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UNIVERSITY OF GONDAR FACULTY OF VETERINARY MEDICINE

A STUDY ON PREVALENCE AND ECONOMIC SIGNIFICANCE OF BOVINE HYDATIDOSIS IN DEBIRE BIRHAN MUNICIPAL ABATTOIR, CENTERAL ETHIOPIA

DVM THESIS

BY

ERMIAS GEBEYEHU

JUNE, 2015

GONDAR, ETHIOPIA

UNIVERSITY OF GONDAR

FACULTY OF VETERINARY MEDICINE

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A thesis submitted to the Faculty of Veterinary Medicine, University of Gondar in Partial Fulfillment of the Requirements for the degree of Doctor of Veterinary Medicine

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LIST OF ABBREVIATIONS

µm	Micrometer
AE	Alveolar echinococosis
CE	Cystic echinococosis
CSA	Central Statistical Agency
ETB	Ethiopian Birr
FAO	Food and agriculture organization
KG	Kilogram
Km	Kilometer
OIE	World Organization for Animal Health
OR	Odd ratio
PAIR	Puncture and aspiration, injection and re-aspiration
SPSS	Statistical package for social sciences
USD	United States Dollar
WH	Women Health
WHO	World health organization

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ABSTRACT

A cross-sectional study aimed at determining the prevalence and estimating the financial losses due to cystic echinococcosis (hydatidosis) in cattle slaughtered at Debre Berhan municipal abattoir was conducted from November, 2014 to April, 2015. Out of 384 examined animals, 110 (28.6%) were harboring visible hydatid cysts. Significantly higher infection was detected in cattle's with poor (48.40%) than animals with medium (24.10%) and good body condition (20.30%). The prevalence of the parasite in male and female was 29.83 and 29.8% respectively. However, there was no significant variation was between two sexes. The prevalence was not statistically different between local (30.10%) and cross (19.20%) breeds. Regarding organ distribution, infections of the lung, liver, kidney and heart were 52.50, 38.10, 5.00, and 4.40%, respectively. Of the total 160 hydatid cysts counted, 64 (40.00%) were fertile, 80 (50.00%) sterile, and 16 (10.00%) were calcified. Of the 64 fertile cysts subjected for viability test, 24 (37.50%) were viable while 40 (62.50%) were nonviable. Moreover, assessment of annual economic loss due to bovine hydatidosis at Debre Berhan municipal abattoir from offal condemnation and carcass weight loss was about 3,730,328 ETB (186,516.40 USD). As the infection was detected in this study, there seems to be an existing favorable situation for hydatidosis, and hence, it remains one of the most important diseases warranting serious attention for prevention and control actions in the district. Hence, establishment of well-equipped standardized abattoirs, creation of public awareness, and control of stray dogs have paramount importance.

Keywords: Debre Berhan; Bovine; Economic significance; Fertility; Hydatidosis; Prevalence

1. INTRODUCTION

Hydatidosis is a chronic cyst-forming parasitic helminthic disease of human beings as well as domestic and wild ungulates and equines. It is caused by infection with the larval (metacestode) stages of dog tapeworms belonging to the genus *Echinococcus* (family Taeniidae) and is also referred to as echinococcosis. Three broad morphological forms of echinococcosis are recognized clinically: Cystic echinococcosis caused by *E. granulosus*, alveolar echinococcosis caused by *E. multilocularis*, and polycystic echinococcosis caused by *E. vogeli* or *E. oligarthrus* (Craig *et al.,* 2007). Human cystic echinococcosis is the most common presentation and probably accounts for more than 95% of the estimated 2 - 3 million global cases, with human alveolar echinococcosis causing around 0.3 - 0.5 million cases (all in the northern hemisphere), fewer than 150 cases of polycystic echinococcosis have been described, all in Central and South America. Until 2005, only four *Echinococcus* species were recognized, but a fifth species, *E. shiquicus*, has now been described in small mammals from the Tibetan Plateau, although its zoonotic potential is unknown (Ahmadi and Dalimi, 2006).

Hydatid disease has been known as a clinical entity since ancient times. Its parasitic nature was recognized as early as 1684 by Redi, Harmann and others. Goeze in 1782 pointed out that the scolices were of teanial origin and differentiated the hydatid cyst from cysticercus and the coenurus. The adult worm was observed in the intestine of dogs in 1808 by Rudolphi, but it was not until 1850 that it was recognized by Van benden as distinct species which he later named Taenia nana. In 1852 Von Siebold recovered the adult worm from dogs that had eaten echinococcal cysts of cattle (Belding, 2005).

Definitive hosts of *E. granulosus* are domestic dogs and some wild canids. Adult cestodes live attached deep inside mucosal crypts of definitive hosts small intestine of dogs. The parasite is 3 to 6 mm long. It has 22 large hooks and 18 small hooks on scolex and usually has three proglottids, of which only the last is gravid (Acha and Szyfres, 2001). The gravid proglottid contains several hundred eggs, detaches from strobila is expelled with feces, and distintegrates in the environment. Each egg contains an embryo (oncophere) with six hooks (hexacanth), Which is infective when ingested by intermediate host and continue its development. Intermediate hosts are sheep, goats,

bovine, swine, equine, camelids, canids and man. The most common localization of these cysts in the intermediate hosts are the liver (in about two-thirds of the cases) and the lungs (in about fourth of the cases). On rare occasions they may become situated in some other organs such as the kidneys, spleen, bones and brain (Eddi *et al.,* 2004).

