PREVALENCE AND RISK FACTORS OF CYSTIC ECHINOCOCCOSIS IN CATTLE AND HUMANS IN WESTERN PROVINCE OF ZAMBIA

BY

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LUSAKA

DECLARATION

I, **Fredrick Banda**, do hereby declare that this dissertation represents my own work and that it has never been submitted before for the award of a degree or any other qualification at this or any other university.

Signature: ....

Date: ....

DEDICATION

I dedicate this work to my late father Mr. Gatson Nsekozimale Banda who always preached hard work and did not believe in being the second best. May His Soul Rest in Eternal Peace.

APPROVAL

This thesis of **Fredrick Banda** has been approved as fulfilling the requirements for the award of Degree of Master of Science in Veterinary Public Health of the University of Zambia

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Date

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AE	Alveolar echinococcosis
CBPP	Contagious Bovine Pleuropneumonia
CE	Cystic echinococcosis
CI	Confidence Interval
CNS	Central Nervous System
Df.	Degrees of freedom
DALYS	Disability Adjusted Life Years
DNA	Deoxyribonucleic acid
ELISA	Enzyme linked immunosorbent assay
EU	European Union
FAO	Food and Agricultural Organization
Fig.	Figure
GPS	Global Positioning Satellite
HH	Household
IFAT	Immuno-florescence Antibody Test
n	Study population (sample size)
PAIR	Puncture, aspiration, injection and re-aspiration
PCR	Polymerase chain reaction
PE	Polycystic echinococcosis
PM	Post mortem inspection
Spp.	Species
UNEP	United Nations Environment Programme
UNZA	University Of Zambia
WHO	World Health Organization

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ABSTRACT

Cystic echinococcosis or hydatidosis is caused by the larval stage of the tapeworm Echinococcus granulosus, is one of the most important parasitic infestations in livestock worldwide and one of the most important parasitic zoonoses. A cross-sectional study was conducted to estimate the prevalence of hydatidosis in cattle and humans of Western Province of Zambia and determine the risk factors associated with disease occurrence. A retrospective analysis of cattle slaughter data from an eleven year period 1994 to 2007 (except for 1997, 1998 and 2002) was done to determine the presence of hydatid cysts in cattle. A retrospective review of, records of human cystic echinococcosis from Lewanika General Hospital, which is a referral centre for Western Province over four year period (2006 to 2010) was conducted and analyzed to determine the prevalence of the parasite in humans. Disease prevalence in cattle in various districts and camps was done by post mortem examination conducted from October 2007 to November 2008. The viability and fertility tests were done on some of the cysts collected during the study. A questionnaire survey to identify risk factors of transmission was also carried out. Annual losses due to abattoir condemnation of organs were estimated to determine the direct economic loss due to the disease.

A total of 158,456 cattle were slaughtered and inspected out of which, 4689 (3.0%) cases of bovine hydatidosis were recorded. The lung accounted for 93.7 %, followed by liver at 6.26 % and spleen at 0.02 %. Proportion positive in humans was 0.009 % (9 per 100,000 cases attended to). Sixty-seven percent of the human cases diagnosed were in females and 33% in male humans.

Hydatidosis was prevalent in 2.1% of the cattle slaughtered in two abattoirs in Western province during the prospective study. District prevalence were at 2.5%, 2.1%, 1.4% and 0.6% for Mongu, Senanga, Kalabo and Lukulu, respectively. In the questionnaire survey, it was observed that 88% of the households owned at least one dog. Among those that kept dogs (n= 58), the majority (96.6%) of dogs were in the free range system. Most dogs were mostly kept for security (86.2%) and cattle herding (87.9%). Stray dogs in the communities were regularly spotted by 65% of respondents. Most of the HH (94.8%) admitted to feeding offal to dogs and 37.9% of the respondents reported dogs scavenging from abattoirs and local slaughter slabs. It was observed that 98% of the households had dogs that defecated in the immediate surroundings of their dwelling places. It was found that the liver had 43.7% and the lung had 43% fertile cysts. The overall percentage of fertile cysts was found to be 43.1%. The estimated annual loss associated with organ condemnation was evaluated at K 15, 894, 000 (U$3,311) annually. The study has established that hydatidosis is prevalent in cattle and humans in Western Province of Zambia and that the risk factors for its transmission exist. The disease also contributes to economic losses due to organ condemnations and is of public health concern.

CHAPTER ONE

INTRODUCTION

Hydatidosis/cystic echinococcosis (CE) is a severe zoonosis caused by the larval stages of a cyclophyllidean cestode called *Echinococcus granulosus.* Two hosts are involved in the completion of the life cycle of *E. granulosus*. The definitive hosts are carnivores which harbour mature tape worms in the intestine (Khuroo, 2002; Zhang *et al*., 2003). The intermediate hosts of *E. granulosus* include ungulates both domestic and wild animals and humans. The adult worm lives in the small intestine of carnivores (definitive host), and the intermediate larval stage (Hydatid cyst or Hydatid) develops in the internal organs of a wide range of mammalian species such as goats, sheep and cattle, including humans, which acquire the infection through accidental ingestion of the tapeworm eggs (Eckert and Deplazes, 2004).

Cystic Echinococcosis is known to be one of the most important parasitic infections in livestock worldwide and one of the most widespread parasitic zoonoses (Craig *et al*., 2007; Cringoli *et al*., 2007).

The disease has a worldwide distribution and is endemic in many countries of the Mediterranean basin, North and East Africa, Western and Central Asia, China, South America and Australia (Jenkins, 2005; Romig *et al.*, 2006).

However, even if the distribution of *Echinococcus granulosus* is considered worldwide, it is higher in developing countries, especially in rural communities where there is close contact between dogs and various domestic animals (Eckert and Deplazes, 2004). In some western countries, CE is being considered a re-emerging zoonosis, due to recent increases in the observed prevalence (Himsworth *et al.*, 2010; Bushi *et al.*, 2005).

In humans, CE is the most common presentation and probably accounts for more than 95% of the estimated 2 to 3 million global cases (Craig *et al.*, 1996, Budke *et al.*, 2006).

The “sheep” strain (defined as Gl. on mitochondrial genotypic grounds) is generally considered as the most widespread strain of *E. granulosus* in the world and the one mainly involved in CE in humans (Thompson and Lymbery, 1990). At least five out of ten strains of *Echinococcus granulosus* (G1 to G10) are infective to humans in sub-Saharan Africa (Magambo *et al.*, 2006).

The echinococcosis/hydatidosis has considerable socioeconomic impact in both human and animal health in different countries (Rkia Azlaf, 2006). In humans, after a long latency period, the disease consequences may include; poor quality of life (disability adjusted life years [DALYS]); costs of medical treatment, lost opportunity for income generation and mortality in some cases (Budke *et al.*, 2006) while in animals there is reduced productivity and monetary losses due to abattoir condemnations (Torgerson and Heath, 2003; Scala *et al.*, 2006). The DALYS for human CE was recently estimated to be more than that for onchocerciasis and almost the same as that for Africa trypanosomaisis (Budke *et al.*, 2006).

The annual CE-associated economic losses on a global basis have been recently estimated to be at least over US$2 billion (Budke *et al.*, 2006). Transmission and maintenance of echinococcosis is dependent on complex interactions of several factors, including environmental, host and pathogen factors. A number of such factors are of local epidemiological significance and the identification of such factors is important in the effective implementation of control strategies.

In Zambia, like in most sub- Saharan African countries, echinococcosis has been reported to occur in many parts of the country, (Pandey and Sharma, 1987; Magambo *et al.,* 2006). Despite this, much more information is still needed. In Western Province, *Echinococcus* cysts have been diagnosed from cattle carcasses during meat inspection (Anon, 1999). However, there has been no comprehensive study carried out thus far to describe the epidemiology of echinococcosis infections in both the intermediate and final hosts and also to determine its zoonotic importance. Based on circumstantial evidence, it is assumed that the disease has serious public health and socioeconomic implications given the interactions that exist between cattle, dogs and humans; and also the uncontrolled disposal of abattoir wastes and remains from animal slaughters. However, this assertion needed to be supported by well-structured studies.

In Zambia, like in most countries in the region, there are very few published reports on CE (Romig *et al.,* 2011). In Western Province, where the study was conducted, hydatid cysts have been diagnosed from cattle carcasses during routine meat inspection. An abattoir can be a source of valuable information of the incidence of animal diseases and conditions some of which may be zoonotic (Phiri, 2006). Records at Lewanika Hospital also indicated that the disease could be present in humans in the province. Due to the absence of a comprehensive study to quantify the extent of the problem in both humans and cattle, it was necessary for this study to be undertaken. The aim of this study was to determine the prevalence of the disease in cattle through the abattoir survey and humans through the retrospective analysis of hospital records. The study also sought to identify risk factors predisposing infections in both species and further attempted to estimate the economic losses as a result of organ condemnation due to CE.

Based on circumstantial evidence above; it was assumed that the disease had serious public health and socioeconomic implications although this has not been thoroughly investigated. Therefore, this study aimed at determining the prevalence of the disease in cattle, and humans; and identifying the risk factors associated with the transmission and maintenance of the disease from domestic animals to humans. It was anticipated from this study that the extent of the problem would be determined and risk factors associated with the transmission and maintenance of the zoonotic cycle of echinococcosis would be identified.

Zambia is a host to 353,938 domestic dogs, according to the 2009 Ministry of Livestock and Fisheries Development report of which 50,772 are in Western Province. Most of these dogs are kept with little or no care at all. In addition, there is a large population of stray dogs especially in peri-urban areas. Among the common uses of dogs in Zambia are: guarding/security, cattle herding, hunting wild animals and companions.

In Western Province, slaughters are done both at household and abattoir levels. In addition, small scale slaughters for butcheries are done in designated slaughter slabs. In the abattoirs, condemned organs are normally incinerated by fire while the liquid waste is discharged to the sewer ponds. However, most of the abattoirs are not adequately fenced and hence are frequented by stray dogs. These dogs sometimes have access to condemned offal. In most cases it takes time before offal are incinerated after being condemned by meat inspectors. This allows stray dogs to have access to the condemned offal and has the potential of sustaining the parasite in dogs that would in turn infect cattle and humans through contamination of the environment.

The situation is the same with the slaughter slabs where some condemned organs are thrown in pits which are equally frequented by dogs. The situation is worsened by the many illegal backyard slaughters in various communities, especially in rural parts of the country where meat inspectors are not available.

In Western Province, cattle are often kept for commercial purposes. Often most people do not slaughter cattle for domestic consumption but consume meat from cattle that die own their own. Consequently, dogs have regular access to both the carcass and offal of these animals that die on their own.

Movement of live cattle from Western Province to cities along the line of rail was banned in 1998 as a result of the outbreak of Contagious Bovine Pleural Pneumonia (CBPP). Consequently, most cattle that are destined for slaughter are moved to Mongu and Senanga slaughterhouses. Therefore, data obtained from cattle that are slaughtered in at these two abattoirs were a good representation of the true provincial picture.

The general objective of the study was to describe the prevalence of *Echinococcus* spp. infections in the cattle and humans in the Western Province of Zambia and to identify the risk factors associated with infections in cattle and humans.

The specific objectives of the study were:

1. To determine the prevalence of CE in cattle and humans in Western Province of Zambia.

2. To identify the risk factors associated with the transmission and maintenance of CE in cattle and humans in Western Province.

CHAPTER TWO

LITERATURE REVIEW

2.1. Background (General Overview)

Echinococcosis is a disease that has been recognized by humans for centuries. There has been mention of it in the Talmud (Judaism Holly Book). It was also recognized by ancient scholars such as Hippocrates, Aretaeus, Galen and Rhazes. Although echinococcosis has been well known for the past two thousand years, it wasn't until the past couple of hundred years that real progress was made in determining and describing its parasitic origin. The first step towards finding out the cause of echinococcosis occurred during the 17th century when Francisco Redi illustrated that the hydatid cysts of echinococcosis were of "animal" origin. Then, in 1766, Pierre Simon Pallas predicted that these hydatid cysts found in infected humans were actually larval stages of tapeworms. A few decades afterwards, in 1782, Goeze accurately described the cysts and the tapeworm heads while in 1786; *E. granulosus* was accurately described by Batsch. Half a century later, during the 1850s, Carl von Siebold showed through a series of experiments that *Echinococcus* cysts do cause adult tapeworms in dogs. Shortly after this, in 1863, *E. multilocularis* was identified by Rudolf Leuckhart. Then, during the early to mid-1900s, the more distinct features of *E. granulosus and E. multilocularis*, their life cycles and how they cause disease were more fully described as more and more people began researching and performing experiments and studies. While *E. granulosus* and *E. multilocularis* were both linked to human echinococcosis before or shortly after the 20th century, it wasn't until the mid-1900s that *E. oligarthus* and *E. vogeli* were identified as and shown as being causes of human echinococcosis (Tappe *et. al*., 2008; Howorth, 1945).

Echinococcosis is an emerging and re-emerging zoonotic parasitic disease caused by the cestode species of the genus *Echinococcus* and is one of the most important helmintic diseases with a worldwide distribution including Europe, Asia, Africa, South America, Canada and Australia (Altintas, 2003; Budke *et al.*, 2006; Moro and Schantz, 2006).

A number of studies have shown that hydatidosis is a disease of increasingly public health and socio-economic concern. The disease is currently considered an emerging or re-emerging disease and the geographic distribution and extent are greater than previously believed (Thompson and McManus, 2002; Torgerson and Budke, 2003; Dakkak, 2010). The disease is one of the neglected diseases world over.

Studies in different parts of sub-Saharan Africa have shown a varied distribution of cystic echinococcosis (Magambo *et al.*, 2006). CE not only causes severe disease and possible death in humans, but also results in economic losses from treatment costs, lost wages and livestock associated production losses (Budke *et al.*, 2006). Echinococcosis is one of the most important parasitic diseases of livestock that has both economic and public health significance. It is associated with severe morbidity and disability, and is one of the world's most geographically widespread zoonotic diseases (Getaw *et al.*, 2010). The pathogenicity of echinococcosis heavily depends on the extent and severity of infection, and the organ where the cyst is situated. The occasional rupture of the hydatid cyst often leads to sudden death due to anaphylaxis, haemorrhage and metastasis.

Previous studies have shown that CE represented a considerable economic and public health significance in different countries (Azlaf and Dakkak, 2006).

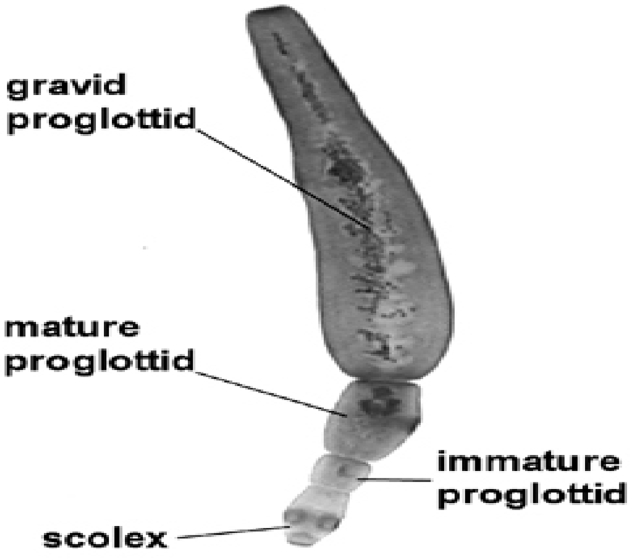
2.2. Aetiology

Echinococcosis is a zoonotic infection caused by the larval stage (hydatid) of tapeworms of the genus *Echinococcus* (Family Taeniidae) found in the small intestine of carnivores. The disease is also referred to as echinococcosis. In carnivores, the worm has very little effect if any. However, in the intermediate host, the effect can be grave depending on the site of infection and the organ involved (Craig *et al*., 2007; Cringoli *et al*., 2007).

Although there are different species of *Echinococcus* described, only four of them namely*; E. granulosus*, *E. multilocularis, E. oligarthrus, E. shiquicus* and *E. vogeli* are recognized as taxonomically relevant and only *E. granulosus* and *E. multilocularis* are the most pathogenic to humans and other domestic animals (da Silva, 2010). Clinically, there are three broad morphological forms of echinococcosis that are recognized; cystic echinococcosis caused by *E. granulosus*, alveolar echinococcosis caused by *E. multilocularis* and polycystic echinococcosis caused by *E. vogeli* and *E. oligarthrus* (Thompson and McManus, 2002, Raether and Hanel, 2003, Khuroo, 2002). To distinguish the diseases caused by the two most pathogenic species, the World Health Organization (WHO) proposed the designation of CE for the disease caused by *E. granulosus* and Alveolar Echinococcosis (AE) for the disease caused by *E. multilocularis*. In addition, a third form of echinococcosis caused by *E. oligarthrus* and *E. vogeli* called polycystic echinococcosis (PE) is also recognised (Raether and Hanel, 2003; Eckert, 2004; Macpherson *et al.*, 2003; Eckert and Deplazes, 2004).

2.2.1 Morphology of Echinococcus.

Echinococcus exhibits certain characteristics that differentiate it from the other major genus in the family *Taenia*. The adult *Echinococcus* is only a few millimetres long (rarely more than 7mm) (Figure 2.1) and usually has no more than six segments, whereas species of *Taenia* can grow to several meters in length and consist of several thousand segments. Like all tape worms, *Echinococcus* has no gut and all metabolic inter change takes place across the synctial outer covering, the tegument (Eckert *et. al*,



2002).

Figure 2.1 Morphology of a mature adult worm of *E. granulosus. (***Source: TMCR <http://tmcr.usuhs.mil/tmcr/chapter3/epidemiology2.htm>**

2.2.2. Scolex and Strobila

The anterior end of the adult worm possess a specialised attachment organ, the scolex, which has four muscular suckers and two rows of hooks, one large and the other small on the rostellum (head). The body or strobila is segmented and consist of reproductive units (proglottids) which vary in number from two to six. The adult worm is hermaphrodite with reproductive ducts opening at a common lateral genital pore. The position of the genital pore varies according to species and strain (Harandi *et al*., 2002, Ahmadi and Dalimi, 2006). For *E. granulosus*, the genital pore is proximal to mid body of the proglottid.

There is a prominent cirrus sac which may be horizontal or tilted interiorly and the vitellarium is globular. The uterus dilates after fertilisation; eventually occupy most of the terminal segments when the eggs are fully developed (Figure 2.1).

2.2.3. Morphology of Echinococcus eggs.

Echinococcus eggs contain an embryo that is called an oncosphere or hexcanth. The name of this embryo stems from the fact that these embryos have six hooklets. The eggs are passed through the faeces of the definitive host and it is the ingestion of these eggs that lead to infection in the intermediate host (David and Petri, 2006).

