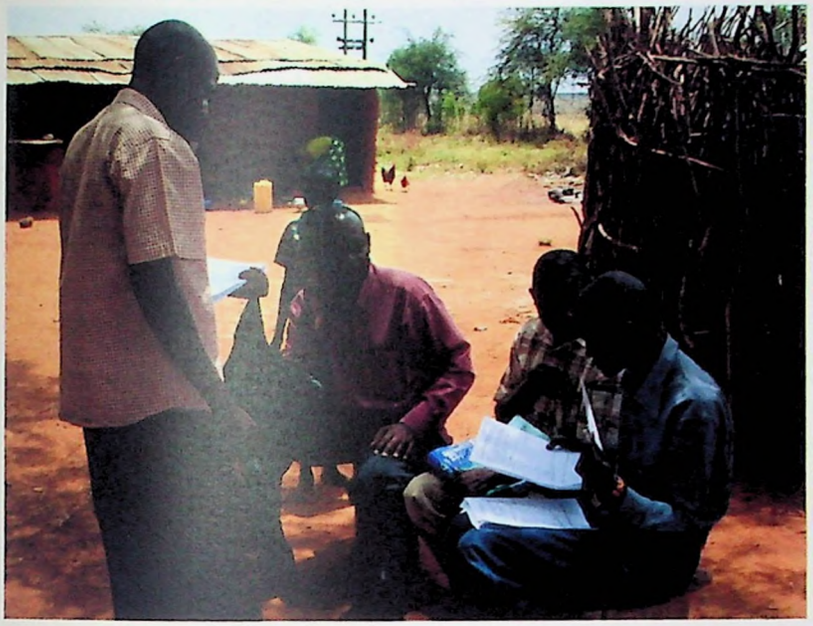
End and thanks for your time.

Figure 8: Dogs freely roaming inside a slaughter slab in Moroto municipality

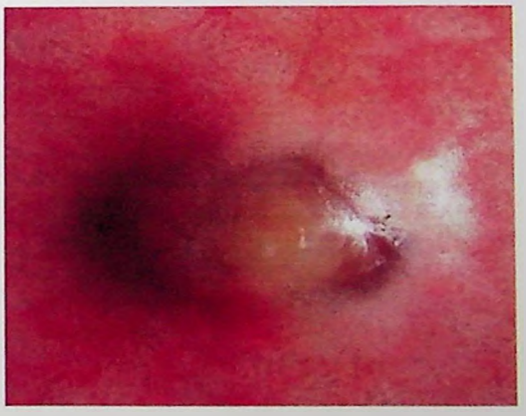
Figure 9: A dog freely consuming a cow carcass inside a slaughter slab at Moroto municipality

Figure 10: Dogs have free access to slaughter facilities (inside a slaughter slab), Moroto municipality

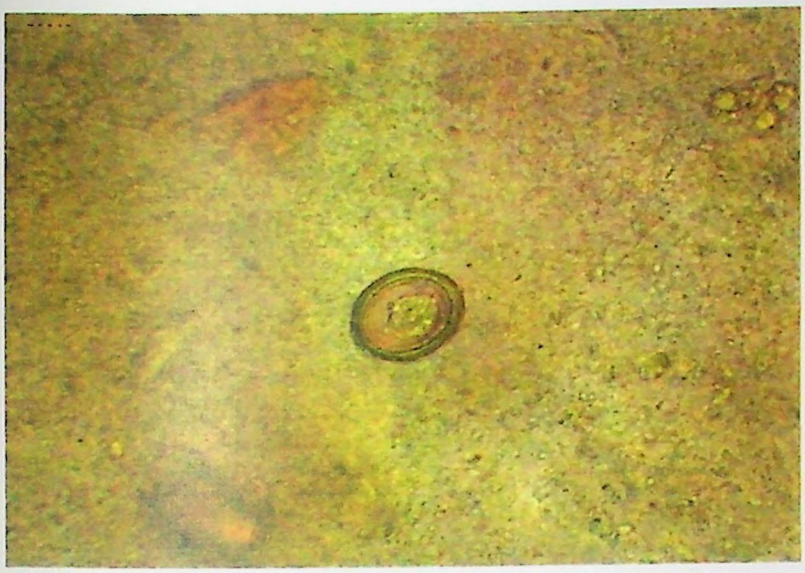
PHOTOS TAKEN FROM THE FIELD



Appendix 5: Research team at Nadunget trading centre, Moroto district with one of the research assistants administering questionnaires



Appendix 6: Hydatid cysts used to guide interviewers and respondents during interviews



Appendix 7: A typical taeniid egg under the microscope Mag x400