In humans, the disease is initially without any symptoms until gradually the cyst increased in size, causing local pressure effects. In animals, the disease does not produce any clinical signs and is usually only discovered during meat inspection at the slaughterhouse where the viscera (mainly liver and lungs) are condemned (Acha and Szyfres, 2001).

In Debre Berhan, there is no recent information about hydatid disease. In spite of this, it is expected to be highly prevalent due to widespread of backyard slaughtering practices, inappropriate meat inspection procedures and the long standing habit of the people to feed their dogs with raw offal's. These were the points which initiated to design a research the prevalence and economic importance of hydatid disease (echinococcosis). Therefore, the objectives of this study were to estimate the prevalence and economic significance of bovine hydatidosis in Debre Berhan municipal abattoir.

2. LITERATURE REVIEW

2.1 General Overview

Echinococcosis is a disease that has been recognized by humans for centuries. It was 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, PierreSimon 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 (Budke *et al.*, 2006). Half a century later, during the 1850s, Carl vonSiebold 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). Echinococcosis is an emerging and re-emerging zoonotic parasitic disease caused by the cestode species of the genus Echinococcusand is one of the most important helmintic diseases with a worldwide distribution including Europe, Asia, Africa, South America, Canada and Australia (Endrias, *et al*., 2010).

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 (Dakkak, 2010).

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 pathogenecity 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, hemorrhage 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 Etiology

Echinococcosis is caused by several species of *Echinococcus*, which is a tiny cestode parasite in the family Taeniidae. The two most important species are *Echinococcus granulosus* and *Echinococcus multilocularis. E. granulosus* causes a type of echinococcosis known as cystic
echinococcosis, unilocular echinococcosis or cystic hydatid disease. Different strains of *E. granulosus* can be found in sheep, cattle, pigs, horses and reindeer. All of the strains, except possibly the horse strain, infect humans. *E. multilocularis* causes a type of echinococcosis known as alveolar echinococcosis, alveolar hydatid disease, multilocular echinococcosis or multivesicular hydatidosis. *Echinococcus vogeli* and *E.oligarthrus* are species of minor importance. These organisms are found mainly in wildlife and rarely affect humans. Human infection with these parasites is known as polycystic echinococcosis. All *Echinococcus* spp. have an indirect life cycle, cycling between a definitive and an intermediate host. Intestinal infections occur in the definitive host, and tissue invasion is seen in the intermediate host. Carnivores are the definitive hosts for *Echinococcus*, and usually have no symptoms of infection. Disease may be seen in the intermediate hosts, including humans (OIE, 2005).

2.3 Morphology of the Cyst

Echinococcus exhibits certain characteristics that differentiate it from the other major genus in the family *Taenia*. The adult Echinococcusis only a few millimeters long (rarely more than 7mm) 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 interchange takes place across the synctial outer covering, the tegument (Eckert, *et al.,* 2002).

2.3.1 Scolex and Strobila

The anterior end of the adult worm possess a specialized 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. 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 fertilization; eventually occupy most of the terminal segments when the eggs are fully developed strain (Ahmadi and Dalimi, 2006).

Figure 1: Scolex and Strobila of Echinococcus (Source; TMCR, 2015)

2.3.2 Morphology of Echinococcus egg

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 (Endrias, *et al*., 2010).

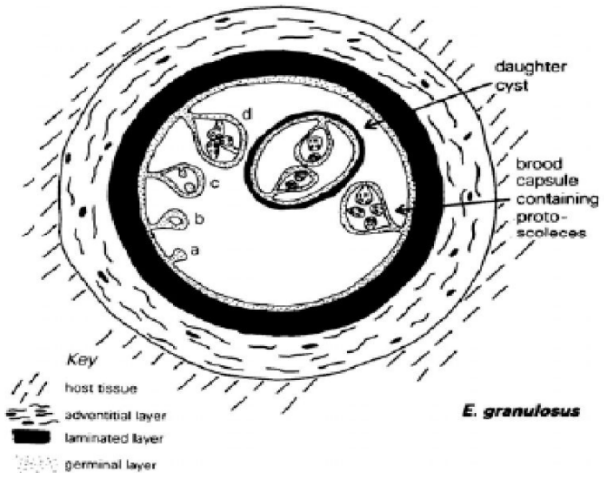


Figure 2: Morphology of *E. granulosus* egg (source: WH, 2015)

2.3.3 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* (Eckert *et al*., 2002).

The hydatid cyst, after 3 weeks, measures 250 μm in diameter and has central cavity. Around fifth months, it measures approximately one- cm and it is apparent that its wall consist of two layers: An external cuticular, or laminar layer, formed by numerous thin lamina that resembles the cross-section of an onion, and another, internal layer germinative or proligerous, which is delicate cellular

syncytium. Larval form of *E. granulosus* typically consists of single cavity (unilocular). The interior of a hydatid cyst is filled with fluid. During the same period, brood capsule buds off from the germinative layer, and forming an invaginatedprotoscolices (Acha and Szyfres, 2001).