2.2.4. Metacestode

The metacestode (second larval stage) basically consists of a bladder with an outer acellular laminated layer and an inner nucleated germinal layer, which may give rise by asexual budding to brood capsules. The metacestode is also called Hydatid or Hydatid Cyst Protoscoleces arise from the inner wall of the brood capsules. The structure and development of the metacestode differs between the four species of *Echinococcus.*

2.2.5. Cystic Echinococcus and characteristics of the causative agents.

The parasite was first characterised by Batsch in 1786. The cestode parasite *Echinococcus granulosus* and its genetic variants are the agents of cystic Echinococcus/ hydatidosis (McManus and Thompson, 2003). To date, studies indicate that there are ten distinct genetic types (Genotypes G1-G10) within *E. granulosus* which vary in their infectivity to humans and livestock (McManus *et al.*, 2002).

Strains of *E. granulosus* may be found in host prey cycles. These strains have a degree of host adaptation and distinct geographic range. A strain that affects one intermediate host may be less able to, or unable to infect other intermediate hosts (Table 2.1). Human cystic echinococcosis accounts for more than 95% of the 2 to 3 million global cases (Craig *et al*., 1996; Budke, 2006).

During the past decade, considerable phenotypic and genetic variability has been observed within the species of *E. granulosus* and several strains have been identified (Thompson and McManus, 2002; van Herwerden *et al.*, 2000; Thompson, 1995). All strains except the lion strain utilize the dog and other canids as definitive hosts, but the strains exhibit several differences in the intermediate host spectra, geographic distribution (table 2.1); adult and metacestode morphology; maturation time in definitive host; organ localization of metacestode and proscolex production (Eckert and Thompson, 1997). It should be emphasized that at least seven of the ten *E. granulosus* genotypes are infective to humans. Globally, most of the human cases of CE are caused by the sheep strain (G1) of *E. granulosus* (Eckert and Thompson, 1997; Thompson and McManus, 2002).

Echinococcus granulosus is a small tapeworm (approximately 2 to 7 mm in length) with typically three segments and other morphological characteristics which allow a species diagnosis (Thompson, 1995).

Echinococcus granulosus has a cosmopolitan distribution and is found worldwide. The definitive hosts are primarily dogs and other canids while the intermediate hosts are primarily ungulates, marsupials and primates including humans. The cysts are unilocular, and have endogenous proliferation with no infiltration of metastasis. These cysts are normally found in the liver and lungs of the intermediate hosts (McManus and Thompson, 2003).

2.2.6. Alveolar Echinococcosis and characteristics of the causative agent.

Alveolar echinococcosis (AE) is a potentially fatal zoonosis caused by *Echinococcus multilocularis*, a helminth that is wide spread in fox populations in temperate regions of the world (Schweiger *et al*., 2007). The life cycle and transmission of *E. multilocularis* is similar to that of *E. granulosus.* A number of carnivores serve as the definitive hosts. The intermediate hosts in the case of AE are usually small mammals, particularly rodents, but domestic animals and humans can also be infected. In both animal and human intermediate hosts, the primary metacestodes are found almost exclusively in the liver. The germinal membrane of *E. multilocularis* proliferates externally rather than internally to form a multilocularis structure with many small cysts. The cysts are usually between 1 to 10mm in diameter but can occasionally grow as large as 20 to 30mm. Cysts of *E. multilocularis* resemble tumours. The cysts are not contained within a capsule and are very invasive. The cysts can metastasize to other sites such as the central nervous system (CNS) or the lungs (Taratuto and Venturiello, 1997). Hundreds of thousands of protoscoleces develop from the germinal membrane in some intermediate hosts.

2.2.7. Polycystic Echinococcosis and characteristics of the causative agents.

Echinococcus oligarthrus and *E. vogeli* cause polycystic hydatid disease (Santivanez *et al*., 2008). The definitive hosts for *E. vogeli* are bush dogs and domestic dogs while the intermediate hosts are rodents. The metacestodes are found primarily in the liver but can also be found in the lungs and other organs. *Echinococcus vogeli* cysts develop both internally and externally, resulting in multiple vesicles. In humans, *E. vogeli* is invasive.

The definitive hosts for *E. oligarthrus* are wild felids, and the intermediate hosts are rodents. The cysts developed in a variety of internal organs and the muscles. The cysts resemble those of *E. vogeli* and can reach up to 5 cm in diameter. Little is known about these organisms and human infections are rare.

Recently, another species of *Echinococcus* called *E. shiquicus* was characterized. *Echinococcus shiquicus* causes unilocular minicysts hydatid disease but humans have not been reported to be intermediate host of this species (Santivanez *et al.*, 2008).

2.3. Transmission and life cycle of *Echinococcus granulosus*.

The parasite's life cycle is almost exclusively domestic, involving dogs as definitive and ungulates (mainly sheep and cattle) as intermediate hosts (Palmer *et al.*, 1998). However, wild canids (dingoes, wolves, jackals, coyotes, red foxes, etc.) can also be involved in the transmission cycle in some areas (Jenkins and Morris, 2003; Jenkins and Macpherson, 2003). This transmission is responsible for the sylvatic echinococcosis cycle. The outcome of infection in livestock is hydatid cysts developing in the lung, liver or other organs (Jenkins *et al.*, 2005).

Echinococcus species have an indirect life cycle and must develop in both an intermediate and the definitive host. In many cases, the parasite cycles through the specific predators and scavengers, and their prey. The dog-sheep cycle is most likely to result in human infections. Other cycles include dog-camel, dog-horse, wolf-deer and coyote-deer.

Under ideal conditions, *E. granulosus* eggs remain viable for several months in pastures or gardens and on house hold items. *Echinococcus granulosus* eggs can survive for weeks under a variety of temperature ranges but they cannot survive for a long time when exposed to direct sunlight and dry conditions.

The intermediate hosts which include cattle and humans are infected by ingestion of eggs within faeces of the definitive host. The eggs may also be found on foods such as vegetables, fruits or herbs, or in contaminated water. They can also stick to hands when a person handles an infected dog, cat, wild animal or its carcass, and may then be transferred to the mouth via the hands.

Hydatid cyst is the common name for the larval phase of *E. granulosus.* Parasites can develop in a variety of organs in the intermediate host but are often found in the liver and lungs. The cysts grow slowly. Most cysts are discovered in humans when they are 1 to 7mm in diameter but they eventually reach 20cm in diameter.

In primary echinococcosis, hydatid cysts develop in various sites from oncospheres after ingestion of *E. granulosus* eggs. In secondary echinococcosis, larval tissue spreads from the primary site and proliferates after spontaneous or trauma-induced cyst rupture or after release of viable parasite material during invasive treatment procedures (da Silva, 2010). Each cyst is filled with fluid and is surrounded by a fibrous laminated outer membrane and an inner membrane called the germinal layer. Brood capsules develop from the germinal membrane. Each brood capsule contains several invaginated heads (protoscoleces) that can develop into an adult worm if they are ingested by the definitive host. Some protoscoleces float freely and are known as "hydatid sand". The hydatid sand has the potential of developing into new cysts. Some cysts are sterile and either never produce brood capsules, or they become sterile after bacterial infection or calcification. The percentages of sterile cysts vary with the intermediate host and play a vital role in transmission of the disease.

Sheep and numerous ungulates (goats, swine, and cattle) are intermediate hosts of CE, harbouring the hydatid cyst (Euzeby, 1991; Garippa *et al.*, 2004). Pigs are infected by different genotypes of *E. granulosus* (Bowles and McManus, 1993; Eckert *et al.*, 1993). Studies on the strain specificities of *E. granulosus* in Tunisia showed that the sheep strain (G1 genotype) was present in sheep, cattle, camels and humans (Lahmar *et al.*, 2004) and that the camel strain (G6 genotype) was only present in camels (M'Rad *et al.*, 2005) with fertile ovine, bovine and cameline cysts being a reservoir for dogs and other canids.

The adult cestode inhabits the small intestines of a carnivore (definitive host) and produces eggs containing infective oncospheres. The eggs remain infective for a period determined by the environmental conditions. Either cestode segments (proglottids) containing eggs or free eggs are released from the intestinal tract of the carnivore into the environment. Eggs have been shown to be dispersed by factors such as wind, flies and others (Eckert *et al.*, 2001). After oral uptake of the eggs by the intermediate host, a larval stage, the metacestode, develops in internal organs. The mature metacestode typically produces numerous protoscoleces, each having the potential to develop into an adult cestode after being ingested by a suitable final host. These protoscoleces in the organs of intermediate hosts may remain viable for up to 36 days depending on ambient temperatures and relative humidity (Diken *et al*., 2007). Accidentally, eggs are also ingested by humans and other “aberrant” hosts that do not play a role in the natural cycle.

Whereas the infection of carnivores with immature or mature intestinal stages does not cause morbidity, the invasion of various organs (mainly liver and lungs) of the intermediate or aberrant hosts by metacestodes can cause severe and even fatal echinococcosis (Eckert and Deplazes, 2004). An important factor influencing the persistence and spread of *E. granulosus* infection is the contamination of dogs by ingestion of the viscera of infected sheep.

Larval/hydatid cyst stage

From the embryo released from an egg develops a hydatid cyst, which grows to about 5–10 cm within the first year and is able to survive within organs for years (Mandell, 2010). Cysts sometimes grow to be so large that by the end of several years or even decades, they can contain several litres of fluid. Once a cyst has reached a diameter of 1 cm, its wall differentiates into a thick outer, non-cellular membrane, which covers the thin germinal epithelium. From this epithelium, cells begin to grow within the cyst. These cells then become vacuolated and are known as brood capsules, which are the parts of the parasite from which protoscoleces bud. Often, daughter cysts will also form within cysts (David and Petri, 2006).

Adult worm

Echinococcus adult worms develop from protoscoleces and are typically 6mm or less in length and have a scolex, neck and typically three proglottids, one of which is immature, another of which is mature and the third of which is gravid (or containing eggs) (David and Petri, 2006). The adult worm only develops to maturity in the definitive host. The scolex of the adult worm contains four suckers and a rostellum that has about 25-50 hooks.

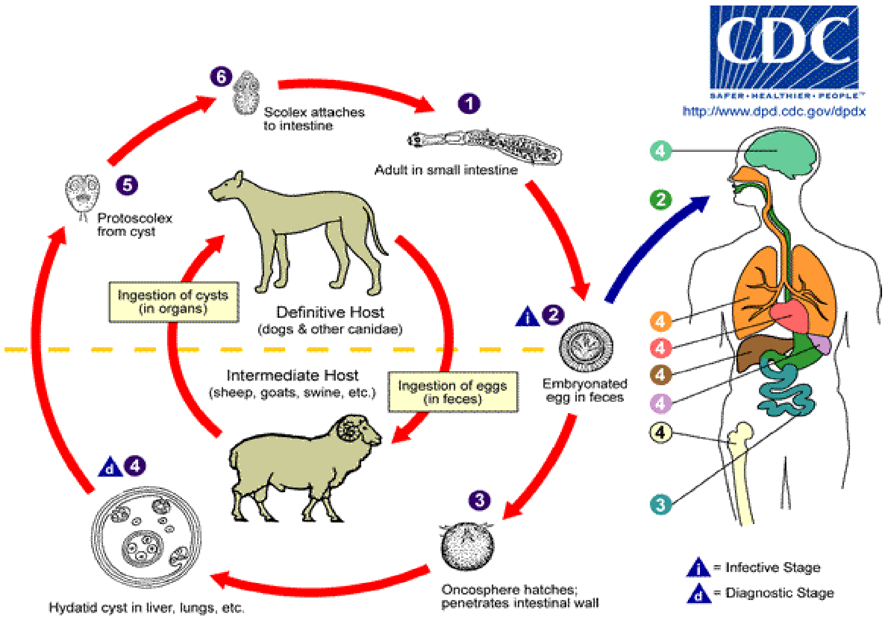


Figure 2.2 Life cycles of *Echinococcus granulosus. (Source: CDC http.www.dpd.cdc.govldpdx)*

2.3.2. Transmission and life cycle of Echinococcus multilocularis.

The typical cycle for this species is sylvatic and involves foxes of the genera *Vulpes* and *Alopex* and rodents, particularly those of the family *Arvicolidae*. Rodents in the families: *Soricidae*, *Talpidae*, *Sciuridae*, *Cricetidae* and *Dipodidae*, and pacas (*Ochotonidae*) may also be involved (Macpherson *et al.*, 2003; Dang *et al.*, 2009). Domestic dogs and cats are also susceptible definitive hosts and may become infected by predating wild intermediate hosts. Such is the case in the Arctic, where a cycle involving dogs and voles occurs. Such cycles may also operate in other areas, where dogs and cats may capture and eat infected rodents; they have been observed in central Europe, Japan and other regions. Cycles involving cats and house mice may also exist in certain areas, although such partially domestic cycles may be of minimal significance in the overall perpetuation of *E. multilocularis*.

2.3.3. Transmission and life cycle of E. oligarthrus and E. vogeli.

Only felids are capable of acting as definitive hosts of this species. The larval stage occurs in large South American rodents such as agoutis (*Dasyprocta* spp.) and pacas (*Cuniculus paca*) (Macpherson *et al.*, 2003; Dang *et al.*, 2009). The principal definitive hosts are the cougar (*Felis concolor*), jaguar (*Panthera onca*), ocelot (*F. pardalis*), jaguarondi (*F. yaguaroundi*) and Geoffroyi's cat (*F. geoffroyi*). The cycle is thus sylvatic, although domestic cats are known to be suitable hosts experimentally and establishment of a partially domestic cycle is therefore possible.

As with *E. oligarthrus*, *E. vogeli* is maintained primarily in a sylvatic predator/prey cycle between the bush dog (*Speothos venaticus*) and pacas, although other rodents such as agoutis and spiny rats (*Proechimys* spp.) are susceptible. Domestic dogs are also suitable definitive hosts and may be involved in cycles in endemic rural areas of South America and would appear to be the only likely source of infection to humans.

2.4. Geographic distribution and prevalence of echinococcosis in selected regions of the world.

Echinococcus granulosus has a world-wide geographic range and occurs in all continents including circumpolar, temperate, subtropical and tropical zones (Craig *et al.*, 1996; Schantz *et al.*, 1995). The highest prevalence of the parasite is found in parts of Eurasia, Africa, Australia and South America. Within the endemic zones, the prevalence of the parasite varies from sporadic to high, but only a few countries can be regarded as being free of *E. granulosus.* The worldwide distribution of the disease is partly due to the easy adaptability of the parasite to several domestic and wild intermediate hosts (Bhatia, 1997). Actually, this wide spectrum of intermediate hosts seems to correspond to genetic variability among *Echinococcus granulosus* strains which can be assessed using nuclear and/or mitochondrial genotypic methods (Raether and Hanel, 2003; Eckert and Deplazes, 2004).

E. granulosus is present virtually worldwide since there are very few countries that are considered to be completely free of *E. granulosus.* An important fact to keep in mind is that the areas of the world where there is a high incidence of infection by *E. granulosus* often coincide with rural, grazing areas where dogs are able to ingest organs from infected animals.

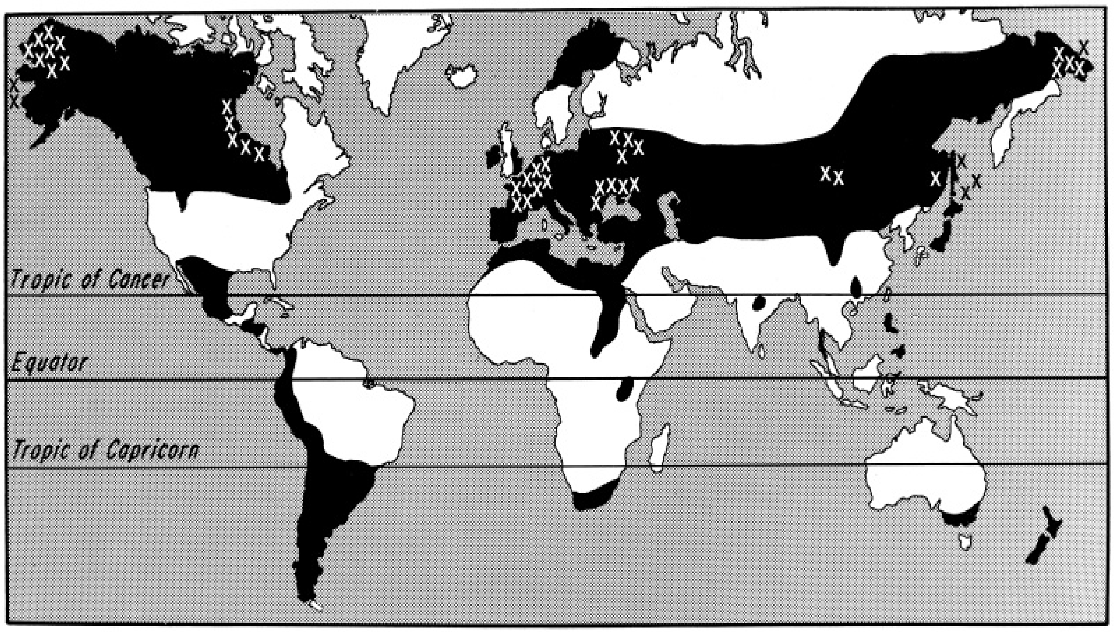


Figure 2.3 Global distribution of *E. granulosus* (black) and *E. multilocularis* (x)

(Source: TMCRhttp://tmcr.usuhs.mil/tmcr/chapter3/geographic.htm)

Table 2.1. The distribution of *Echinococcus* by species and strain.

Echinococcus specie	Strain	Distribution
E. granulosus s.s	G1 sheep strain G2 Tasmanian sheep strain G3 buffalo strain	Mediterranean, South-America, Central-Asia, East Africa (G3- water buffalo – Italy
E. equinus	G4 horse strain	Rare and sporadic (except England) non infective to humans.
E. ortlepi	G5 cattle strain	(Central Europe) infrequent low infectivity to humans
E.canadensis	G6/G7 camel-pig strain G8, American cervid strain G9, Variant pig strain G10, European cervid strain	(Central and Eastern Europe) wide host range; pigs, goats and camel not sheep. (G8, G10- northern hemisphere) wildlife cycles moderate infectivity to humans.
E. felidis	Lion strain	

Echinococcus multilocularis mainly occurs in the Northern hemisphere, including central Europe and the northern parts of Europe, Asia, and North America. However, its distribution was not always like this. For instance, until the end of the 1980s, *E. multilocularis* endemic areas in Europe were known to exist only in France, Switzerland, Germany, and Austria. But during the 1990s and early 2000s, there was a shift in the distribution of *E. multilocularis* as the infection rate of foxes escalated in certain parts of France and Germany. As a result, several new endemic areas were found in Switzerland, Germany, and Austria and surrounding countries such as the Netherlands, Belgium, Luxembourg, Poland, the Czech Republic, the Slovak Republic, and Italy. While alveolar echinococcosis is not extremely common, it is believed that in the coming years, it will be an emerging or re-emerging disease in certain countries as a result of *E. multilocularis*' ability to spread (Tamás *et al.,* 2003).