2.4 Geographic distribution

E. granulosus is the most widespread of the species with areas of high endeminicity in southwestern Asia, northern Africa, Australia, Kenya and Uganda. The distribution of *E. multilocularis* is limited to the northern hemisphere (Meresie, 2006). The most important endemic areas are northern tundras of Europe, Asia, America and central Siberia*, E. oligarthrus* and *E. vogeli* are present only in South and Central America. Although the areas of infection coincide, since the definitive host of *E. vogeli* exists only from Panama to northern Argentina, cases of polycystic hydatidosis outside this area are probably imported or due to *E. oligarthrus.* (Acha and Szyfres, 2001).

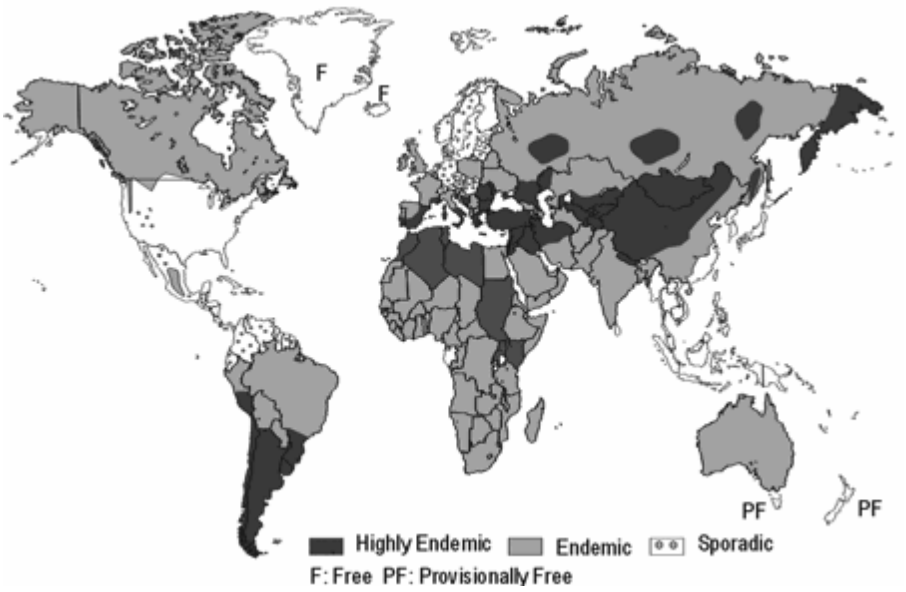


Figure 3: Global distribution of *Echinococcus granulosus (*Apelo, 2015)

2.5 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. However, wild canids can also be involved in the transmission cycle in some areas. 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. *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 (Palmer, 2011).

The dog-sheep and dog-cattle cycle are 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 feces of the definitive host (Jenkins *et al.,* 2005).

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 candevelop in a variety of organs in the intermediate host but are often found in the liverand lungs. The cysts grow slowly. Most cysts are discovered in humans when they are1 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-inducedcyst rupture or after release of viable parasite material during invasive treatment procedures. Each cyst is filled with fluid and is surrounded by a fibrous laminated outer membrane and an inner membrane called the germinal layer (da Silva, 2010).

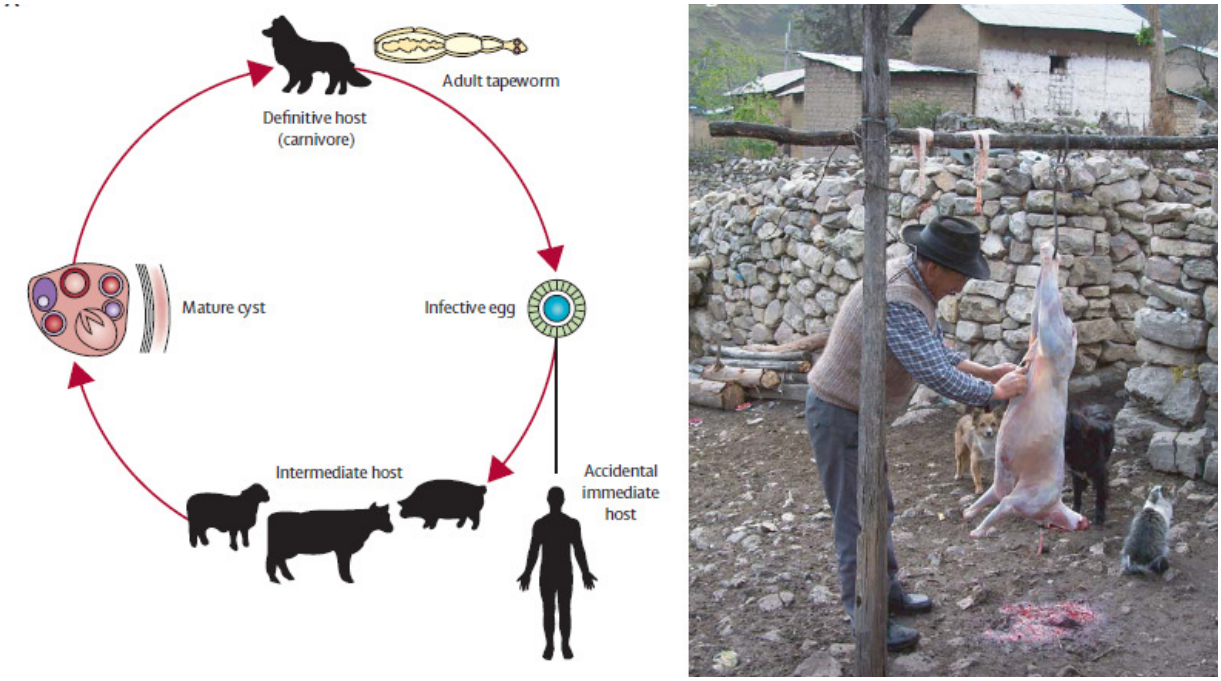
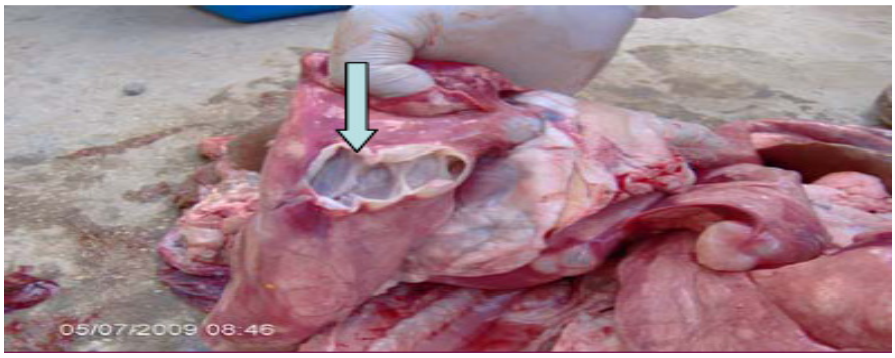


Figure 4: Life cycle of *Echinococcus species* (Craig *et al.,* 2007)

2.5.1 Larval stage

From the embryo released from an egg develops a hydatid cyst, which grows to about5–10 cm within the first year and is able to survive within organs for years. Cysts sometimes grow to be so large that by the end of several years or even decades, they can contain several litters of fluid. The larval stage of *Echinococcus granulosus* is a fluid filled bladder or hydatid cyst that is unilocular, although communicating chamber occurs (OIE, 2005). Once a cyst has reached a diameter of 1cm, 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 (Cabrera, *et al.*, 2002).

A.



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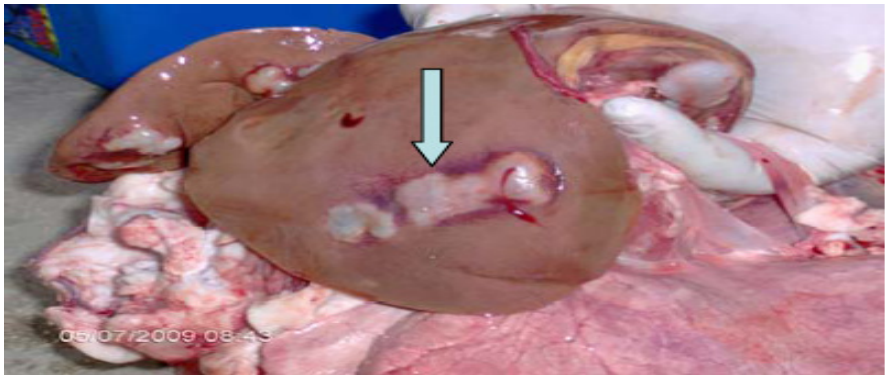

Figure 5: Hydatid cysts in lung (A) and liver (B) (El-Ibrahim, 2009)

2.5.2 Adult worm

Echinococcus adult worms develop from protoscoleces and are typically 6mm or lessin 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). 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 (Torgerson and Budke, 2003)

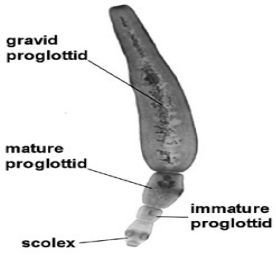

Figure 6: Morphology of a mature adult worm of *E. granulosus* (Source; TMCR, 2015)

2.6 Pathogenesis

The pathogenesis of hydatidosis heavily depends on the extent and severity of infection and the organ on which it is situated (Biffa *et al.*, 2006). Infection with the adult stage of *Echinococcus granulosus* is generally asymptomatic and non-pathogenic to the canid host. Infection with the larval stage of *echinococcus granulosus* can be pathogenic depending on the localization, size of the cyst, and intensity of the infection in the cervid or human intermediate host. Most hydatid cysts reside in the lung parenchyma but they are also found in the liver parenchyma, just below the capsule (Getaw *et al.*, 2010).

2.7 Diagnosis

Diagnosis of human hydatidosis is suspected based on the clinical symptoms and epidemiological circumstances. Imaging methods such as radiography, computerized tomography, ultrasonography and scintigraphy are usually used. While they do not confirm the diagnosis, they are very helpful to the specialist. Ultrasonography is the first choice because it is economical, noninvasive, simple, and accurate and reveals developing cysts that generally cannot be found with X-rays. Numerouimmunobiologic tests have been used in the diagnosis of human hydatidosis by *E. granulosus*, among them Casoni's intradermal test, complement fixation, indirect hemagglutination, latexagglutination electrosyneresis, and double diffusion to detect antibodies against the arc5 antigen. Practically all have been displaced by Enzyme linked immunosorbent assay (ELISA) and the immunoelectro transfer or Western blot test. Casoni's intradermal test is not very sensitive and is nonspecific for the diagnosis. While it was once used for epidemiological surveys, the collection of drops of blood on filter paper now makes it possible to use serologic techniques that are much more sensitive and specific on a large scale. More recently, the polymerase chain reaction (PCR) has also been used to detect nucleic acids from the parasite in patients' bloodstreams (Acha and Szyfres, 2001).