Unlike *E. granulosus* and *E. multilocularis, E. vogeli* and *E. oligarthus* are limited to Central and South America. Furthermore, infections by *E. vogeli* and *E. oligarthus* (polycystic echinococcosis) are considered to be the rarest form of echinococcosis.

Table 2.2 Distribution of other species of *Echinococcus.*

Echinococcus specie	**Distribution**
E. multilocularis	Primarily in the Northern Hemisphere; Canada, USA, Europe, Japan, Russia, India, Turkey, Iraq, China and North Africa (tinny pocket)
E. shiquicus	Tibetan plateau in China
E. oligarthrus	Central and South America
E. vogeli	Central and South America

2.4.1. Prevalence of echinococcosis (CE) in Africa.

Studies conducted in North Africa have shown wide significant variation in infection to cattle and sheep depending on the location (Azlaf and Dakkak, 2006). The variation in infection is as a result of several factors which aide transmission of *Echinococcus spp*. The infection rates in cattle are especially high in Middle Atlas (8.72%) and in the Loukkos (37.61%) (Azlaf and Dakkak, 2006). A recent study in Ngorongoro District of Tanzania showed an overall prevalence of 47.9% and species prevalence of 48.7%, 34.7% and 63.8% in cattle, goats and sheep respectively (Kazwala, 2008).

Countries around the Mediterranean region, have exhibited high prevalence of CE in both humans and livestock. Egypt has recorded human cases between 1.34- 2.6 cases per 100,000 people through hospital surveys and 6.4% prevalence in cattle and buffalo through abattoir surveys (Table 2.4).

Table 2.3 Prevalence of echinococcosis (CE) in Sub-Saharan Africa.

Country	Species	Prevalence (%)	Type of survey	Reference
Kenya				
	Dog	72.4	Sero-survey	Wachira *et.al*,
	Dog	38	Sero-survey	1994.
	Sheep	20.4	Abattoir survey	Macpherson *et.al*,
	Goats	16.7	Abattoir survey	1998.
				Nginyi *et.al*,
				1995.
				Nginyi *et.al*,
				1995.
Sudan	Camel	4.3 – 8.2	Abattoir	Haridy *et.al*. 1989
Uganda	Dogs	66.3	Post-mortem	Inangolet *et.al*
				,2010
Tanzania	Cattle	4.2	Abattoir survey	Nonga and
	Sheep	6.0	Abattoir survey	Karimuribo 2009.
	& Goats	48.7	Abattoir survey	Nonga
	Cattle	34.7	Abattoir survey	Karimuribo 2009
	Goats	63.8	Abattoir survey	Kazwala 2008
	Sheep			Kazwala 2008
				Kazwala 2008
Zambia	Cattle	14.3	Abattoir survey	Pandey G.S.
				(1987).

Table 2.4. Prevalence of echinococcosis (CE) in North Africa.

Country	Species	Prevalence	Type of survey	Reference
Morocco	Bovine	23.%	Abattoir survey	(Azlaf and Dakkak, 2006)
	Sheep	10.6%		
	Goats	1.9%		
	Camel	12.0%		
	Equines	17.8%		
Libya	Cattle	6.4%	Abattoir survey	(Al-Khalidi, 1998)
	Sheep	8.7%		
	Goats	5.4%		
	Camels	35.0%		
	Humans	4.2 / 100,000	Surgical	(Tashani *et al*, 2002)
Egypt	Dogs	5%	Necropsy	(Haridy *et al*, 2006)
	Cattle& Buffalo	6.4%	Abattoir Survey	
	Humans	1.3-2.6 / 100,000	Hospital survey	(Ahmed *et al*, 2004)
				(Behe, 2009)
Ethiopia	Cattle	32.1%	Abattoir survey	

Human cystic echinococcosis occurs predominantly in poor pastoral communities that raise sheep and other livestock, and keep dogs for guarding and/or herding animals. *Echinococcus granulosus* is mainly transmitted in a cycle between dogs (definitive hosts) that harbour the small intestinal tapeworm and livestock after the latter ingest the microscopic eggs while grazing pastures that are contaminated with dog faeces (McManus *et al.*, 2003). Dogs usually acquire infection from hydatid carrying livestock as a result of their deliberately feeding of infested offal (liver and lungs) by owners who practice home slaughter (McManus *et al.*, 2003, Moro *et al.*, 1997). Thus the domestic cycle of *E. granulosus* is perpetuated by human behaviour. Humans get exposed to eggs of the tape worm through contact with an infected dog or its contaminated environment (Seimenis, 2003, Eckert and Deplazes, 2004).

Studies in different parts of sub-Saharan Africa have shown varied distribution of CE in humans. A recent review of hospital records among the Karamojong community in western Uganda has shown an average of 20 surgical cases of CE per year reported in each of the hospitals in Karamojang and Mbarara (Macpherson *et al.*, 2004). These findings indicate that CE is a public health and an economic problem not only in Uganda but also in countries where animal infections are prevalent. In Turkana in Kenya, the prevalence of CE in humans is about 2.5% (Zeyhle *et al.*, unpublished data, 2005). In central Sudan, a recent ultrasound survey that included 300 and 651 people in two different areas showed prevalence between 0.3% and 0.8%, respectively (Elmahdi *et al.*, 2004). In southern Sudan the prevalence of CE among Bouya people was 2% (Magambo *et al*, 1996) and 3.5% among Toposa (Magambo *et al.*, 1998). Records of human CE in western and southern Africa are rare. Nevertheless, a survey in Karaye hospital in Nigeria showed that 5.1% scans had sonar features consistent with intra-abdominal CE (Magaji *et al.*, 2004). Cystic echinococcosis in humans is fairly common in north-western Nigeria but this fact is probably unrecognized by clinicians in this area, and therefore is not reported (Magaji, 2004). In the nearby country of Burkina Faso, the prevalence in humans seem to be very negligible (0.007%) (Coulibaly, 2000).

2.4.2. Prevalence of echinococcosis (CE) in America and Europe.

In some western countries such as France, the CE prevalence figures are low (Table 2.3). This is due to public awareness and other control measures. However, CE is considered as a re-emerging disease in some western countries due to relaxations in control. In Spain for instance, high incidences of CE have been recorded in cattle and sheep (23%) in central and western regions of the country with 1.1 to 3.4 cases per 100,000 inhabitants in humans (Table 2.3).

Table 2.5 Prevalence of echinococcosis (CE) in America and Europe.

Country	Species	Prevalence	Type of survey	Reference
Italy	Sheep	70-92.8%	Abattoir survey	(Garippa 2004,2006)
	Cattle	9.4%	Abattoir survey	
Chile	Sheep	6.5-36.5%	Abattoir survey	(Garippa 2004,2006)
Canada	Cattle	10.4	Abattoir survey	(Garippa 2004,2006)
	Sheep	5-28%	Abattoir survey	
	“	22%	Abattoir survey	
	Livestock	47%	Abattoir survey	Ascota Jamett(2010)
	Humans		Surgical	
	Humans		Serosurvey	Himsworth *et al* 2008
Spain	Sheep-dogs	8.0%	Coproantigen ELISA,	(Sobrino *et al* 2006)
Madrid	Iberin wolves	15%	Abattoir Survey	(Sobrino *et al* 2006)
	Sheep	2.88%	Abattoir Survey	
	Sheep and cattle	23%		(Rodrigal *et al* 1997)
North, Central and Western Spain	Humans	1.1-3.4 cases/ 100,000	Surgical	(Jimenez *et al* 2002)
				(Pardo *et al* 2005)
France	Cattle&	< 0.5%	Abattoir survey	(OIE 2004)
	Humans	< 0.3% cases/100000	Surgical	(Bitchet and Dorcines)

2.5. Zoonotic importance and risk factors

Cystic echinococcosis is a public health problem in different geographical areas of the world, particularly in Asia, South America, Central America and Africa (McManus and Smyth, 1986). Spain and other Mediterranean countries are considered as hyper endemic areas (McManus and Thompson, 2003). *Echinococcus granulosus* of carnivores and its metacestode in herbivores and man have been recognized as the most important helminth zoonosis and of great economic and public-health significance in developing countries (Eckert, 1981). Echinococcosis due to *Echinococcus granulosus* which occurs at high prevalence in both dogs and livestock and also accounts for the highest number of condemned lungs in slaughterhouses is of major public health concern in Zambia (Pandey and Sharma, 1987).

Dogs are the most successful canids adapted to human habitation world-wide (Robertson *et al.*, 2000; Dohoo *et al.*, 1998; Ugbomoiko *et al.*, 2008). They have contributed to physical, social and emotional well-being of their owners, particularly children who are often at greatest risk of exposure. However, despite the beneficial effects, close bonds of dogs and humans (in combination with inappropriate human practices and behaviour) remain a major threat to public health, with dogs harbouring a bewildering number of infective stages of parasites (including *Echinococcus*) transmissible to man and other domestic animals (Molyneux, 2004; Ugbomoiko *et al.*, 2008).

Certain deep-rooted traditional activities have been described as factors associated with the spread and high prevalence of the disease in some areas. These factors include; the wide spread backyard slaughter of animals, the corresponding absence of rigorous meat inspection procedures, the long standing habit of feeding domesticated dogs with condemned offal and the subsequent contamination of pasture and grazing fields (Getaw *et al.*, 2010). This can facilitate the maintenance of the life cycle of *Echinococcus granulosus* which is the causative agent of cystic hydatidosis and consequently the high rate of infection of susceptible hosts (Biffa *et al.*, 2006). Risk factors for human hydatidosis include: a pastoral occupation, a history of dog ownership, poor education background, eating habits, age, sex and drinking water source (McManus *et al.*, 2003).

2.6. *Economic importance.*

Echinococcosis in humans and animals is both an economic and public health problem in many parts of the world (Budke *et al.*, 2005; Moro and Schantz, 2006). For example, in the North African countries, the cost to human health treatment and animal losses was estimated at US$ 60 million per year (Budke *et al.*, 2006; Moro and Schantz, 2006). In Jordan alone, a more recent estimate was reported at an equivalent of twenty one million United State of America dollars (US$) (Conteh *et al* 2010). Hydatidosis in animals is equally an economic problem and results in growth delays; the qualitative and quantitative production loss of meat, milk, wool; the fall in fertility as well as the seizures of viscera (offal) during meat inspection (Torgerson and Budke, 2003; Torgerson *et al.*, 2000). In Uruguay, the annual losses were estimated at US$ 6.2 million from the organs seizure and the loss of livestock productions (Torgerson *et al.*, 2000). In Queensland Australia, hydatid disease was thought to cost the meat industry, conservatively about U$2.7 million annually through lost offal sale (McManus and Thompson, 2003).

The economic importance of echinococcosis in livestock is due to the condemnation of the whole edible carcasses and offal such as liver, lung and heart (Torgerson *et al.*, 2000). In severe infection, the parasite may causes retarded performance and growth and reduced quality and yield of meat and milk (Getaw *et al.*, 2010). For instance, in Yugoslavia, a 10% reduction in milk yield and 5% in carcass weight due to hydatidosis has been described (Torgerson, 2003; SarIözkan and YalçIn, 2009).

2.7. Diagnosis.

2.7.1. Parasitological methods

In cattle, diagnosis of cystic echinococcosis is mainly through post-mortem findings during meat inspection. The presence of hydatid cysts in internal organs is a very important tool of diagnosis in that it actually confirms the disease.

The most reliable method for diagnosis of *Echinococcus* spp. in definitive hosts is by necropsy, because worm burdens can be accurately estimated and parasites collected for identification (Eckert, 1997). However, necropsy usually results in a biased sample, in that only unwanted dogs can be necropsied.

For many years, treatment and diagnosis of *E. granulosus* infection in dogs relied on the use of a single drug Arecoline hydro bromide, a substance obtained from an extract of nuts from the betel palm (*Areca catechu)(*Jenkins, 2005*).* The drug causes rapid and strong contraction of the smooth muscle of the intestine. These contractions dislodge the tapeworms and they pass out in faeces in a rapidly expelled purge. However, the drug has several major disadvantages among them are; it can be lethal to very young or old dogs, it may cause the pregnant females to abort, dogs with small infections may not pass the worms, a proportion of Arecoline –treated dogs may purge incompletely or not at all, the procedure is very time consuming and purging results are always an under-representation of the true picture of infection (Jenkins, 2005).

The use of arecoline salts as a purgative to remove the worms from the intestine for identification thus lacks sensitivity. A single treatment with the drug has been shown to diagnose less than 50% of *Echinococcus* infections (Wachira *et al.*, 1990).

Faecal examination does not differentiate *E. granulosus* from other taenid species and thus cannot be used to diagnose *E. granulosus* in dogs and other definitive hosts. The morphology of taeniid eggs is similar and therefore it is very difficult to differentiate on the basis of presence of eggs in faeces of dogs and other intermediate hosts.

2.7.2. Immunodiagnostic techniques.

In the 1980s an immunological method for detecting circulating antibodies against *E. granulosus* in serum was devised (Jenkins and Rickard, 1986). Specific circulating antibodies against *E. granulosus* were readily detectable in dogs raised worm-free and mono-specifically infected with *E. granulosus* in Australia, but in populations of rural dogs in Kenya with natural infections of *E. granulosus* and other cestodes, detection of antibodies was unreliable (Jenkins *et al.*, 1990). The presence of antibodies in serum did not mean the dog was infected at the time of testing because the antibodies against taenid cestodes remain detectable for weeks or months after worms have been lost (Jenkins and Rickard, 1985).

In the early 1990s, an immunological method of detecting substances (Coproantigen) released in the faeces of tape-worm infected definitive hosts was devised independently in England and Switzerland (Allan and Craig, 1989; Deplazes *et al.*, 1990). This method was adapted to detect Coproantigen of taenid cestodes including *E. granulosus* (Deplazes *et al.*, 1990; Deplazes *et al.*, 1992).

2.7.2.1. ***Coproantigen detection ELISA diagnostic test.***

In dogs, the diagnosis of *Echinococcus* species using coproantigen-detection ELISA method has a number of advantages over the use of Arecoline purgation as a diagnostic test. Among the advantages is that the Coproantigen-detection ELISA has easier sample collection, is faster to do, and requires less personnel. These factors make it suitable for surveillance of large dog populations (Abbasi *et al.*, 2003). Faecal samples for coproantigen testing can be collected in the field for some dogs hence eliminating the need of transporting the dogs to specific locations, unlike Arecoline purgation which requires taking dogs to specific purge sites and concentration of dogs in specific locations (Lopera *et al.*, 2003). The other advantage of coproantigens test is that it enables early detection in the course of infection (Jenkins *et al.*, 2000; Lopera *et al.*, 2003). The important advantage of coproantigen test over the antibody detection is that those coproantigen-positive dogs are infected at the time of the testing, Coproantigen become undetectable a few days after removal of *E. granulosus* (Jenkins *et al.*, 2000) and the test can be carried out at room temperature. The coproantigen test can be used to test *E. granulosus* in wild carnivores which are not easy to capture. The detection of coproantigen of *E. multilocularis* has been performed in wild fox populations in Japan (Sakai *et al.*, 1998).

A commercial ELISA kit (Genzyme-Virotech GmbH, Rüsselsheim, Germany), which was mainly designed for the detection of *E. granulosus* in dogs, cross-reacts with *E. multilocularis*, but the sensitivity for this parasite is low (53%) (Manufacturer’s information). Another commercial ELISA (Checkit®-Echinotest, Dr Bommeli AG, Liebefeld-Berne, Switzerland) has a higher sensitivity of approximately 90% for both *E. multilocularis* and *E. granulosus* (manufacturer’s information). In addition, several ELISA systems are available in various laboratories. There is a potential for refinement of the coproantigen ELISA. An interesting approach was described by Nonaka *et al*. (1996), who developed a sandwich ELISA based on a polyclonal capture antibody against excretory/secretory antigens of intestinal stages of *E. multilocularis* and a monoclonal detecting antibody (Kohno *et al.*, 1995) directed to a homologous antigen. This assay can detect coproantigen in material from heat treated (70°C for 12 h) or formalin-fixed (1%) fecal samples. However, variable results have been obtained in various laboratories with formalin-fixed material.

Recently, the crude cystic fluid has been used as antigen for serological diagnosis in cattle (Golassa *et al.*, 2011). However, this is not as sensitive as the post-mortem method due to the fact that CE may not in some cases elicit an immune response in the host and thus some infections could be missed.

2.7.3. DNA technology (PCR).

Great potential for diagnosis of canine echinococcosis has been shown by DNA amplification through the development of stool based polymerase chain reaction (PCR) (copro-PCR) tests for both species-specific and strain-specific pre-patent and patent detection of adult *Echinococcus granulosus* infections (Naidich *et al.*, 2006; Zhang *et al.*, 2003).

Mitochondrial DNA-based detection of *Echinococcus* species has been shown to be an excellent tool for analysis of strain/genotypic variation in the genus, determining phylogenic relationship and informing taxonomic species questions (Thompson and McManus, 2002).

2.7.4. Ultrasound imaging technology (US).

Ultrasound imaging has been used in small ruminants like sheep in some studies and followed up with post-mortem examination.

2.7.5. Diagnosis of CE in humans.

In humans, the diagnosis of hydatidosis is highly dependent on imaging techniques (e.g. computed tomography scans, magnetic resonance imaging, ultrasound and radiography) to detect the space occupying lesions caused by the developing, dying or dead metacestodes of *Echinococcus spp.* (Raether and Hanel, 2003; Eckert, 2004; Macpherson *et al.*, 2003; Eckert and Deplazes, 2004). Most cases of CE are discovered as incidental findings in routine diagnostic imaging procedures since most infected humans do not show any clinical signs unless the cysts enlarge sufficiently to have pathological effects. International classification of ultra sound images has been produced by the WHO expert working group and should, in principle, be used whenever ultrasound diagnosis is done.