2.8 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, 2000). 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. 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 Ethiopia (Meresie, 2006). Dogs are the most successful canids adapted to human habitation world-wide. They have contributed to physical, social and emotional well-being of their owners, particularly children who are often at greatest risk of exposure (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). Humans should avoid handling fecal matter of canines and avoid consuming infected animals and home slaughtering animals (Eckert and Deplazes, 2004). 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).

2.9 Economic importance

Echinococcosis in humans and animals is both an economic and public health problem in many parts of the world. 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).The economic importance of echinococcosis in livestock is due to the condemnation of the whole edible carcasses and offal such as liver, lung kidney and heart. In severe infection, the parasite may cause retarded performance and growth and reduced quality and yield of meat and milk (Getaw *et al.*, 2010).

2.10 Treatment, Prevention and control of Echinococcosis

In animals, prevention and control of echinococcosis is achieved by sanitary disposal of slaughter house 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 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 (Hegglin and Deplazes, 2008).

The 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 to10 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 dewormers 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 (Zerihun, 2006). In summary, the control programs against cystic echinococcosis have traditionally relied on anthelmintic dosing of dogs, improved slaughter hygiene and surveillance, and health education relating to human–dog behavior (Cabrera, *et al.*, 2002).

3. MATERIALS AND METHODS

3.1 Study Area

The study was conducted at Debre Berhan town municipal abattoir from November, 2014 to April, 2015. The town is found in Amhara regional state, situated at 130 km northeast of Addis Ababa. It is geographically located at 09^0 31' N latitude and 39^0 28' E longitude with an altitude of 2780 meter above sea level. The area is mountainous with large plane grazing lands and dissected by two rivers, namely Dalicha and Beriesa (Zerihun, 2006). The rainy season of this area extends June to September while the dry season extends from November to January. Sporadic rainfall also occurs from February to April, The mean annual temperature of Debre Berhan is 12.9^0c where the minimum and maximum temperature is 6.1 and 19.9^0c, respectively. The average annual rainfall is 905.4 mm and relative humidity is 62.3%. The livestock population in the area comprises of cattle (2984), goat (115), sheep (5912), horse (169) and poultry (5190) (CSA, 2008).

3.2 Study Population

The study animals comprise local zebu and cross breed cattle slaughter at Debre Berhan municipal abattoir.

3.3 Study Design and Methodology

A cross-sectional study was conducted at Debre Berhan municipal abattoir on randomly selected animals. Of the animals brought for slaughter in three selected days, 30% them were randomly sampled.

3.4 Sample Size Determination

The required sample size for this study was estimated by considering 50% hydatidosis prevalence since there was no study on hydatidosis in the area before. Thus, the sample size was calculated based on the formula given by Thursfied (2005) using 95% confidence interval and 0.05 absolute precision as follows:

$$n = \frac{1.96^2 \times P_{exp}(1-P_{exp})}{d^2}$$

Where n= required sample size

P_{exp}= expected prevalence

d^2= desired absolute precision (0.05)

1.96^2 = z- value for 95% confidence interval

As a result, 384 animals will be sampled.

3.5 Sample collection

3.5.1 Antemortem examination

Regular visits were made to conduct ante-mortem examination of slaughter animals. A total of 384 cattle were examined in the Debre Berhan abattoir during the survey period which extended from November 2014 to April, 2015. During the antemortem inspection, the age, sex, breed, and body condition of each animals were recorded. The age of animals was determined by incisors of animal's teeth (De-Luata and Habel, 1986). Body conditions of each animal was assessed and recorded depending on their body condition score, were ranked as poor, medium or good (Nicholson and Butterworth, 1986).

3.5.2 Postmortem examination

During the postmortem examination, visual inspection, palpation and systemic incision of each visceral organs were performed particularly the liver, lungs, kidneys and heart. In parallel, serial number, date, infection, infected organ and number of cysts were recorded. Infected organs were collected in polyethene bags and taken to Debre Berhan veterinary clinic laboratory to conduct cyst count, cyst fertility and viability of protoscoleces.

3.5.3 Laboratory examination of cyst

The fertility of cysts was examined microscopically. Each cyst was cut, opened with scissor and the content of the cyst was poured into a clean petri dish. A drop of cyst fluid was put in a clean slide and then examined under the microscope (40×) for the presence of protoscolices. For clear vision a drope of 0.1% aqueous eosine solution was added to to equal volume of protoscolices in Hydatid fluid on the microscopic slide .The viability of protoscolices was determined by flame cell motility. The cyst which contained no protoscolices as well as calcified, or degenerated was considered as unfertile cyst .Whenever and wherever the cysts were present, they were removed and incised. The shrunk, evacuated, pus formatted cysts were classified as degenerated cysts, while the solid and sands contained ones were considered as calcified cysts, while the fluid filled ones and had no protoscolices by direct microscopic examination were considered as sterile cysts.

3.5.4 Economic Analysis

To study the economic losses due to hydatidosis in cattle, both direct and indirect losses were considered. The calculation of the direct losses is based on condemned organs (lung, liver, heart and kidney) and the indirect losses were assessed on the basis of live weight reduction due to hydatidosis. Average price was drawn out from that data and this price index was later used to calculate the meat loss in terms of ETB or USD. Based on description of polydorous (1981): Total loss=LOC+LCWL

Annual cost of offal condemned= (CSR x P_{HLu} x C_{PLu}) + (CSR x P_{HLi} x C_{PLi}) + (CSR x P_{HHc} * C_{PHe}) + (CSR x P_{HKi} x C_{PKi})

Annual Cost of Carcass Weigh t loss=5% x CSR x P_H x CPB x126 Kg

Where:

LOC=loss due to organ condemnation (*Annual cost of offal condemned*)
LCWL=loss from carcass weight loss (*Annual Cost of Carcass Weigh* t loss)
126 KG = Average Carcass Weight of Zebu Cattle
5%= Carcass Weight Loss in Individual Animal due to Hydatidosis (Polydorous, 1981).
CPB = Average Market Price of One Kg of Beef in Debere Berhan
C_{PHe} = Mean Cost of One Bovine Heart in Debere Berhan
C_{PKi} = Mean Cost of One Bovine Kidney Condemned
C_{PLi} = Mean Cost of One Bovine Liver Condemned
C_{PLu} = Mean Cost of One Bovine Lung Condemned
CSR = Average Number of Cattle Slaughtered Per Year
P_H = Prevalence of Hydatidosis at that Town
P_{HHc} = Percentage of Heart Condemned
P_{HKi} = Percentage of Kidney Condemned
P_{HLi} = Percentage of Liver Condemned
P_{HLu} = Percentage of Lung Condemned

3.6 Data management and Analysis

Data obtained from ante mortem and postmortem findings, characterization of cysts were coded and uploaded into Microsoft Excel 2007 spreadsheet computer program in a pre-designed format and analyzed by using SPSS-16 for windows software. Chi-square (X^2) test was applied to compare the infection status with regard to the hypothesized risk factors like age and body condition.

4 RESULTS

4.1 Overall Prevalence

Out of the total 384 heads of cattle slaughtered and examined, 28.6% were infected with hydatid cyst, harboring one or more cysts involving different visceral organs (lung, liver, heart and kidney).

The risk factor of bovine hydatidosis infection between age groups showed that adults have had lower odd of becoming affected than old age groups [OR=0.219] which was statically significant (0.000). In the study population, the occurrence of bovine hydatidosis infection was more likely in poor body conditioned animals than in medium body condition animals [OR=3.565], than good body condition animals [OR=1.291] (Table 1).

Table 1**:** Prevalence of hydatidosis based on different variable categories

Variables	**Category**	**Number examined**	**Number positive**	**Prevalence (%)**	**χ2**	**p- value**	**Odd ratio**
Age	Adult	193	28	14.50	37.94	0.000	0.219
	Old	191	82	42.90			
Breed	Local	332	100	30.10	2.61	0.106	0.375
	Cross	52	10	19.20			
Sex	Female	94	28	29.80	0.08	0.778	1.064
	Male	290	82	28.30			
Body condition	Poor	93	45	48.40	23	0.000	3.565
	Medium	158	38	24.10			1.291
	Good	133	27	20.30			
Total		384	110	28.60			

Table 2: Proportion of fertile, sterile and calcified hydatid cysts

Condition of cyst	Category	Number	Percent (%)
	Viable	24	37.50
Fertile	Non-viable	40	62.50
	Sterile	80	83.33
Non-fertile	Calcified	16	16.67

Table 3: Viable and Fertile cyst with respect to the organ involved

Type of organ	Condition of cysts					
	Viable	Non-viable	Sterile	Calcified	Total	Percent (%)
Lung	16	21	43	4	84	52.5
Liver	7	16	30	8	61	38.1
Heart	1	2	3	1	7	4.4
Kidney	0	1	4	3	8	5
Total	24	40	80	16	160	100

4.2 Economic Loss Evaluation

In Debre Berhan municipal abattoir 50 bovines were slaughtered in average in each days of the week. Based on this record 14000 bovines are expected to be slaughtered annually at that abattoir. By taking this amount, the annual economic loss due bovine hydatidosis in Debre Briham municipal abattoir was calculated as follows by using the formula given by polydorous (1981).

4.2.1 Economic loss due to organ condemnation

This was calculated from mean market price of cattle heart and liver (25 birr), kidney (20 birr) and lung (5 birr)

Annual cost of offal condemned= (CSR x P_{HLu} x C_{PLu}) + (CSR x P_{HLi} x C_{PLi}) + (CSR x P_{HHc} x C_{PHe}) + (CSR x P_{HKi} x C_{PKi})

(14,000 x 0.52 x 5)+ (14,000 x 0.38 x 25) + (14,000 x 0.044 x 25) + (14,000 x 0.05 x 20) = 198,800 ETB (9940 US$)

4.2.2 Economic loss due to carcass weight loss

Annual Cost of Carcass Weigh t loss=5% x CSR x P_H x CPB x 126 Kg

(0.05 x 14,000 x 0.286 x 140 x 126) = 3,531,528 ETB (176576.4 US$)

For calculating the indirect loss due to carcass weight reduction, a 5% carcass weight loss brought by hydatidosis as described by polydorous, (1981) and 126kg, an average carcass weight of an Ethiopian zebu, was considered here to estimate the economic loss and computed result showed a loss of 3,531,528 ETB (176576.40 US$) annually.