Additionally, laboratory based diagnosis such as histology; cytology and serology provide confirmation of clinical infection and can be applied to aid epidemiological surveys of cystic hydatidosis in endemic regions. Such methods include serology where specific serum antibodies are detected and can be used in mass screening programs (Dottorini *et al.*, 1985; Wen and Craig, 1994; McManus *et al.*, 2003.). However, serological techniques are not hundred percent sensitive and specific and some cystic hydatidosis patients might not produce a marked antibody response (Dottorini *et al.*, 1985; Lorenzo *et al.*, 2005). Specificity for cystic hydatidosis serology can be a problem because of cross- reaction with inter-taeniid species which are not infrequent (Shepherd and McManus; 1987; Siracusano *et al.*, 1991; Ito *et al.*, 1999). Serological positive sera can further be tested for confirmation of Echinococcosis spp. and differentiated by western blotting (Logar *et al.*, 2008).

2.8. Treatment Prevention and control of echinococcosis.

In animals, prevention and control of echinococcosis is achieved by sanitary disposal of slaughterhouse waste to prevent access by dogs and also regular de-worming of dogs. Ruminants acquire infection by grazing on contaminated pastures. Contamination of pastures is as a result of using dogs for herding cattle. Therefore, limiting the use of dogs in herding cattle can help in the control of CE. Fencing off of the grazing area can also help in prevention of transmission of CE to cattle and other ruminants by preventing dogs from defecating on pastures where cattle graze.

Control of hydatidosis in animals will result in reduced risk of human exposure. Children are particularly at risk of zoonotic infections because of their close and regular contact with dogs and these also require regular de-worming (Hegglin and Deplazes, 2008).

Diagnosis and treatment of CE is very difficult and the disease can be asymptomatic in many patients. Because of this, the disease is under reported and can take up to 5 to 10 years for the cysts to cause problems. Treatment of CE in humans is through medical and surgical means and also puncture and aspiration, injection and re-aspiration (PAIR). Medical treatment is cheaper and is done by administration of de-wormers to the infected humans. Medical treatment is usually used when surgery is not possible due to anatomical location which can cause difficulties in the removal of the cysts. Medical treatment is used even in surgical treatment as a pre-surgical chemotherapy to reduce the possibility of rescinding of scoleces and thus reoccurrence of the cyst.

In some EU countries such as Denmark and Belgium, *E. granulosus* infection in animals is a notifiable disease. In other countries such as Greece, a dog control program has been in effect since 1985 with emphasis on dog registration and stray dog collection, with preventive treatment of all owned dogs testing of sheep-herd dogs and treatment with praziquantal.

Public attitudes towards zoonoses play a major role for a successful implementation of prevention, control and management measures. The development of strategies to inform the public on risk and prevention of zoonoses must be based not only on the results of scientific research of risk factors but also on the analyses of the perception of the problem by the public. This can differ from region to region and population-specific situations (Hegglin *et al.,* 2008). Data on control of hydatidosis is lacking in most sub-Saharan Africa. This may be explained by the low priority given by governments to arid pastoral areas where the disease is prevalent (Magambo *et al.*, 2006). According to World Bank statistics, the region is one of the poorest with more than 46% of people surviving on less than 1US$ per day.

Turkana District of Kenya is one of the few areas where extensive data has been obtained and a hydatid control program has been in place since 1983, managed by the African Medical Research Foundation (Magambo *et al.,* 2006). In this region, the hydatid control program consists of three components namely human treatment (surgery, puncture, aspiration, injection and re-aspiration [PAIR] and chemotherapy), dog population control (killing of stray dogs, sterilization of female dogs and de-worming of all owned dogs) and community education.

In summary, the control programmes against cystic echinococcosis have traditionally relied on anti-helmethic dosing of dogs, improved slaughter hygiene and surveillance, and health education relating to human – dog behaviour (Cabrera *et al.*, 1996; Cabrera *et al.*, 2002; Kachani *et al.*; 2003; Eckert and Deplazes, 2004). In principal therefore, *echinococcus* vaccine would ideally prevent oncosphere development to hydatid cysts in sheep and other intermediate hosts, and thus stop the development of adult gravid tapeworms in dogs which are the definitive hosts (Lightowlers *et al.*, 2000; Zhang *et al.*, 2003). A defined recombinant vaccine for ovine CE (called EG95) was developed in 1996 by the groups of Marshall Lightowler and David Heath in Australia and New Zealand. Field trails in Australia, New Zealand, Argentina, Italy and China in the following 8 to 10 years demonstrated more than 95% protection of at least 12 months in sheep (with colostral transfer of immunity) following two injections (Dempster and Harrison, 1995; Lightowler *et al.*, 1996).

Although the EG95 vaccine against ovine hydatidosis is a reality that now requires innovative delivery strategies, no similar effective vaccine exists against canine echinococcosis. This is so not because of lack of potential for application, because such a vaccine for dogs would be of enormous benefit in further reducing the infective period (currently more than 10 years) required to stop transmission of *E. granulosus* to people and their livestock (Herd, 1977). Studies undertaken in China on dogs vaccinated with recombinant proteins from mature adult worms of *E. granulosus* resulted in substantial repression of worm growth and suppression of egg production (Zhang *et al.*, 2006).

Theoretically, CE is an eradicable disease, but numerous factors are involved in the maintenance of the transmission cycle including behavioural and cultural factors that are often difficult to regulate or modify (Dakkak, 2010).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study Area

The study was conducted in Western Province of Zambia. This area was selected because previous reports had suggested that *Echinococcus* spp. infections could have been a problem in cattle and humans. Western province lies between and longitudes 22 degrees and 25 degrees East and latitude 13 degrees 30 minutes and 17 degrees 45 minutes South (Figure 3.1). The Province covers an area of 126,386 Km^2, which represents about 17% of the total land surface of Zambia. The Province consists of a vast sandy upland and a lower flood plain that covers an area of 12,950 Km^2, which is about 10% of the total land area of the province. The Zambezi River effectively bisects the Province into two distinct regions known as the Zambezi flood plain and the Upper land.

The Province has seven districts namely; Kaoma, Kalabo, Lukulu, Mongu, Senanga, Sesheke and Shangombo (Figure 3.1). Mongu town is the provincial capital and acts as the main centre for cattle trade. Senanga and Mongu have a total of eight abattoirs and several slaughter slabs were most of the cattle slaughters take place.

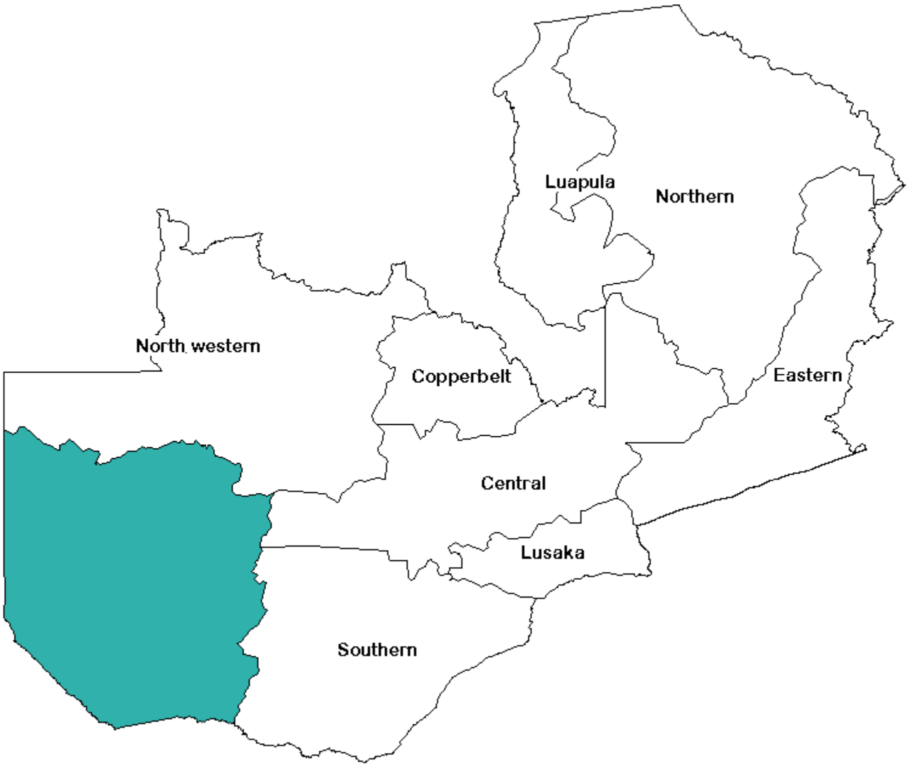
Western Province falls in Region I and II b in the southern part of the equator. The province has a dry and cold winter (April to July), hot and dry season (August to October) and hot and wet summer (November to March). The province has four main ecosystems as represented by the Zambezi flood plain, the upland river valleys and wet lands, and the upland forests.

The annual flooding of the Zambezi flood plains controls the pattern of life for the people and livestock. The largest population is concentrated along the edges of the flood plains. The people follow the transhumant subsistence economy. The level of the Zambezi and its cycle of flooding control the ecological balance and land use of the province.

The most important agricultural zone of Western Province is the Zambezi flood plain, which is suitable for producing cereals such as maize and rice. The district with greatest potential for crop production in the province is in Kaoma. Currently, Shangombo district is coming up as an agricultural district. The rest of the province generally is not suitable for crop farming and is thus mainly used for livestock rearing.

The province is covered by a deep mantle of Kalahari sands with peculiar indigenous flora. These Kalahari sands are pure, loose, and coarse grained in nature extending to great depths of up to 105 meters. The type of soil seldom shows any clear profile and is found both in the flood plain and the upland.

The Zambezi flood plains contain considerable deposit belt of bleached alluvial sands with dark loams while lower areas carry valuable brown silt clay loams often with surface peat in flood sites.



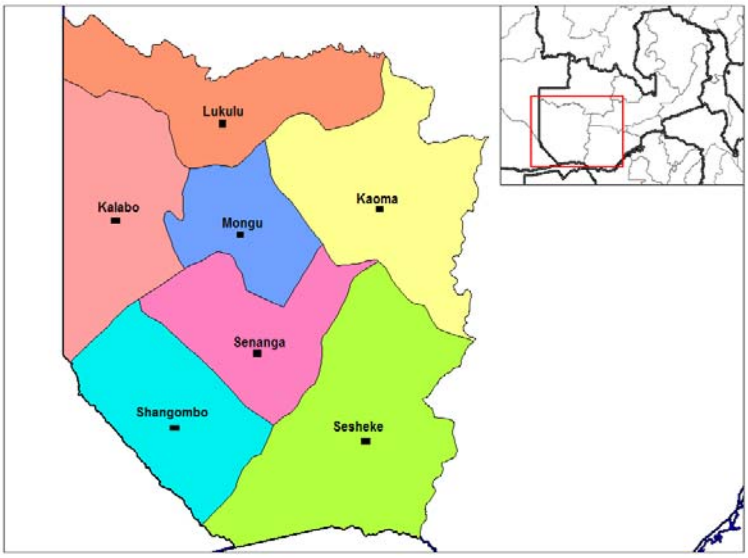


Figure 3.1 Maps of Zambia; Western Province (Shaded area) and districts of Western Province (Insert).

Western Province has a large number of livestock due to the agro-ecological zones that make the area suitable for animal production. The cattle population is approximately 452,400 (Appendix Two) which is comparatively high than other provinces of Zambia. (Appendix Three). The dog population in Western Province is estimated at 65,315 with Mongu accounting for the highest (n=16,210) followed by Kalabo (n=13,496), Shangombo (n=11,732), Sesheke (n=8,638), Kaoma (n=6,254), Senanga (n=4,750) and Lukulu with 4,236 dogs (Anon, 2010). Generally, most dogs could be described as family dogs, implying they belong to a specific household where feeding is supplemented, but the dogs often have freedom to roam and scavenge in the neighbourhood. In Western Province, dogs are used as working animals, mainly guarding and helping in cattle herding (Figure 3.2). In this cultural setting, dogs tend to be close to young male members of the household who often play with the dogs. The use of dogs for herding cattle could facilitate transmission of *Echinococcus* to this species and other animals sharing grazing land. Western province has a human population of 820,000 according to the 2010 national census.

Figure 3.2. Cattle grazing on the Zambezi flood plain with a dog being used for herding.

3.2. Study design.

The cattle study was conducted as a two-tier study involving a retrospective review of meat inspection reports and a prospective abattoir survey which also included testing some collected cysts for fertility and viability.

The human study was conducted as a retrospective review of human cases at Lewanika General Hospital in Mongu district. Lewanika hospital is the referral hospital in Western Province.

Lastly, a questionnaire was administered in order to identify risk factors responsible for transmission and maintenance of CE in Western province.

3.2.1. Retrospective cattle study

A retrospective study was carried out based on a review of post-mortem findings during meat inspection at the abattoirs in the last eleven years (1994-2007, with exemption of 1997, 1998 and 2002). Data was obtained from District Veterinary Offices in Senanga and Mongu that included abattoir reports on meat inspection and movement of livestock carried out in the previous 11 years in Western Province.

Data had been previously recorded by veterinary officers who conducted daily inspections of cattle carcasses at all the abattoirs in the two districts. Further information was obtained from monthly and annual report summaries for the period 1994 to 2007 which was categorized by affected organ. An organ and an animal were classified as positive when at least one hydatid cyst was detected on PM examination. Where multiple cysts occurred in different organs, more than one organ was reported.

Information collected included; number of cattle slaughtered, type of organs condemned, number and weight of condemned organs. The purpose of this was to provide baseline information and a retrospective understanding of the prevalence, dynamics and spatial distribution of the disease in Western Province and also to estimate the annual economic loss due to organ condemnation. Though the dataset did not allow the identification of individual animals, all the cattle slaughtered were from within the province. Therefore, the prevalence calculated represents the provincial prevalence.

3.2.2. Retrospective human survey

The purpose of this study was to estimate the prevalence of disease in human and identify associated risk factors. The human study was ethically conducted as a retrospective review of human cases from 2006 to 2010 at Lewanika General Hospital which is the referral hospital in Western Province. Diagnostic imaging (radiography and ultra sound) records for the period 2006 to 2010 of diagnosed human CE cases were obtained and reviewed for each of the positively diagnosed cases. For each patient, information on the place of origin, age, sex of patient and where possible, the household of origin was collected using a specially designed Excel® data sheet. The purpose of entering the data on excel sheet is for easy analysis and transfer to any statistical software. The results from the human survey were correlated with the disease in cattle.

3.2.3. Slaughterhouse survey

This survey was conducted between October 2007 and October 2008 at the Zambeef® and Starbeef® abattoirs in Mongu district. Cattle that were slaughtered at the two abattoirs originated from all the seven districts of Western province except Sesheke district

An individual animal was considered to be the sampling unit. Therefore, all the cattle that were slaughtered during the period were inspected both at ante-mortem and post-mortem inspection and samples with lesions were collected. Prior to commencement of the study, meat inspectors at the two slaughterhouses were trained in recognition of hydatid cysts in various organs of the carcasses according to the procedures recommended by FAO/UNEP/WHO (1994). Each animal that was slaughtered was uniquely identified using stock movement permits and by interviewing the owner. Therefore, each animal was traced back to its area of origin.

During ante-mortem examination, all cattle were examined for any abnormalities and the owner and place of origin was determined at the same time. At this stage, age of the particular animal was determined by asking the owner or where the animal was presented by the buyer (middleman), the age of the animal was estimated by checking the teeth eruption and wear (mouthing) as described by Jenny (2009).

A thorough post-mortem examination was conducted on slaughtered cattle. Visceral organs including lungs, liver, heart, spleen and kidneys were examined visually, through observation, palpation and systematic incision in each carcass according to procedures recommended by FAO/UNEP/WHO (1994). Some hydatid cysts that were found during inspection were removed whole and collected in polythene bags. Each polythene bag was used for hydatid cysts obtained from one animal and one organ. These bags were labelled appropriately to show the animal identification. The polythene bags were stored on ice to preserve the cysts and transported to the nearest laboratory where they were examined within one hour.

All organs were examined and special attention was paid to the liver, lungs and kidneys. Each lung and liver was visually inspected and palpated to check for any cysts which could have been imbedded in the organs. The data obtained from the study was subjected to statistical analysis. Basing on the results obtained, areas with high incidences were selected for further follow-ups and administration of questionnaires to the farmers and dog owners.

3.2.4. Examination of cyst and viability of protoscolices.

Some cysts identified during meat inspection in the prospective study were randomly collected from different organs and taken to the laboratory. Individual cysts were grossly examined for degeneration and calcification as described by Oostburg *et al.* (2000). In the laboratory, each cyst wall was carefully incised with a scalpel blade and the contents poured into a clean glass petri dish.

The contents were examined under a microscope (40×) for the presence of hydatid protoscoleces. The germinal layer was put in glycerine between two microscopic glass slides and examined for the presence of protoscoleces which were seen as white dots on the germinal epithelium or brood capsule or hydatid sands within the suspension in fertile cysts. Cysts that did not contain protoscoleces, contained pus or were calcified were considered as sterile or not fertile. Based on the presence or absence of brood capsules containing protoscoleces, the cysts were classified as fertile or infertile. The infertile cysts were further classified as sterile if they were filled with fluid without protoscoleces or calcified if they were calcified based on the procedure described by Macpherson (1985).

Viability of the protoscoleces was checked under the microscope. A drop of the sediment from the hydatid cysts was placed on a glass slide and covered by a 22 X 22 mm cover slip. Amoeboid-like peristaltic movement (flame cell activity) were checked under a microscope at X40 (Smyth and Barrett, 1980). Doubtful results were further examined after being stained with 0.1% aqueous eosin solution mixed with equal volume of hydatid fluid containing protoscoleces and allowed to stand for fifteen minutes on a microscopic glass slide. The protoscoleces were classified as dead when they took up the stain and viable when they did not (Macpherson, 1985). Thus viability of protoscoleces was determined by exclusion of eosin dye and flame cell motility (Macpherson and Smyth, 1985; Dalimi *et al.*, 2002).

3.2.5. Questionaire survey.

Using information obtained from the retrospective and prospective studies described above, a trace back system was applied to follow up the areas that had a history of echinococcosis/hydatidosis in the previous 12 months based on abattoir reports. Questionnaires were administered in these areas and evaluated for risk factors associated with the transmission of echinococcosis in cattle and humans. The questionnaire was orally administered by veterinary assistants in charge of the respective areas using the native language (Lozi). Households in each village were randomly selected for questionnaire administration but participation was voluntary depending on the willingness of the farmers to participate in the study.

Questions were designed so that majority of the responses could either be circled or answered in only a few words to minimize any misunderstanding during translation. All questionnaires were identified by date, house-hold identification number and GPS coordinate.

Data collected included (i) population structure of the affected communities (ii) social, ecological and epidemiological factors associated with the transmission and maintenance of echinococcosis.

Residents were interviewed by the interviewer using a closed-end questionnaire that lasted approximately 30 minutes. It covered questions on; (i) dog ownership and number of dogs owned (ii) livestock owned per household (iii) diet of dogs, (iv) offal disposal, (v) public health awareness of the disease, (vi) use of anti-parasitic treatment of dogs in the household (vii) co-mingling between dogs and other livestock, (viii) measures taken to control the disease and other relevant details. Information on stray dogs having access to domestic animal offal was also collected.

After the questionnaire was completed, information regarding *Echinococcus spp.* life cycles and mode of transmission was provided to the respondent.

3.2.6. Estimation of economic loss as a result of organ condemnation.

The loss attributed to condemnations of offal due to Echinococcosis was estimated using a modification of the formula as described by Yamene 1990 and cited by Getaw (Getaw *et al.*, 2010).

Formula for estimating annual economic loss resulting from abattoir organ condemnation:

$$\text{Annual loss} = (N_{ps} \times I_{lu} \times C_{lu}) + (N_{ps} \times I_{li} \times C_{li}) + (N_{ps} \times I_{he} \times C_{he}) + (N_{ps} \times I_{ki} \times C_{ki})$$

Where N_{ps} was the total number of positive animal slaughtered, $I_{lu:}$ prevalence of lung hydatidosis, $I_{li:}$ prevalence of liver hydatidosis, $I_{ki:}$ prevalence of kidney hydatidosis, C_{lu}: cost of lung, $C_{li:}$ cost of liver, $C_{he:\ cost}$ of heart and C_{ki} : cost of kidney.

Financial loss was based on the average price of wholesome and intact visceral organs obtained from Zambeef® Mongu.

3.2.7. Data analysis.

Information was stored in Microsoft® Excel® spread sheets and transferred to Stata® statistical packages for analysis. Prevalence of *Echinococcus* spp. infections in cattle and human populations was determined as a proportion of the test-positive subjects against the total number tested in the study population. Apparent prevalence estimates was converted into true prevalence values by taking into account the sensitivity and specificity of the test methods as described by Dohoo (2003). The Fisher's exact test was used to test the significance of association between categorical variables. The relation between continuous variables, were evaluated using the t-test. Further, risk factors associated with transmission and maintenance of *Echinococcus* spp. infections were identified using the Fisher's exact test in univariate analysis. Two way contingency table analyses were also used for univariate analysis of data to assess the effects of area, age and sex on CE positivity.

The risk-ratio and attributable risk analyses were used to assess the effect of specific risk factors on the occurrence of *Echinococcus* spp. infections in a given study population.

Furthermore, the economic loss as a result of organ condemnation due to the disease was estimated by calculating the economic loss as a result of condemnation of organs.

CHAPTER FOUR

RESULTS

4.1. Retrospective cattle study (Abattoir survey).

4.1.1 Prevalence of echinococcosis in cattle from the retrospective study.

During the period under review, 158,456 cattle were slaughtered and inspected out of which, 4, 689 (3.0%) cases of bovine hydatidosis were recorded.

The abattoir meat inspection records that were reviewed only had the number of cases and organs involved. Data on the age of cattle, sex and area of origin, were not captured in the data set. It was therefore not possible to analyse the influence of these missing variables on disease occurrence.

Annual prevalence ranged from the lowest at 1.6 % (n = 12641) in 2006 to the highest at 4.7 % (n = 2633) in 2001 (Figure 4.1). The prevalence of bovine echinococcosis during the period under review was found to be 3.0 %.

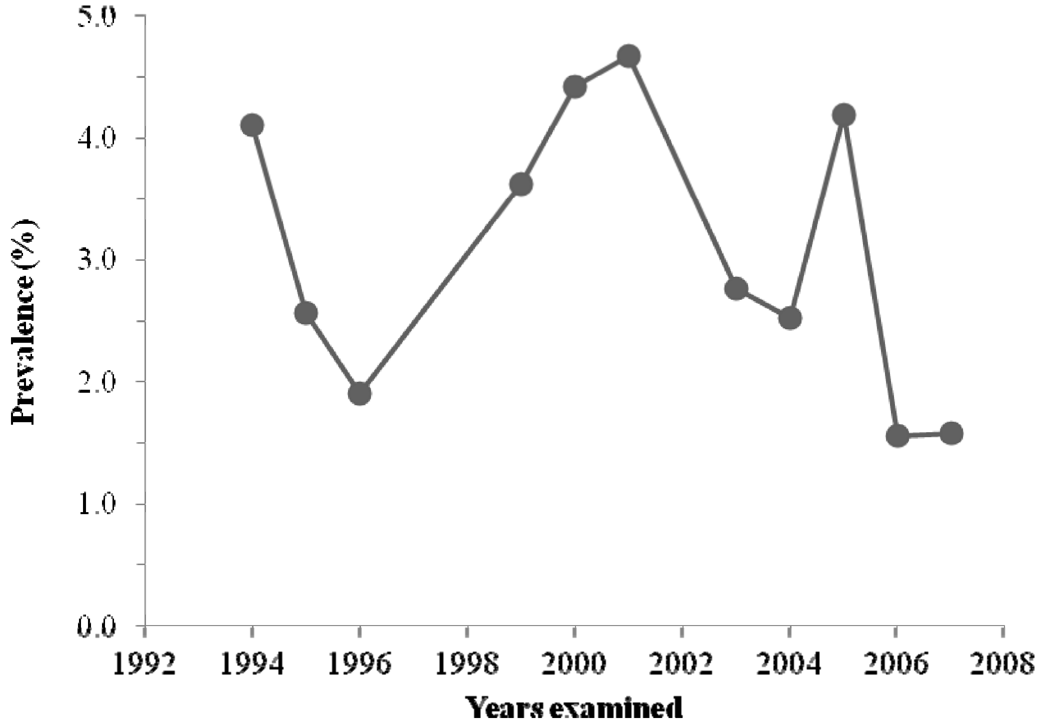


Figure 4.1. Prevalence (%) of Echinococcus in cattle from 1994 to 2007 (except for 1997, 1998 and 2002) in Mongu.

4.1.2. Distribution of bovine hydatidosis in different body organs on retrospective study

The distribution of hydatid cysts in organs from the slaughtered cattle were analysed by reviewing the meat inspection records both at the abattoirs and veterinary offices from 2004 to 2007 (with exception of 1997 and 1998).

It was observed that the distribution of hydatid cysts in bovine was highest in the lung at 93.7 %, followed by liver at 6.3 % and lowest in the spleen at 0.02 % (Figure 4.2).

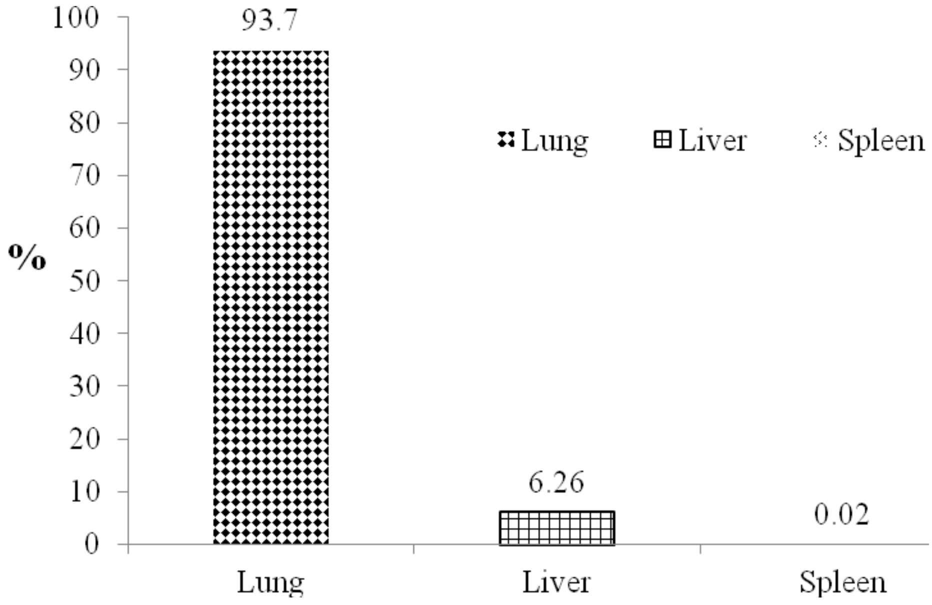


Figure 4.2. Distribution of cases of hydatidosis by different organs of cattle found positive at slaughter (1994-2007).

4.1.3. *Estimation of economic loss due to organ condemnation attributed to bovine CE*

The annual economic loss as a result of condemned organs was estimated by taking into account the average number of cattle slaughtered per annum at the Zambeef® and Starbeef® abattoirs and the percentage of condemned organs using the formula described by Yemane (1990) and cited by Getaw *et al.*, (2010).

Annual loss= $(N_{ps} \times I_{lu} \times C_{lu}) + (N_{ps} \times I_{li} \times C_{li}) + (N_{ps} \times I_{he} \times C_{he}) + (N_{ps} \times I_{ki} \times C_{ki})$

Where $N_{p:}$ the average number of animals slaughtered, $I_{lu:}$ prevalence of lung hydatidosis, $I_{li:}$ prevalence of liver hydatidosis, I_{ki}: prevalence of kidney hydatidosis, C_{lu}: cost of lung, C_{li}: cost of liver, $C_{he:}$ cost of heart and C_{ki} : cost of kidney.

The average retail prices of wholesome and intact visceral organs were obtained from Zambeef® butchery in Mongu (2009 to 2010) and used in the estimation of the annual loss due to condemned organs. While the average weight of the various organs was calculated from the data obtained from the abattoir study. The average weight of a lung was calculated as 2.92 Kg, that of liver was 3.34 Kg and that of the spleen was 2.00 Kg.

The average cost of lung was K12, 000 per Kg, liver K18, 000 per Kg and spleen K12, 000 per Kg.

The estimated cost of one lung = average weight x cost/kg (2.92 @ K12, 000) = K35, 040, cost of liver = average weight x cost/kg (3.34 @ K18, 000) = K60, 120, cost of spleen = average weight x cost/kg (2 @ K12, 000) = K24, 000. The average annual slaughter was K14, 405; and the condemnation rates for lung, liver and spleen were 2.8%, 0.2% and 0.001% respectively. The average total annual loss as a result of organ condemnations was estimated at K15, 894,039 (Table 4.1)

Table 4.1 Estimated average annual loss due to organ condemnation attributed to bovine cystic echinococcosis in Western province.

Organ	Average annual slaughters	Prevalence (%)	Total organ condemnations (14,405 x rate)	Total weight (Kg) (14,405 x 0.028 x2.92)	Loss in (K) = total weight x price /kg	Total value (K)
Lung	14,405	2.8	14,405 x 0.028 = 403	403 x 2.92 = 1,177	1,177 x 12,000	14, 124,000
Liver	14,405	0.2	14,405 x 0.02 = 29	29 x 3.34 = 97	97 x 18000	1, 746,000
Spleen	14,405	0.001	14,405 x 0.00001 = 1	1 x 2 = 2	2 x 12,000	24, 000
Average Total.						**15, 894, 000**

The estimated annual loss associated with organ condemnation was K15, 894,000 (USA $3,311) annually: **[**Average exchange rate during the study was 1US$= K4800, Source Bank of Zambia]. This was a function of the average annual slaughters; condemnation rate and average price of the condemned organs.

4.2. Retrospective Human Survey

4.2.1 Prevalence of human cystic echinococcosis at Lewanika Hospital between 2006 and 2010.

A retrospective survey of cases of human hydatidosis at Lewanika General Hospital in Mongu district revealed that 9 cases out of 95,064 people that were attended to, were diagnosed with CE between 2006 and 2010 (Table 4.2). This represents a group prevalence of 9 cases per 100,000 humans scanned when the total number of people attended to is taken into consideration. All the nine cases were clinical cases and none was an accidental finding. Diagnosis was made using clinical signs and confirmed by ultra-sonography.

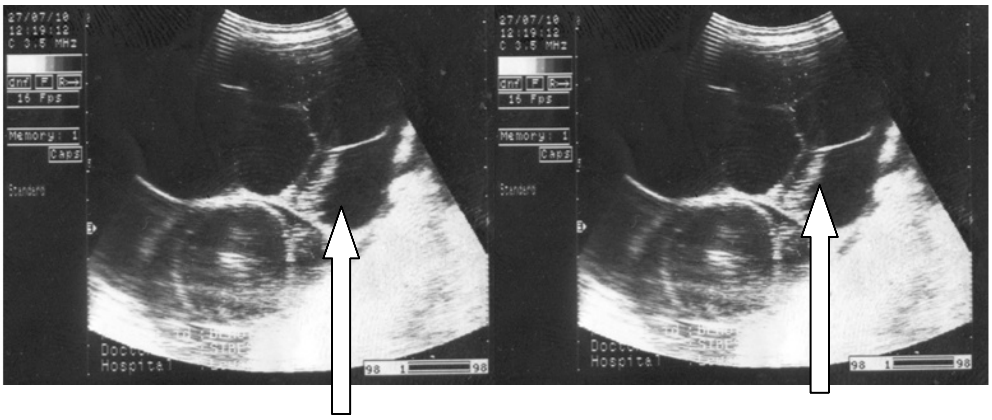


Figure 4.3. Ultrasound images of human hydatidosis, showing hydatid cysts (dark circular zones as indicated by the arrows) in human liver. (**Source**: Ultra Sound Department at Lewanika Hospital, 2010)

Table 4.2 Total number of patients attended at Lewanika General Hospital during the period 2006 to 2010.

Year	**n**	**No of +ve cases**	**No. of +ve cases per 100,000**
2006	25,730	1	4
2007	22,607	3	13
2008	23,915	2	8
2009	22,813	2	9
2010	24,812	1	4
Total	**95,064**	**9**	**9**

4.2.2. Prevalence of human cystic echinococcosis at Lewanika Hospital by sex.

Sixty-seven percent (67) of the cases diagnosed were females and 33% (3/9) male (Figure 4.3). The records were showing that the main clinical symptom observed in all the cases was ascites. Other clinical signs included jaundice, chronic cough and abdominal pain.

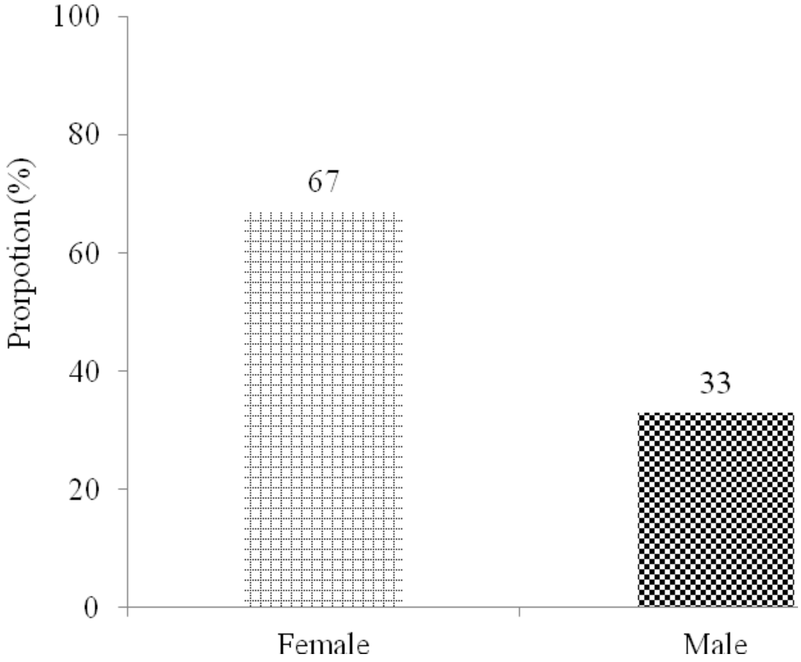


Figure 4.4. Proportion of positive human cystic echinococcosis cases by sex recorded at Lewanika General Hospital in Western province of Zambia between 2006 and 2010.

4.2.3 Prevalence of human cystic echinococcosis at Lewanika Hospital by age.

The age range for positive human CE observed at Lewanika Hospital was 17 to 78 years with a mean of 52 years. A total of 9 cases out of 95, 064 were recorded (Table 4.3) All the cases were found in the liver. Eight of the cases were adults (age range: 43-61 years) whereas the other case involve a 17 year old female (Table 4.3).

Table 4.3 Cases of human hydatidosis at Lewanika General Hospital by age, sex and place of origin (2006-2010).

Gender	Age	Origin	District	Hospital Attendance
Female	57	Mulambwa	Mongu	25,730
Male	46	-	-	-
Female	43	Mongu	Mongu	-
Female	52	Mupwisa	Mongu	22,607
Male	78	Mangango	Kaoma	
Male	61	Lububa	Kaoma	23,915
Female	55	-	-	-
Female	60	Lububa	Kaoma	22,813
Female	17	Kaoma Town	Kaoma	24,812

Key: -‘-‘data not available

4.3. Prospective cattle study

4.3.1. Overall prevalence of bovine echinococcosis in Western Province.

Four thousand and sixty one (4061) Barotse breed cattle were slaughtered and examined at the Zambeef® and Starbeef® abattoirs in Mongu between October 2007 and November 2008. Out of these, 2441(60.1%) cattle came from Mongu, 577 (14.2%) from Senanga, 653 (16.1%) from Kalabo, 335 (8.2%) from Lukulu, 47 (2.1%) from Shangombo and 8 (0.2%) from Kaoma district (Table 4.4). Eighty-four (2.1%) of the carcasses were diagnosed positive for hydatidosis during post-mortem inspections. Spatial variations in prevalence of hydatid cysts in cattle were found. Cattle coming from Mongu had the highest prevalence of cyst positive cases (2.5%)

compared to Senanga (2.1%), Kalabo (1.4%) and Lukulu (0.6%). No hydatid case was recorded in cattle coming from Shangombo and Kaoma (Table 4.4).

Table 4.4 Prospective abattoir survey results of hydatidosis in slaughtered cattle from Western Province (2007 to 2008).