Estimation of Total Financial Loss: The total annual financial loss attributed to hydatidosis was estimated to be 3,730,328 ETB (186516.40 US$).

4. DISCUSSIONS

The current study revealed a prevalence of 28.6% which was 14.50% in adult, 42.90% in old, 30.10% in local and 19.20% in cross breeds, 29.8% in female, 28.30% in male, 48.40% in poor, 24.10 in medium and 20.30% in good body condtions (Table 1). This result was in agreement with previous work of Yetnayet (2010), who reported a prevalence of 27.2% in the Gondar town and Zewdu *et al.* (2010) who reported a prevalence of 29.69% in Ambo area and very lower prevalence were also reported by Kebede *et al.* (2009) in Shire (7.5%). Higher prevalence was registered in other areas of which 72.44% in Asella (Fikadu, 1997), 59.9% in Bahir Dar (Nebiyou, 1990), 62.96% around Bale Robe (Woubet, 1987), 52.69% around Hawassa (Regassa *et al.,* 2010). However, the extent to which results were documented from different locations tends to show variable scales. The variation in prevalence from different areas of a country might be attributed mainly due to the difference in strains of *E. granulosus* that exist in different geographical situations (Garrippa *et al.,* 2004) and other factors like difference in culture, social activity and attitude to dog in different regions (Oostburg *et al.,* 2000).

In this study, a significant variation was observed in the rates of infections between age groups ($p<0.05$) where animals above 5 years of age were highly infected. This is in agreement with the findings of Azlaf and Dakkak (2006) and Regassa *et al.* (2010). This could be mainly due to the fact that aged animals have longer exposure time to eggs of *E. granulosus* in addition to weaker immunity to combat against the infection (Himonas, 1987). In addition most of the slaughtered animals were culled animals due to less productiveness and hence were exposed to the diseases (parasitic ova) over long period with an increased possibility of acquiring the infections.

The prevalence of hydatidosis was slightly higher in cattle having poor (lean) (48.4%) followed by medium body condition (24.1%) and good body condition (20.3%) (Table 1).This result may be due to weaker immunity of poor condition animals to combat against the infection (Himonas, 1987). Polydorous (1981) explained that in moderate to severe infections, the parasite may cause retarded performance and growth, reduced quality of meat and milk, as well as live weight loss.

In this study, no significant variation was noticed with regard to sex and breed of animals. This may be explained by indiscriminate exposure to risk irrespective of sex and breed in the management system of the area. In addition to this, 90% of the samples used are male and local breeds so that no need of comparison by these two factors.

In this study, it has been shown that hydatid cysts occurred most commonly in the lung (52.5%) followed by the liver (38.1%), kidney (5%) and heart (4.4%) (Table 4). This is in agreement with the findings of Njoroge *et al.,* (2002) and Eckert and Deplazes, (2004), which show that the lung and liver are the most common sites of hydatid cyst in domestic animals. It is due to the fact that the lung and liver posses first great capillaries encountered by the migrating echinococcus oncosphere (hexacanth embryo), which adopt the portal vein route and primarily negotiate hepatic and pulmonary filtering system sequentially before any other peripheral organ is involved. Likewise, due to older age of slaughtered cattle, during which time the liver capillaries are dilated and most oncosphere pass directly; additionally, it is possible for the hexacanth embryo to enter the lymphatic circulation and be carried through the thoracic duct to the lungs in such a way the lung may be infected before or instead of the liver (Eckert and Deplazes, 2004). However, development of hydatid cysts occurs occasionally in other organs like spleen, kidney and heart and other organs and tissues when onchospherers escaped into general systemic circulation (Urquhart *et al.,* 2003).The reason behind for many cysts develop in the lung might be the softer consistency of lung tissues which allows easier development of the pressure of the cyst (Hubert, 1999).

In this study 40 % of the cysts were fertile and 15% of the fertile cysts were proved to be viable (Table 3). This was relatively different as compared with the findings of Zelalem (2008) and Alemante (2009) who reported the fertility rate of 26.9 % and 19.3%) respectively. And also it is lower as compared to the reports from Niger Delta 70.0% (Arene, 1995) and much higher than report of 1.76% around Wolayita Soddo (Nigatu *et al.,* 2009), 9.85% in Nekemte (Berssisa, 1994)and 6.2% in Bahir Dar (Nebiyou, 1990). The variation in fertility, sterility and calcification in different areas could be strain difference (Arene, 1995).

In the current study, an assessment was done on annual economic loss due to bovine hydatidosis at Debre Berhan municipal abattoir. Losses from organ condemnation and carcass weight loss (meat

production loss) in infected cattle were assessed and estimated at 3,730,328 ETB (186516.40 US$). The current estimate is greater than estimates done by Kebede *et al.* (2009) (25,608 ETB) in Tigray region. However, it is lower than 1,791,625.89 ETB that estimated by Regassa *et al.,* (2010) in Hawassa municipal abattoir. The difference in economic loss estimates in various abattoir or regions may be due to the variations in the prevalence of disease, mean annual number of cattle slaughtered in different abattoirs, and variation in the retail market price of organs. Considering the current result, hydatidosis is an important disease of cattle in Debre Berhan and its surroundings, causing substantial visible and invisible losses. It causes considerable economic loss in livestock due to condemnation of organs and denied weight gain of infected stocks.