District	**n**	**Organ**			**Total +ve (%)**
		Lung (%)	**Liver (%)**	**Kidney (%)**	
Mongu	2441	29 (1.2)	35 (1.4)	1 (0.1)	2.7
Senanga	577	5 (0.8)	3 (0.5)	0	1.3
Kalabo	653	8 (1.2)	1 (0.2)	0	1.4
Lukulu	335	1 (0.3)	1 (0.3)	0	0.6
Shangombo	47	0	0	0	0
Kaoma	8	0	0	0	0
Total	**4061**	**43 (1.1)**	**40 (1)**	**1 (0.02)**	**2.1**

4.3.2. Prevalence of bovine echinococcosis in Western Province from prospective study by age.

Of the 4061 cattle analysed by age, the mean age among the positive was 7.8 (95% CI: 7.4 - 7.6) while that among the negative was 7.5 (95%CI: 7.3 - 8.3) (Table 4.5). On t-test analysis, there was no significant association between age and cattle infection with hydatid cyst (P= 0.31).

Table 4.5 Prevalence of cystic echinococcosis in cattle by age in traditional cattle (n = 4061) in Western Province (2007-2008).

Disease status	Number cattle	Mean age in years	95% Confidence Interval (Years)
Positive animals	84	7.8	7.4 - 7.6
Negative animals	3977	7.5	7.3 - 8.3
Total	**4061**	**7.5**	**7.4 - 7.7**

4.3.3. Prevalence of bovine echinococcosis by sex.

Sex was found to be positively associated with hydatidosis (P = 0.035) with female cattle being more likely to test positive than males (Odds ratio = 1.6) (Table 4.6).

Table 4.6 Positive bovine CE cases by sex in cattle slaughtered in Mongu (2007 – 2008)

Sex	# slaughtered	Positives	Prevalence (%)	95% CI
Female	2215	36	1.6	(1.1 – 2.1)
Male	1845	48	2.6	(1.9 – 3.2)

4.3.4. **Prevalence** *of bovine echinococcosis by veterinary camp.*

Seventeen veterinary camps out of thirty-six camps where the slaughtered cattle originated from had positive cases of bovine hydatidosis while nineteen camps did not have any positive cases observed. This meant that 47% of the camps had at least one case of bovine hydatidosis observed. Prevalence of bovine hydatidosis was found to vary in different veterinary camps within districts.

Mukukutu camp in Senanga district, which had four out of nine camps with observed cases of bovine hydatidosis (44%), accounted for the highest prevalence at 4.0% (95% CI: 3.8-11.8%) while Lukulu Central camp in Lukulu district had the lowest at 0.3% (95% CI: 0.2- 0.9%).

The comparison of bovine CE in camps in different districts; revealed that in Mongu, seven out of the nine (78%) veterinary camps that were sampled had bovine hydatidosis with the highest prevalence of bovine hydatidosis observed in Limulunga at 2.9% (95% CI: 1.4 – 4.4%) and the lowest in Luandui camp at 1.5% (95%CI: 0.5 – 3.7%) (Table 4.7). Mongu district overall prevalence was 2.7%.

In Senanga district, the highest prevalence was in Mukukutu camp at 4.0% (95%CI: 3.8- 11.8%) and the lowest was in Mouyo camp at 1.6% (95%CI: 0.04 – 3.2%) (Table 4.8). The overall prevalence for Senanga was 1.3%.

In Kalabo district, the highest prevalence was observed in Sikongo camp at 3.3% (95%CI: 1.2 – 8.0%). Kalabo district had four camps out of ten (40%) with cases of bovine hydatidosis (Table 4.7). The overall prevalence for Kalabo was 1.4%.

In Lukulu district, which had two camps out of three (67%) with positive bovine hydatidosis, the highest prevalence was observed in Mbanga camp at 1.8 % (95%CI 1.7- 5.3%) and lowest in Lukulu central camp at 0.3% (95% CI: 0.2 – 0.9%) (Table 4.9). The overall prevalence for Lukulu was 0.6%.

Table 4.7 Prevalence of hydatid cysts in slaughtered cattle according to veterinary camps (2007-2009) in Mongu district.

Camp	Proportion positive%	95% Confidence Interval (CI)
Luatembo	2.8	1.1 – 4.6
Mongu Central	2.5	1.4 – 3.6
Namushakende	2.3	0.5 - 4.2
Ndanda	1.9	1.8 – 5.6
Limulunga	2.9	1.4 – 4.4
Kama	2.8	1.0 – 4.6
Luandui	1.5	0.5 – 3.7
Litawa	0	-
Ushaa	0	-

Table 4.8 Prevalence of hydatid cysts in slaughtered cattle by veterinary camps in Kalabo district (2007-2009).

Camp	Proportion positive%	95% Confidence Interval
Kalabo Central	2.0	0.05 – 4.0
Mapungu	2.6	0.3 – 5.6
Lueti	3.0	2.9 – 8.9
Sikongo	3.3	1.2 – 8.0

Table 4.9 Prevalence of hydatid cysts in slaughtered cattle by veterinary camps in Senanga district (2007-2009).

Camp	Proportion positive%	95% Confidence Interval
Mwandi Ukolo	1.9	1.8 – 5.8
Nande	0%	-
Senanga Central	0%	-
Nanjucha	0%	-
Nande	0%	-
Senanga Central	0%	-

Table 4.10 Prevalence of hydatid cysts in slaughtered cattle by veterinary camps in Lukulu district (2007-2009).

Camp	Proportion positive%	95% Confidence Interval
Lukulu Central	0.3	0.2 – 0.9
Lupui	0%	-
Mbanga	1.8	1.7 – 5.3

4.3.5. Distribution of hydatid cysts in different body organs.

In the study, distribution of hydatid cysts by organ was found to be 51.2% in the lungs, 47.6% in liver and 1.2% in the kidneys (Figure 4.6). Cysts were classified as either external (Figure 4.4) or embedded (Figure 4.5) depending on their location in the organs. Some organs had both internal and external cysts.

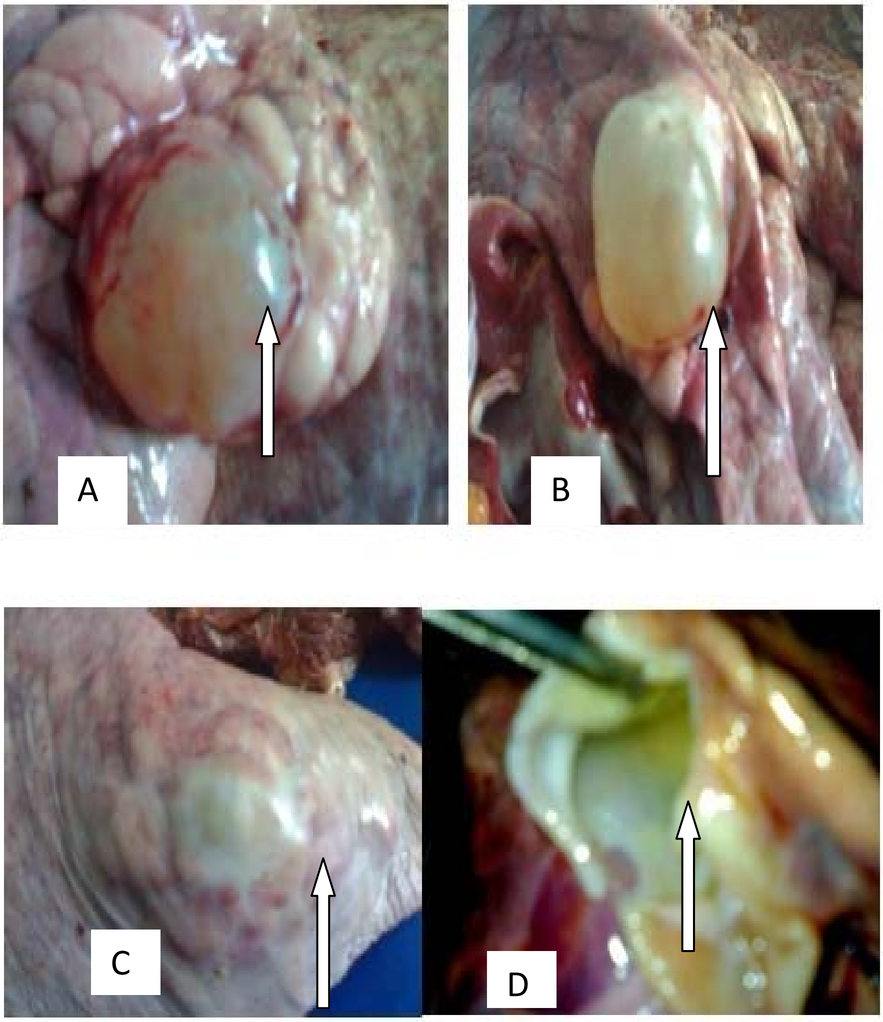


Figure 4.5: *Echinococcus* cysts on bovine lungs as shown by arrows in A and B, and an imbedded *Echinococcus* cyst in a bovine lung in C and incised hydatid cyst showing the germinal layer in D.

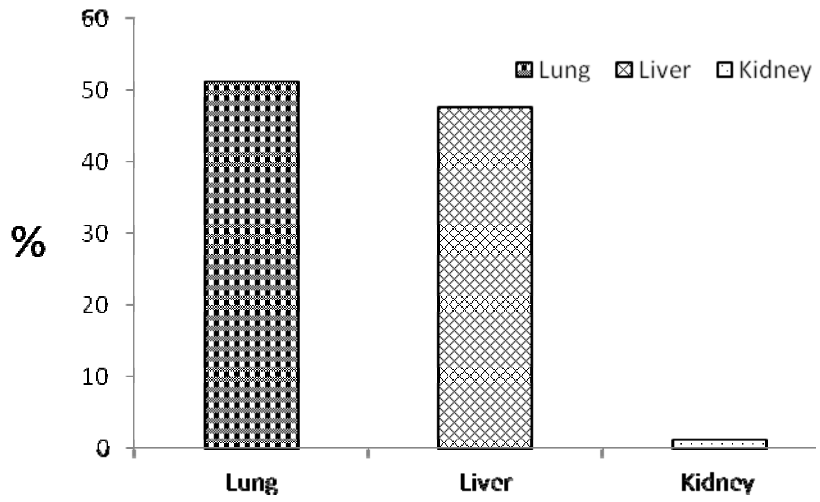


Figure 4.6. Proportion of positive cattle hydatidosis cases by organ from Mongu abattoirs (2007 to 2008).

4.4.1. Viability of hydatid cyst.

Nineteen hydatid infested organs were investigated (15 lungs and 4 livers) for cyst viability and density per organ. The overall percentage of fertile cysts was 43.1% (Table 4.11). Table 4.11 shows the median number of live cysts in the lungs and liver are not significantly different. The percentage of live cysts in the liver was 43.7 and in lung was 43. The values of average percentage of live cysts between the two organs were similar and hence there was no significant difference in the fertility rate of cysts.

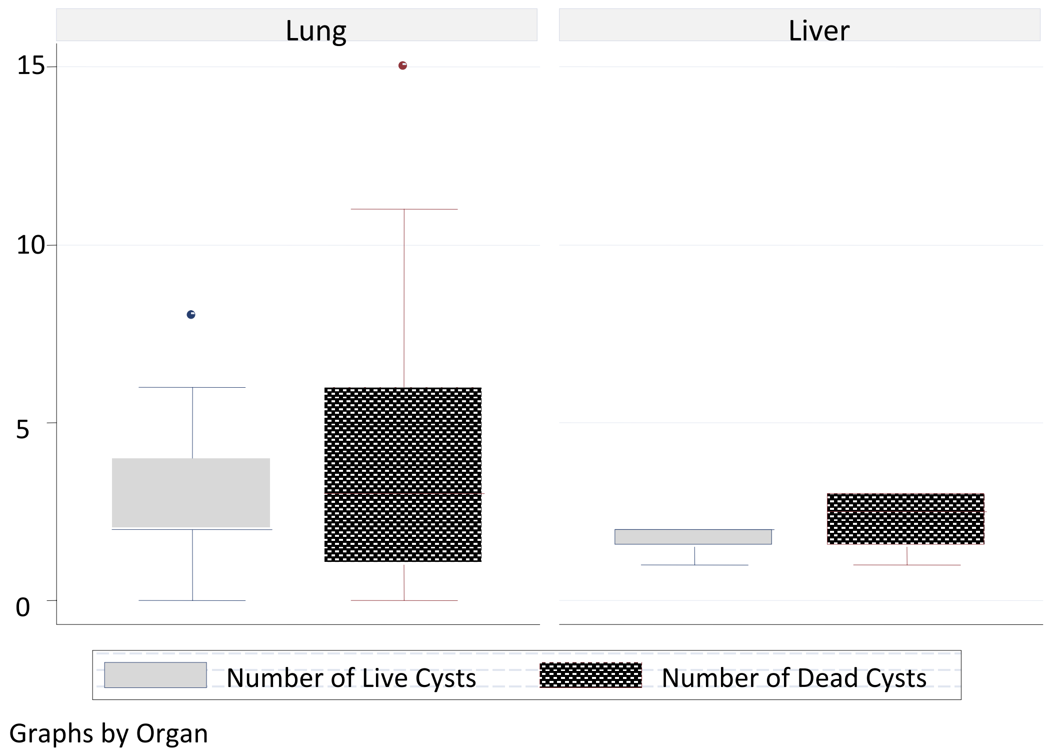


Figure 4.7: Box plots showing the median (central line) and quartile ranges of live and dead cyst counts per organ**.**

4.4.2. Hydatid cyst density.

The overall median number of cysts in an organ was 6 (range 2-21), in the lungs the median count was 6 (range 2-21) and liver the median was 4 (range; 3-5). The number of hydatid cysts that were examined in the lung was 108 while in the liver it was 16. The lung had a higher density of cysts per organ compared to the liver (Table 4.11).

Table 4.11 Average values of mean cysts per organs, the 95% CI and the live dead cysts ratios.

Organ	**# organs examined**	**Average # Cysts per organ**	**Range of # of cysts**	**Mean # of live cysts (95% CI)**	**Mean # of dead cysts (95% CI)**	**Average % of live cysts**	**Average % of dead cysts**	**Average Ratio live/dead cyst**	**Ratio dead/ live cyst**
Lung	15	7	2-21	3 (2-4)	4 (2-6)	43.0%	57%	0.8	1.3
Liver	4	4	3-5	2 (1-2)	2 (1-3)	43.7%	56.3	0.8	1.3
Overall	19	6	2-21	4 (2-4)	3 (2-5)	43.1%	56.9%	0.8	1.3

4.5. Investigation of risk factors.

4.5.1. Sample description (Socio-demographics).

A total of 66 households were investigated in Senanga (n =20), Mongu (n = 33) and Kaoma (n =13) districts of Western province. The median household (HH) size was 8 persons (range 3 to 17), while the median number of dogs kept per household was 2 (range 1-7 dogs).

4.5.2 General dog and livestock management system

During the study, it was observed that 88% (95% CI: 77.5-94.6) of the HH owned at least one dog. Among those that kept dogs (n = 58), the majority (96.6%) of dogs were managed using the free range system.

Figure 4.8. Backyard unsupervised slaughters in Senanga Western Province (Free access by scavenging dogs to cattle viscera).

These dogs were mostly kept for security (86.2%) and cattle herding (87.9%) although some dogs were also kept for other purposes such as companionship and hunting. In addition, 65% of respondents admitted that stray dogs were regularly spotted in their communities. Most of the HH (94.8%) admitted to feeding offal to dogs and 37.9% of the respondents observed dogs scavenging from abattoirs and local slaughter slabs.

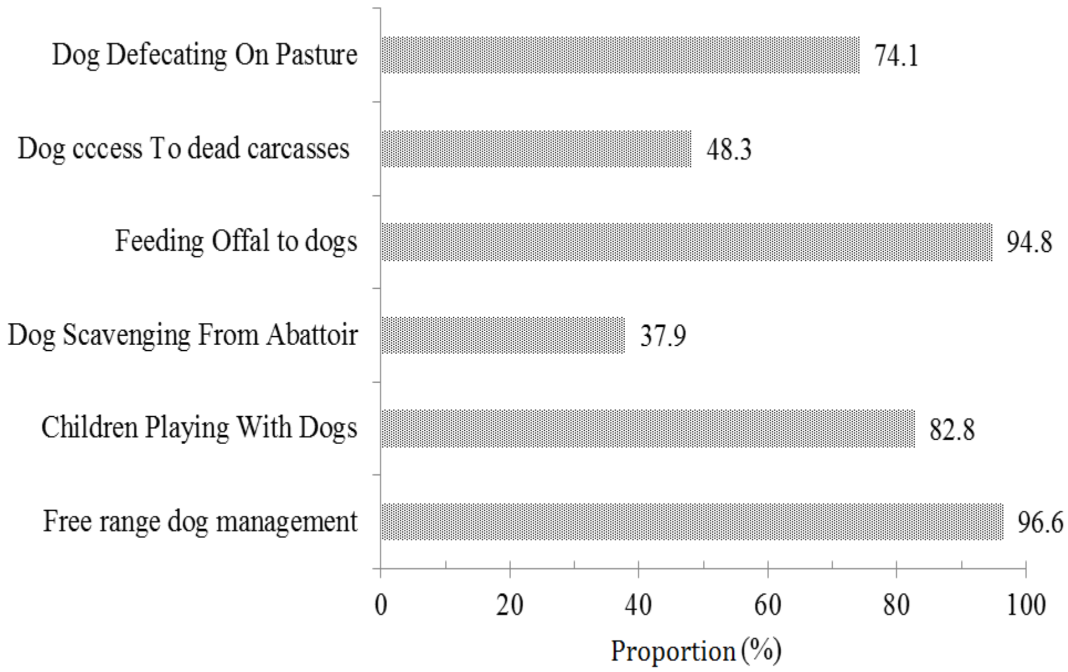


Figure 4.9 Some risk factors perpetuating the transmission and maintenance of hydatid echinococcosis in Western province.

4.5.3. Awareness of cystic echinococcosis by the community.

Whilst a larger proportion (95.4%) of the surveyed households were aware about the risk of contracting rabies from dogs, fewer (39.4%) HH knew about the possibility of dogs transmitting helminth and other diseases (Figure 4.10).

It was observed that 98% of the households had dogs that defecated in the immediate surroundings of their dwelling places. An investigation into the presence of *Echinococcus* associated clinical signs such as ascites and chronic cough showed that 75% of the respondents knew at least one person who exhibited such clinical sings.

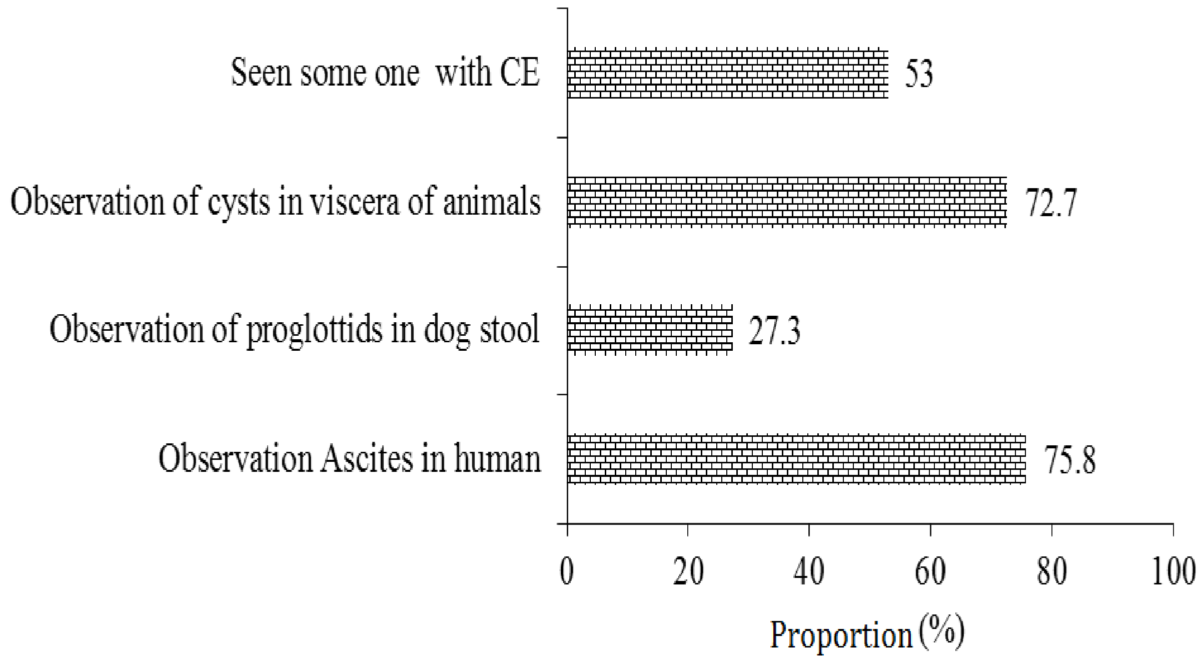


Figure 4.10. Disease awareness by the interviewed households of Cystic Echinococcosis.

On presence of hydatid cysts in viscera of slaughtered livestock or those that died on their own, 73% of the respondents reported having seen hydatid-like cysts in one or more visceral organs.

It was further observed that only 16 % of the surveyed households had at least one person having some form of tertiary education. Twelve percent of the respondents kept dogs for hunting while most (86.2%) kept them as watch dogs. 84.8% of humans had never had any antihelmintic treatment.

In addition, 72% of respondents were aware that cyst infested meat or offal were not safe to eat.

CHAPTER FIVE

DISCUSSION

5.1. Retrospective abattoir survey.

Like it is a common practice in most sub-Saharan African countries and other regions, the research data was obtained from slaughter records of cattle at abattoirs. Both the retrospective review and prospective surveys were carried out to assess the prevalence of bovine CE and evaluated the associated economic losses due to organ condemnations.

There was a significant difference in the annual prevalence of hydatid cysts between carcasses slaughtered in 2007 and 2008 (P = 0.024). The reason for this is not easily discernible but could be due to more animals having come from areas of higher prevalence of bovine hydatidosis such as Mongu and Senanga in 2008 than in 2007. However, this could not be ascertained due to absence of trace back information during the period under review.

The lung was found to be the most affected organ at 93.7% of the total positive bovine hydatidosis cases while the liver accounted for 6.3% of the positive cases and the spleen 0.02%. This is in agreement with what was reported by Getaw *et al*., (2010) who observed that the lung had a higher prevalence at 55.2% and the liver at 37.1% while the kidney was the least affected organ. Unlike other researchers who found higher prevalence in the liver than in the lung (Kassem, 2006), Azlaf and Dakkak (2006), Ibrahim and Craig (1998) and Dalimi *et al.,* (2008), this study showed that the lung has a higher likelihood of being infected and this was in agreement with findings by other researchers, (Kebede *et al.,* 2009; Getaw *et al.,* 2010,). In contrast, a study carried out in Libya by Al-khalidi (1989) showed that 75% of the positive bovine hydatidosis cases were in the liver and 37.5% in the lung, and 12.5% in the spleen. Ibrahim (2009) also found that the liver had higher prevalence than the Lung. The reason why the lung and liver are mostly affected could be due to the fact that the lungs and livers are the first capillary beds encountered by migrating Echinococcus oncospheres via the portal vein route before any other peripheral organs. The lungs however have a larger capillary bed than any other organs and this may account for the higher prevalence than the other organs (Getaw *et al.*, 2010). In humans however, the liver is most commonly affected (Tashani *et al.*, 2002; Ahmed *et al*. 2004). The results of this study are in agreement with these findings. The explanation to the difference in the predilection sites between cattle and human is however beyond the scope of this study.

5.2. Retrospective human survey.

In the human hydatidosis survey, the positive proportion of human hydatidosis was found to be low at 9 cases per 100,000 of those who attended the ultra sound and x-rays section at Lewanika Hospital in the reviewed period. Due to the limitation of time, only a few cases were reported and therefore the reported disease estimate may not indicate the true situation. The recording of 9 cases at Lewanika hospital indicated that CE poses a public health problem in Western Province of Zambia. Studies in different parts of sub-Saharan Africa have revealed different infection levels and varied distribution (Magambo *et al.*, 2006). Hospital reports in Uganda have shown an average of 20 surgical cases per year in each hospital in Karamojang (Macpherson *et al.*, 2004). These findings indicate that CE is a public health and an economic problem in Uganda (Magambo *et al.*, 2006). In Sudan, a recent ultrasound survey with 300 and 651 people in two different areas showed prevalence in human CE of 0.3% and 0.8% respectively (Elmahdi *et al.*, 2004). In southern Sudan the prevalence was found to be

2% among the Bouya people and 3.5% among the Toposa people (Magambo *et al.*, 1996). In Burkina Faso, the prevalence of human CE has been reported at 0.007% (Coulibaly and Yameogo, 2000) this is in agreement with the observation in current study (0.009% or 9 cases per 100,000 attended to at the hospital). The differences in the prevalence of human CE in different regions are mainly due to the human animal interaction and ethnic beliefs which expose the humans to different risk factors. There is also an element of under reporting of human cases of CE as a result of clinicians not being able to recognize the disease.

Like in cattle, the results in humans show that there is a higher prevalence in females than in males. Females accounted for 67% of the positive human hydatidosis cases while males accounted for 33% of the cases on ultra sound scanning. The cases were mainly from Kaoma and Mongu Districts. This observation is despite the fact that Kaoma had no bovine cases from the cattle survey results. This could mean that in Kaoma, there could be other intermediate hosts rather than cattle. The most likely intermediate hosts for Kaoma could be goats and pigs which are kept in bigger numbers than in other districts. In Mongu, Kalabo, Lukulu and Senanga, the most likely strain of CE could be *Echinococcus ortlepi* which is normally not very effective to humans and is characterized by high cystic fertility in cattle as observed in this study. However, no equivocal statement can be made regarding the infection *Echinococcus* since this requires characterisation of the infecting species.

The fact that the disease has been diagnosed in both cattle and humans, notwithstanding the fact that some slaughters are not supervised, is an indication that there is a possible environmental contamination with ova of *Echinococcus granulosus* in Western Province of Zambia. Both human and animal cases of CE could be higher than the figures obtained in this study. This calls for effort to curtail the contamination of the environment by the definitive host which in this case is the domestic dog (Gemmell, 1990).

5.3. Prospective abattoir survey.

In this study, the prevalence of hydatidosis in cattle was investigated using abattoir survey. It should therefore be noted that the prevalence estimates provided here could have some bias. This is so because the abattoir sample population is not always representative of the reference population from where animals are drawn. This could be due to the fact that animals brought for slaughter were mainly those that were old or out of production. Further, the disease prevalence was estimated based on PM diagnosis of cattle brought to the abattoirs, and not those that were slaughtered in the back yards and were not inspected. During PM inspection there is always a possibility that some positive cases could be missed resulting in under-reporting the disease burden. Despite these short-comings, abattoir survey data is routinely used to estimate disease burden because of easy feasibility of conducting abattoir surveys compared to field surveys based on random study designs. To improve on accuracy, all people that were involved in conducting PM inspections received brief training before the commencement of the study.

The observed prevalence of hydatid cysts in cattle sampled at the two abattoirs in Mongu was found to be 2.1% and was comparatively close to that observed during the retrospective survey (3.0%). The observed prevalence in this study is in agreement with that observed in a study done in Sudan which reported a prevalence of 3% in cattle (Elmahdi *et al.*, 2004). In Arusha Tanzania, a study by Nonga and Karimuribo found the prevalence of 4.2% in cattle (Ernest *et al.*, 2009). Similarly, low levels of bovine hydatidosis were reported by Njoroge *et al*. (2002). In contrast to this, some studies done elsewhere in Africa have reported higher prevalence. For instance, Azlaf and Dakkak (2006) reported prevalence of 23.0% bovine hydatidosis in Morocco and so did Kebede in Ethiopia who reported a prevalence of 16 % (Kebede *et al.*, 2009). More recently, studies in Ngorongoro district of Tanzania reported bovine CE prevalence of 48% and 16.85% at Wolayita Sodo abattoir in Southern Ethiopia (Sekele, 2010).

The spatial distribution of hydatidosis varied according to district with Mongu reporting the highest compared to other districts. The reason for the high prevalence in Mongu could be due a high number of cattle and dog populations coupled with a high number of back-yard slaughters which in some cases are not inspected by the veterinary department staff. In general, the difference in prevalence of hydatidosis in different districts and camps could be as a result of difference in animal husbandry practices, unsupervised slaughter of animals, improper treatment of infected carcass which dogs easily scavenge and the dog population in a particular area.

Unlike in the study conducted in Saudi Arabia in slaughtered cattle in Al Baha region by Ibrahim (2009) where age was found to have a significant effect on prevalence of CE in cattle, the current study did not find age to have any significance influence on the prevalence of bovine hydatidosis in the prospective cattle survey. The results of this study are in agreement with the results obtained by Berhe (2009) in Ethiopia who equally did not find any significant association between age of cattle and prevalence of CE.

The possible reason why apparently age had no effect on the prevalence was that, most cattle were slaughtered when they were old (Median 7 years) with very few cattle slaughtered when they were less than three years old. The skewed distribution of age among slaughtered cattle in this study is likely to distort the relationship between CE and age that could be found in a population where age is normally distributed.

Considering the fact that bovine echinococcosis has a long course stretching into several years after infection, it is likely to be a problem in old animals.

Sex was found to be positively associated with hydatidosis [P= 0.035] with female cattle being more likely to test positive than males. This is in agreement with the findings by Darayani *et al.*, (2007) in Iran, who reported that the prevalence was higher in females than males. The other reason for a higher prevalence of CE in female cattle than in male cattle could be due to the fact that female cattle remain longer in the herd for reproduction purposes than males and therefore have a higher probability of having more encounters with the ova of *E. granulosus* during their longer life span than their male counterparts.

5.4. Viability of hydatid cysts form slaughtered cattle.

Cysts viability study revealed that the overall percentage of fertile cyst in this study was 43.5% which is comparable to findings by other researchers like Ibrahim (2009) who found cyst fertility of 47.8 % in sheep and 24 % in goats. However, different observations were reported by Rinaldi *et al.*, (2008), who did not observe any viable cyst from their survey and Berhe (2009) who found a lower fertility rate of 10.7% in cattle in Tigray region of Ethiopia. The possible reason why no viable cysts were observed by Rinaldi *et al.* (2008) could be due to the differences in immunological responses by different individual hosts or de-worming of the animals by use of anti-helmethics Daryani *et al.* (2009).

Out of a total of 19 hydatid cyst infested organs that were investigated (15 lungs and 4 livers) for cyst fertility , viability and density, it was found that the lung had a higher average density of cysts infestation (7 cysts per lung), while the liver had a low hydatid cyst density (4 cysts per liver). This was however different from findings by

Mohamed (2009) in Saudi Arabia who observed that the liver had a higher cyst density .The difference in the cyst density could mainly be attributed to the higher vascularisation of lung tissue compared to liver (Kebede *et al.,* 2009). The other reason in the difference in cyst density could be as a result of the soft texture of the lung tissue in comparison to liver (Getaw *et al*., 2010), which has a harder texture thus restricting hydatid cyst development.

The median number of cysts in an organ was 6 (range 1-21). The number of hydatid cysts that were examined in the lung was 108 while in the liver was 16.

Most of the dead cysts in the liver were found to be calcified compared to the lung. This is in agreement with the findings by Dalimi *et al.* (2002), Kebede *et al.* (2009) and Berhe (2009) who reported a higher fertility rate of pulmonary and lower fertility rate in hepatic cysts. This could probably be due to the various metabolic reactions that take place in the liver as compared to lungs. However, Ibrahim (2009) found a higher fertility rate in liver at 38.8 % than in the lung at 25.1 % and so did Dalimi (2002) who reported a higher fertility rate in the liver than in the lung.

The high percentage of viable cysts indicates that there is a high risk of dog exposure in situations where offal are carelessly disposed off and dogs have access to the infected offal, like the situation is in Western province of Zambia.

5.5. Investigation of risk factors.

Cattle acquire CE infection by ingesting fertile and viable eggs of *E. granulosus* excreted by infected dogs. So for the infection to be maintained in a population, both the definitive and the intermediate host should be present.

The study revealed that 88 % of the interviewed households owned dogs while 98.3% owned cattle thus basing on the knowledge of the transmission of CE between the dog which is a definitive host of *Echinococcus granulosus* and cattle which are the intermediate host in the transmission cycle of CE, it can be concluded that the ownership of the two animal species pose a high risk of exposure to CE in dogs and cattle. It is known that the abundance of infected definitive host and a high stocking rate of livestock contribute to the transmission of CE and to the differences in prevalence in different areas (Macpherson *et al.*, 1985; Njoroge *et al.*, 2002).

The median number of dogs kept by the interviewed household was 2 (range 1-7) of which 96.6% were managed under the free range system with the dog owners having no control of where the dogs scavenged. This poses a serious risk to transmission of *Echinococcus granulosus* from infected cattle organs to the dogs which would in this case have access to infected offal due to their uncontrolled scavenging.

The presence of stray dogs in the communities as it was stated by 65% of the respondents has a significant impact on transmission and maintenance of CE in cattle since these dogs are scavengers and have a higher chance of accessing condemned offal.

The common practice (87.9%) of allowing dogs to accompany cattle as they graze on pasture is a known risk factor as this exposes cattle to dog feaces on the pasture and thus raises the possibility of cattle ingesting viable ova of *E. granulosus* (Eckert and Deplazes, 2004*)*. Most of the households that keep dogs do not de-worm them or take the dogs for treatment when they are sick, this implies that vaguely every dog that has *E. granulosus* infestation has a high possibility of transmitting the worm to cattle and other susceptible hosts including humans.

Humans acquire primary CE by oral uptake of *E. granulosus* eggs excreted by infected dogs (Eckert and Deplazes, 2004). Since 88 % of the interviewed households owned dogs, which are known to transmit echinococcosis, owning a dog is a known risk factor for human exposure to echinococcosis.

In this study, it was generally observed that children in most households played with dogs. Being a child (age) is a known risk factor to acquiring of CE (Wang *et al.*, 2001; Larrieu *et al.*, 2002; McManus *et al.*, 2003). The fact that the majority of these dogs were neither de-wormed nor received any kind of treatment may imply that they are most likely to harbour helminthes which could include *Echinococcus granulosus*.

Furthermore, drinking of untreated water from shallow water wells commonly practiced by many households is a known risk factor for exposure to helminths infestations (Macpherson, 2005).

It was also discovered that dogs defecate in the immediate surroundings and this coupled with the fact that both the children and dogs were never de-wormed could imply an increased risk of exposure to helminthes including CE.

Since 53% of the respondents acknowledged knowing someone in their family or community who had suffered or had been diagnosed with CE, it implies that there was a real risk of exposure to echinococcosis. Generally, it was observed that helminth associated clinical signs such as ascites and chronic coughs were prevalent in the communities investigated implying that helminth infestation could be a huge public health problem in the investigated communities. However, no equivocal statement can be made over this as data was not collected to investigate the association between clinical signs and helminth infestation.

The majority of the people in the communities had low literacy levels with only 6 % of the respondents having some form of tertiary education. It has generally been shown that ignorance and poor sanitary conditions are risk factors for exposure to infectious diseases including helminths (McManus *et al.*, 2003; Macpherson, 2005).

Low education in most cases means reduced/ or inadequate knowledge of disease and risk factors that may be associated with disease transmission in communities. It may also implied poor knowledge by the respondents to recognise that the cysts they observed on some visceral organs had the potential to transmit CE to dogs and thus to humans and cattle.

The fact that a large proportion of respondents noted that cysts infested organs/ meat were unsafe to eat could be for aesthetic reason and not knowledge that these have a potential to transmit CE.

The presence of several known risk factors in the communities meant that unless control measures were implemented, the transmission cycle of CE could easily be sustained.

5.6. The economic losses due to organ condemnation.

In this study, the annual economic loss as a result of condemnation of organs due to bovine hydatidosis was moderate at K15, 894,039 (U$3,311) per annum. The loss was found to be moderate due to the low prevalence of hydatidosis in cattle in Western Province of Zambia. However, this figure of the annual economic loss is comparable to that which was recently calculated by Getaw in Ethiopia who found an annual economic loss due to organ condemnation at U$5,059 (Getaw *et al.*, 2010). Considering the low income levels in addition to poor nutrition experienced by most rural families, the annual economic loss from organ condemnation in Western Province of Zambia could be considered significant if we take into account the local economic environment.

Back-yard slaughters are quiet rampart in the communities as shown by the high number (40%) of respondents who admitted to carrying out home slaughter as shown from the questionnaire survey from this study and this could mean that the actual economic loss due to CE is higher than what is recorded during meat inspection.

Furthermore, the economic impact of CE could be higher than the calculated figure due to the fact that other indirect losses such as loss of carcass weight and other reproduction related losses were not captured in this study. It is generally accepted that there is an estimated 5% reduction in carcass weight attributed to CE in infected cattle (Budke *et al.*, 2006). The annual total loss is thus greater than the calculated figure taking into account the fact that the present study only took into account the direct losses. Indirect losses associated with weight loss due to CE as well as losses due to reduced milk production and reproduction efficiency were not accounted for in the present study.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

The main thrust of the study was to describe the disease situation so as to increase public health awareness, identify the risk factors of transmission and to suggest possible mitigation measures. The determination of prevalence of echinococcosis in cattle and humans has some practical implications. Observational studies such as the current study help detect the disease and estimate prevalence and other parameters easily. It should be noted however, that echinococcosis is a disease of multiple hosts and the objective of this study could only be addressed by the application of conventional observational studies.