5. CONCLUSIONS AND RECOMMENDATIONS

Hydatidosis is one of the highly prevalent parasitic diseases of cattle in Debre Berhan municipal abattoir and incurring huge economic loss due to organ condemnation and indirect weight loss. The disease is difficult to control due to backyard slaughtering, lack of adequate meat inspection and habit of raw offal give for their dogs. The result of the study shows that the disease is highly distributed. Therefore, it is necessary to carry out special control program to combat the disease. However, for the program to be successful, further epidemiological investigations which break the possible chain of infections between the final and intermediate hosts and the role of wild life cycles under local condition are necessary. Based on the above conclusions the following recommendations are forwarded:

- The government should give attention towards building standard abattoirs with good facilities and control backyard slaughtering houses.
- Awareness generating/creating programs should be given for butchers and dog owners as to the dangers of hydatidosis to human and animal health.
- Appropriate control measure should be taken to stop the sale of contaminated meat for pet animal's consumptions.
- Dog proof garbage collection and waste disposal system should be applied, particularly around slaughter places and abattoirs.
- Control of stray dogs should be practiced. Preventing of dogs access to raw offal is an effective measure.
- The authority should supervise slaughtering practices of carcass in order to prevent the illegal slaughtering of animals.
- The most important indispensible point is registration of dogs and the unregistered dogs should be liable to collect, eliminate unwanted stray dogs, Euthanasia of unwanted puppies and kennelling, tying up or restricting working dogs to fence premises.
- Regular deworming dogs for tape worm infections.

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7. ANNEXES

Annex 1: Description of body condition score

Score	Feature
1	Marked emaciation
2	Transverse process project prominently
3	Individual dorsal spines are pointed to touch, hips, pins, tail head and ribs are prominent transverse process visible, usually individual
4	Ribs, hips,and spines clearly visible muscle mass between hook and pins slightly concave, slightly more flesh above the transverse process
5	Ribs usually visible. Little fat cover, dorsal spines barely visible
6	Animal smooth and well covered, dorsal spines cannot be seen, but are easily felt
7	Animal smooth and well covered, but fat deposits are no marked. Dorsal spines can be felt with firm pressure, but rounded rather than sharp
8	Fat cover in critical areas can be easily seen and felt, transverse processes cannot be seen or felt
9	Heavy deposits of fat clearly visible on tail head, brisket, dorsal spines, ribs, hooks and pins fully covered and cannot be felt even with firm pressure

(Source: Nicholson and Butterworth (1986))

N.B:- 1, 2, 3, are poor

4, 5, 6, are medium

7, 8, 9, are good.

ANNEX-2: age determination based on dental formula

Age (year)	**characteristic change**
1 ½-2	I1 erupts
2-2 ½	I2 erupts
3	I3 erupts
3 ½ -4	I4 erupts
5	all incisor and canine are in wear
6	I1 is level and the neck has emerged from the gum
7	I2 is level and the neck is visible
8	I3 is level and the neck is visible I4 may be level
9	I4 is level and the neck is visible
10	the dental stare is square in I2 and in all teeth by 12 years
15	the teeth that are not fallen out are reduced small round.

Remark: - canine of ruminants is usually considered as fourth incisor.

(Source: (De-Luata and Habel, 1986))

Annex-3: Method for the determination of fertile and viable of cystic hydatidosis

1. Obtain/collect non-degenerated hadatid cyst from infected organs of slaughtered animals.
2. Take the cysts to laboratory in cool box.
3. Aspirate hydatyid fluid from the cyst by a sterile 18 gauge needle and transfer to a test tube.
4. The protoscolices allowed to sediment in the fluid for 20-30 minutes which indicates fertility and the supernatant discarded.
5. Confirm the fertility of the cyst by microscope examination of sediment protoscoliices.
6. A drop of sediment contained protoscolices on microscopic glass slide and cover with the cover slip; observe for amoeboid like peristaltic movements with high power objective.
7. For clear vision a drope of 0.1% aqueous eosine solution added to equal volume of protoscolices in Hydatid fluid on the microscopic slide with the principle that viable protoscolices should completely or partially exclude the day while the did ones take it up.
8. Furthermore, infertile cysts where further classified as sterile and calcified. Sterile Hydatid cysts were characterized by their smooth inner lining usually with slightly turbid fluid in its content while typical calcified cysts produce a gritty sound feeling up on incision.

(Source: Macpherson et al., (1985))

Annex 4: Laboratory data collection format for fertility and viability test.

No.	**Id. No**	**Organ from which cyst is taken**	**Cyst characterization**				
			Fertile	**Sterile**	**Viable**	**Non-Viable**	**Calcified**

Annex 5: data collection format

No	sex	Bred	Age group(years)		Body condition			result	Infected organ				
			Adult (3-5 yrs)	old(≥5 yrs)	P	M	G		Lung	Liver	Heart	Spleen	Kidney

REMARKS: P=Poor M=Medium G=Good

Annex 6: Location of Debre Berhan town

(Source: TTGC, 2015)

8. DECLARATION

I under signed, declare that the information presented here in my thesis is my original work, has not been presented for degree in any other university and that all source of materials used for the thesis have been dully acknowledged.

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Date of submission: 05/10/07

This thesis has been submitted for examination with my approval as university advisor

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