This study demonstrated that hydatidosis is an important disease and is endemic in Western Province. The disease also caused considerable economic losses as a result of offal condemnations. Despite the low infection rate demonstrated by the current study, there are certain socioeconomic conditions that are favourable for the existence of CE, and therefore CE still remains one of the most important helminth zoonotic disease hence the need for increased attention in control and prevention of the disease. Among the important socioeconomic conditions include; pastoral occupation as evidenced by a high percentage of people keeping cattle, lack of adequate health education and poor hygiene practices as evidenced by the consumption of untreated well water and carcasses from cattle that die on their own.

The prevalence of hydatidosis in cattle and humans varied from one District and camp to another. In cattle the level of infestation was found to be 3% on retrospective study and 2.1 % on prospective study. Domestic animal species Echinococcosis prevalence studies and surveillance can help map out CE risk landscape profiles that will determine communities at greatest risk to human CE.

The human study also demonstrated that human hydatidosis is present in Western Province with 0.009% (9 per 100,000) of people who were attended to between 2006 and 2010 having been diagnosed with human hydatidosis at Lewanika Hospital. Human cases seemed to be higher in females than in male suggesting that sex was a risk factor that predisposed human beings to infection.

A human sero-survey in conjunction with follow-up diagnostic imaging procedures could reveal the true extent of human CE. A molecular analysis of human and animal hydatidosis would be desirable in order to effectively map out the epidemiology of the disease and determine the spread of the disease. A specific study of the disease in dogs could help in knowing the prevalence in the definitive host. Wildlife species have not been shown to harbour *E. granulosus* in Zambia. In view of the extensive livestock/wildlife interface areas in the province, a study in these would also be useful.

Furthermore, a study in small ruminants such as sheep and goats may improve the epidemiological understanding of the disease in Zambia, since worldwide; sheep are reported to be more prone to CE.

It is recommended that public health measures such as control of stray dogs and provision of and strengthening of meat inspection services at abattoirs be encouraged in Western province. Home slaughter of cattle should be discouraged. These measures would definitely contribute towards effective control of CE and reduce the risk of transmission to humans.

With the current study having been done in humans and cattle, a further study is imperative in dogs so that the epidemiological picture is complete. It is also suggested that in areas where there is a presence of large numbers of wildlife, definitive hosts such as observed in some parts of Kalabo, Lukulu and Kaoma districts, increased effort should be made to sample some of the possible wildlife definitive host of *Echinococcus* spp. Efforts should be made to ascertain the host specificity of local strains of the parasite in respect to cattle and other domestic animals. To effectively come up with a control program, possible wildlife reservoirs and the survival of eggs under the local climatic and soil conditions have to be investigated.

The study brought out some key factors associated with the persistence of the disease in human and animal populations which could be summarized as follows: A close association of dogs and other animals, especially cattle in grazing areas; Lack of health education; Easy access of dogs to offal which could be infected with *E. granulosus*; Un inspected home slaughters; Feeding of offal from cattle to dogs; Drinking of untreated water from shallow wells due to poor living condition; Children playing with dogs; Presence of large numbers of dogs, including stray dogs which could be infected by *E. granulosus* due to the fact that they are not dewormed; and A high percentage of fertile hydatid cyst in cattle as shown by a high percentage of fertile and viable cysts.

It would be a good idea to consider declaring hydatidosis a modifiable disease, if Zambia is to contemplate an effective control of the diseases. In summary, any attempt at the control of cystic echinococcosis, should consider three main interventions namely; human treatment (which should include surgical, PAIR and chemotherapy), dog population control (killing of stray dogs, sterilization of dogs and de-worming of owned dogs) and community sensitization (in all high risk areas and schools).

CHAPTER SEVEN

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APPENDICE

A : Echinococcosis Questionnaire

A. RESPONDENT DETAILS

A1. Name: ……………………………………………………………………………………………

A2. Age: ………………………………A3. Sex: …………………………………………………

A4. Position in the household: ……………………………………………………………........

A5. Highest education level in the household: …………………………………………………

A6. Occupation: ……Farmer……Fisherman…………Businessman.............Office worker..........

A7. Monthly average income...

B. HOUSEHOLD DESCRIPTION

B1. Household identification number: …………………GPS coordinates:……………………........

B2. Name of household head: …………………………………………………………..…

B3. Ethnic background: ……………………………………………………………………………

B4. Main household income: ……………………………………………………………...

B5. Number of members of the household: ……………………………………………........

B6. Is it a dog keeping house hold? (Y/N)....................... No of Dogs..

B7. Where do the dogs roam? Confined to dog house on compound,…….. . (1) Inside House ….. (2)Within the Compound… (3)Anywhere within and outside the compound.

How are the dogs kept? (1) Free range …….(2) Housed………(3) tied:…………………………

B8 How do the dogs leave the house premises? (1) Accompanied,….. (2) Occasionally accompanied … (3) Never accompanied………

B9 Reason for keeping dogs (1) Hunting,…..(2) Watch dog….., (3)Companion,……(4) No specific reason….

B10 Approximate ages (months) (I) 0-6… (ii) 7 -11, (iii) >12

Sex of your dog (s) (1) M…..(2) F……

Are there stray dogs in your community? (1) No…… (2) Yes……

B11 Other species of animals kept Cats(Y/N)( ) Goats (Y/N)( ) Sheep (Y/N)( ) Donkeys (Y/N)( ) Pigs (Y/N)( ) (Y/N) ( ) Cattle

C. POSSIBLE TRANSMISSION FACTORS

C1. Do you or any of the dogs consume offal? (Y/N):………………………………

C2. If yes, how is the offal prepared? (1) Boiled …..(2) Fried …..(3) Roasted …….(4) Raw ……. (5) Others specify ………

C3. Have you slaughtered cattle at home in the last 12 months? (Y/N):……………………

Have you slaughtered any other livestock at home in the last 12 months? (Y/N) Specify

C4. Is there a slaughter house nearby? (Y/N) ………………………………………………

C5. If "Yes", is the meat inspected done by a meat inspector? (Y/N):………………………........

C6.What do you do with livestock that die on their own? (1) bury (2) burn (3) skin and eat/sell

C7.What do you do with offal of animals that die on their own (1) bury (2) burn (3) skin and eat

C8.Do your dogs have access to dead carcasses and their offal?

C9. Do stray dogs have access to dead carcasses and their offal?

C10. Do your dogs go out to pasture with the cattle when the animals are being herded or they are kept purely as guard dogs?

C11.Do your dogs hunt small mammals in the bush when they go out? (Y/N) ……………

C12.Do your animals graze areas where dogs defecate? (Y/N) ……………

C13. Where do your dogs usually defecate? Within the house, Within/ Outside house premises

C14.Do children play with dogs? (Y/N) …………… ……………………………………………

C15.Are your dogs ever treated by veterinary staff when they are sick (1) No…. (2) Yes….(3) Sometimes……

C16.Have your dogs ever been de-wormed? (Y/N)………………………………………

C117. If YES when (………………. and how often? (1) <12 months, …… (2) >12 months……?

C18. Source of drinking water. (1) River; ……..(2) Borehole; …..(3) Well; …..(4) Others (specify):………….

D. AWARENESS OF HYDATIDOSIS IN MAN

D1. Are you aware of possible diseases/ conditions that are caused by dogs? (1) Rabies….., (2) Wounds from dog bite …….. (3)Scabies……… (4) Worms…… (5) Dysentery…….(6) Other bacterial/viral infections?……………………………………

D2. Have you ever heard of tapeworm infections in humans? (Y/N)……….. (If "NO", go to D7):

D3. Have you heard or met anyone who has been diagnosed with a cyst at any hospital in the village/ your household/yourself? Y/N): ………………………………………………………………

D4. How does one know that he/she has a cyst? ....

D5. How can people acquire a cystic infection? ....

D6. What should people with cysts infection do? (1) Go to hospital; ………(2) Use traditional medicine; …….(3) Do nothing:…………………………………………

D7. Have you heard of anyone saying or complaining of the following diseases in the village? (1) Skin nodules (Y/N); ……. (2) Chronic cough (Y/N); ……..…(3) Ascites (Y/N); ………….(4) Madness(Y/N):………………

D7. Have taken any de-wormer in the past one year (Y/N)………………………………………

E. AWARENESS OF ECCHINOCOCCOSIS IN DOGS AND LIVESTOCK

E1. Have you observed "proglottids" (*Echinococcus*) in dog stool? (Y/N): …………………………

E2. If "YES", do you know what these "proglottids" are? (Y/N): …………………………………..

E3. If "YES", do you know how a dog acquires this infection? ....

E4. When you see the "proglottids' in the dog stool; (1) Do you de-worm the dog? (Y/N); ………… Do you play with the dog? (Y/N); ……….

E5. Have you observed cysts in the abdominal viscera of slaughtered livestock? (Y/N)……………

E6.Which organs did you observe these cysts? ....

E7.If "YES", do you know what these "cysts" are? (Y/N) ……………………………………………..

E8.If "YES", do you know how livestock acquire this infection? ....

E9.Is the meat from Livestock with these cysts safe to eat? ....

E10.Are the offal/organs from. Livestock with these cysts safe to eat? ....

B . Estimated Livestock Population in Western Province.

District	Cattle	Goats	Pigs	Poultry	Donkeys	Dogs
Kalabo	67,159	681	1583	39,575	34	13,496
Kaoma	28,645	2,855	3,014	37,124	19	6,254
Lukulu	55,432	511	10,342	80,000	137	4,236
Mongu	64,749	5,749	24,273	153,685	556	16,210
Senanga	85,001	1,200	1,112	40,121	418	4,750
Sesheke	70,414	9,058	1,775	51,281	464	8,638
Shangombo	81,000	4,500	8,356	85,379	58	11,732
Total	452,400	24,554	50,455	487,165	1,686	65,316

C . Prevalence of cattle hydatidosis from 1994 to 2007 in cattle slaughtered in Mongu and Senanga.

Year	**# slaughtered**	**Organ**			**# Positive**
		Lung	Liver	Spleen	
1994	3,500	142	2	0	144
1995	3,973	99	2	1	102
1996	791	15	0	0	15
1999	30,497	1035	68	0	1103
2000	14,268	616	14	0	630
2001	2,633	121	2	0	123
2003	36,124	953	44	0	997
2004	25,604	539	106	0	645
2005	10,893	433	23	0	456
2006	12,641	182	15	0	197
2007	17,352	246	31	0	277
Total	158,456				4,689

D. Annual abattoir prevalence of hydatid cysts by infected organ in cattle from 1994 to 2007 in Mongu.

Year	No. Slaughtered(n)	Positives	Condemned Organ	Combined Prevalence (%)
			Lung (142)	
1994	3500	144	Liver (2)	4.11
			Lung (99)	
			Liver (2)	
1995	3973	102	Spleen (1)	2.57
			Lung (15)	
1996	791	15	Liver (0)	1.90
			Lung (1035)	
1999	30497	1103	Liver (68)	3.62
			Lung (616)	
2000	14268	630	Liver (14)	4.42
			Lung (121)	
2001	2633	123	Liver (2)	4.67
			Lung (953)	
2003	36124	997	Liver (44)	2.76
			Lungs (539)	2.52
2004	25604	645	Liver (106)	
			Lungs (433)	4.19
2005	10893	456	Liver (23)	
			Lung (182)	1.56
2006	12641	197	Liver (15)	
			Lung (246)	1.58
2007	17532	277	Liver (31)	
Total	158,456	4,689		2.95

E. Possible risk factors of hydatidosis in cattle and dogs (n=58)

Variable	Level	Number	Proportion	Standard error
Dog Management	Free range	56	96,6%	2.4
	Housed	1	1.7%	1.7
	Tied	1	1.7%	1.7
Major Reason For Keeping Dogs	Hunting	7	12.1%	4.3
	Watch dog	50	86.2 %	4.6
	Companion	1	1.7%	1.7
Feeding Offal To Dogs	Yes	55	94.8%	29.3
	No	3	5.2%	29.3
Dog Scavenging From Abattoir	Yes	22	37.9%	6.4
	No	36	62.1%	6.4
Dog Access To Dead Carcasses	Yes	28	48.3%	6.6
	No	30	51.7	6.6
Defecating On Pasture	Yes	43	74.1%	5.8
	No	15	26.9%	5.8
Use In Cattle Herding	Yes	51	87.9%	4.3
	No	7	12.1%	4.3

E. continues

Variable	Level	No.	Positive proportion	Standard error
Children Playing With Dogs	Yes	48	82.8%	5.0
	No	10	17.2 %	5.0
Dogs De-Wormed	Yes	51	87.9%	4.3
	No	7	12.1%	4.3
Presence Of Stray Dogs	Yes	38	65.5%	6.3
	No	20	34.5%	6.3
Owned Cattle	Yes	57	98.3%	1.72
	No	9	1.7%	1.72
Owned Sheep	Yes	0	0	0
	No	58	100%	100%
Owned Goats	Yes	22	37.9%	6.4
	No	36	62.1%	6.4
Owned Donkey	Yes	6	10.4%	4.0
	No	52	89.6%	4.0
Owned Pigs	Yes	8	13.8 %	4.6
	No	50	86.2%	4.6

F. Possible risk factors for the transmission of hydatid cysts in humans n = 66 (2011)

Variable	Level	Number	Proportion	Standard error
Children playing with dogs	Yes	50	75.8%	5.3
	No	16	24.2%	5.3
Owning dogs	Yes	58	87.9%	4
	No	8	12.1%	4
Medical attention of dogs.	Yes	15	22.7%	5.2
	No	51	77.3%	5.2
Source of water	River	10	15.2%	4.4
	Borehole/ Tap	15	22.7%	5.2
	Well	35	53.0%	6.2
	Others	6	9.1%	3.6

G. Public awareness of echinococcosis and other zoonoses transmitted by dogs.

Variable	**Level**	**Number**	**Proportion**	**Standard error**
Worms	Yes	26	39.4%	6.1
	No	40	60.6%	6.1
Where do dogs defecate?	Within the house	1	1.5%	1.5
	Outside the house premises	65	98.5%	1.5
Any one diagnosed with CE	Yes	35	53.0%	6.2
	No	31	47.0%	6.2
Awareness of the disease	No idea	21	31.8%	5.7
	Weight loss	4	6.1%	2.9
	Diagnosis at Hospital	16	24.2%	5.3
	Clinical signs	25	37.9%	6.0

G. continued

Variable	**Level**	**Number**	**Proportion**	**Standard error**
Ascites	Yes	50	75.8%	5.3
	No	16	24.2%	5.3
Observation of proglottids in dog stool	Yes	18	27.3%	5.5
	No	48	72.7%	5.5
Observation of cysts in viscera	Yes	48	72.7%	5.5

of animals				
	No	18	27.3%	5.5
Is cyst infested meat safe to eat	Yes	19	28.8%	5.6
	No	47	71.2%	5.6
Is cyst infested offal safe to eat	Yes	17	25.8%	5.4
	No	48	72.7%	5.5
	Don't know	1	1.5%	1.5

G. continued

Variable	Level	Number	Proportion	Standard error
Highest education	Primary	10	15.2%	4.4
	Secondary	45	68.2%	5.8
	College	7	10.6%	3.8
	University	4	6.0%	2.9
Major reason for keeping dogs	Hunting	7	12.1%	4.3
	Watch dog	50	86.2 %	4.6
	Companion	1	1.7%	1.7
Dog management	Free range	56	96,6%	2.4
	Housed	1	1.7%	1.7
	Tied	1	1.7%	1.7
Presence of stray dogs in the community	Yes	38	65.5%	6.3
	No	20	34.5%	6.3

H. The 2009 National Livestock Census Figures by Province.

Province	Bulls	Cows/ heifers	Oxen/ steers	Calves	Total Cattle	Sheep	Goats	Pigs	Donkeys	Dogs	Poultry
Central	35,456	206,093	98,908	94,032	434,489	215,293	12,992	169,709	48,453	105,782	4,094,870
Copperbelt					74,357	6,312	72,517	85,245	297	44,363	2,236,900
Eastern	12,979	138,467	103,967	51,255	306,668	168,449	17,445	242,470	47,458	425	812,565
Luapula	867	6,537	275	1,245	8,924	6,421	55,516	26,028	7	1,863	232,267
Lusaka					65,838	1,794	41,557	9,208	180	17,189	1,608,594
Northern	4,081	42,851	32,648	22,446	102,025	18,693	127,322	61,659	140	23,332	842,401
North-western					60,498	2,420	43,553	8,123	25	17,854	236,677
Southern	175,790	393,842	190,298	170,951	930,881	45,340	377,295	94,957		92,358	1,374,639
Western					463,183	-	25,373	43,205	1,876	50,772	431,187
Total	193,717	581,697	327,188	245,897	2,446,863	464,722	773,570	740,604	98,436	353,938	11,870,100

I. Fertility and intensity of hydatid cysts in selected liver and lung of cattle

Organ	Number of cysts per organ	Number of live cysts	Number of dead cysts	% of live cysts	% dead cysts	Ratio live/ dead cyst	Ratio dead/ live cyst
Lung	6	3	3	50%	50%	1.0	1
Lung	2	1	1	50%	50%	1.0	1
Lung	2	2	0	100%	0%	0	0
Lung	2	0	2	0%	100%	0	0
Lung	2	2	0	100%	0%		0
Lung	6	5	1	83%	17%	0.5	2.2
Liver	4	2	2	50%	50%	1.0	1
Liver	3	2	1	66.7%	32.3%	2.0	0.5
Lung	8	3	5	37.5%	62.5%	0.6	1.7
Lung	21	6	15	28.6%	71.4%	0.4	2.5
Lung	6	2	4	33%	67%	0.5	2
Lung	12	8	4	66.7%	33.3%	4.0	0.25

I. Continued

Organ	Number of cysts per organ	Number of live cysts	Number of dead cysts	% of live cysts	% dead cysts	Ratio live /dead cyst	Ratio dead /live cyst
Lung	9	2	7	22.2%	77.8%	0.3	5
Liver	5	2	3	40%	60%	0.7	.5
Lung	3	2	1	66.7%	33.3%	2.0	.5
Liver	4	1	3	25%	75%	0.3	3
Lung	8	4	4	50%	50%	1.0	1
Lung	14	4	10	28.6%	71.4%	0.4	
Lung	6	2	4	33.3%	66.7%	0.5	2
Lung	108	47	61	43.5%	56.5%	0.7	1.2
Liver	16	7	9	43.8%	56.2%	0.8	1.2