

**"Evaluation of Ultrasonographic Diagnosis, Treatment Methods and
Epidemiology of Cystic Echinococcosis in Sheep and Goats"**

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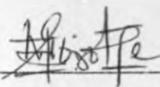
**A thesis submitted in fulfillment of the requirements for the degree of Doctor of
Philosophy (Ph.D.) in Veterinary Clinical Studies of the University of Nairobi**

June 2001

Declaration

This is my original work and has not been presented for a degree in any other university. All photographs and illustrations, unless acknowledged, were taken developed and printed by the author.

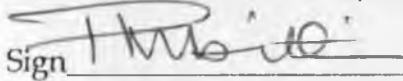
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Dedication

This thesis is dedicated to my wife Watiri and my son Njoroge

"Researchers working with Parasites may have the satisfaction of winning a scientific race but, unless they are in close contact with the problems in the field, they will become frustrated and disillusioned if their elegant diagnostic tools and synthesized vaccines remain on the shelf whilst the parasites continue to take their toll in the countries which cannot afford or are not prepared to use the new tools."

Professor George S. Nelson (1986)

Acknowledgements

My deep appreciation goes to my supervisors – Prof. P.M.F. Mbithi, Prof. J.M. Gathuma and Dr. T.M. Wachira. They devoted most of their time and energy in the development of this project, its implementation and finally development of the thesis. They were patient with me in all aspects of my research and even took time to come to the field to experience the difficult circumstances that I was working under. For this I am extremely grateful.

Both DAAD (Germany) and AMREF (Kenya) played very crucial roles in implementation of this project. DAAD provided all the funds for this project for which I am very grateful. AMREF Kenya facilitated my research by providing the necessary equipment (ultrasound scanner etc.) and a conducive environment for me to carry out the research in Lokichoggio. For this I am extremely grateful to the two organizations. I would specifically want to mention Richard Jacobs (Kenya Country Director, DAAD), Mette Kjaer (Kenya Country Director, AMREF), Jumbe Sebunya (Former Country Director, AMREF) and Dr. John Nduba (Deputy Country Director and former acting Director, AMREF).

Eberhard Zeyhle (AMREF, Kenya) worked tirelessly towards completion of this work. He spent long hours with me during fieldwork, offered positive criticism during drug trials and arranged for my study visit to Germany to do the

molecular biology part of my work. Without his contribution, this work would not have been complete. He indeed is a great scientist.

Dr. Stephen Njiru (University of Nairobi, Kenya) assisted me in preparation and examination of histological samples from the animals used in drug trials for which I am grateful. Dr. Thomas Romig (University of Hohenheim, Germany) and Dr. P.B. Gathura (University of Nairobi, Kenya) spent time to read the initial project proposal and the manuscript. Additionally, Dr. Romig hosted me in Germany when I was doing molecular biology work. For this I am very grateful.

Dr. John Wachira and Alex Gikandi (AMREF, Kenya) assisted me to acquire *Echinococcus* cyst samples from humans during surgery at Kakuma Mission Hospital. Dr. Ngetich and Ben Musungu were very helpful in acquiring *Echinococcus* samples from camels in Kakuma. Martine Obore and Eric Nyongesa (Lodwar District Hospital, Kenya) assisted me in collection of *Echinococcus* cysts from slaughter slabs in Central Division. Dr. Abby Maxson (Pennsylvania State University, USA) participated in the initial part of ultrasound scanning in this project while Dr. Ronald E. Blanton (Case Western Reserve University, USA) was part of the team in the initial drug trials. Their input was highly appreciated.

Prof. Utte Mackensted allowed me to carry out PCR work in her department (Department of Parasitology, University of Hohenheim). Anke Dinkel

introduced me to PCR techniques and allowed me to use her primers. Rainer Oehme was very patient with me when I was learning gene-sequencing techniques in his laboratory. Anja Zimmerman spent most of her time with me in the laboratory when carrying out the PCR work. To all of you I say thank you.

Prof. J.K. Magambo (Jomo Kenyatta University of Agriculture and Technology) provided valuable information, constructive criticism and was always with me to remind me there are 24 hrs in a day to work. His enthusiasm to see me complete the project was a source of encouragement when the work seemed overwhelming. For this I am very grateful.

Mette Kjaer (Director, AMREF Kenya Country Office) facilitated field visits of my supervisors from Nairobi to Lokichoggio and for this I am extremely grateful. Dr. Eliab Some (AMREF headquarters) offered constructive criticism on this project at the beginning of my research in Lokichoggio. Thank you Eliab. Rose Muli (AMREF hydatid office) was very efficient in ensuring that all the administrative matters that pertained to this project were done on time and for this I am grateful. AMREF library staff (Jane Ireri, Lucy Thiongo, Ann Mwikali), AMREF information office staff (Rosemary, Brenda Muchiri) and University of Nairobi Library staff in Kabete were very helpful when I needed information to compile this thesis.

The office of the Chairman, Clinical Studies Department, University of Nairobi, was very helpful in logistics of my research. I specifically want to thank the chairman (Prof. Peter M.F.Mbithi) who facilitated logistics for my research and the secretary (Jane Wairimu) and her team who were always there for me in time of need. For this I am very grateful.

The AMREF Turkana team was always a constant source of encouragement when I felt like quitting. These included Edward Losinyono, Francis Kaleli, Albanus Muiya, John, Kener, Lucy Githaiga, Cecilia Mbae, Beatrice Asukul, Margaret Ngari (CBHC project leader), and Akaran Napakiro.

When this work was completed, Prof. C.N.L. Macpherson (St. George's University School of Medicine, Grenada, West Indies) took time to proofread the whole thesis. Also, he offered valuable information and constructive criticism on use of ultrasound in diagnosis of cystic echinococcosis. For this, I am extremely grateful.

Lastly, I wish to thank my wife Alice who comforted me in difficult times of my research. I had to spend long periods of time away from her while carrying out this work. Her patience with me was truly commendable.

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 POSTMORTEM FINDINGS OF ANIMALS USED IN ALBENDAZOLE AND OXFENDAZOLE DRUG TRIAL

Abstract

Cystic Echinococcosis, caused by *Echinococcus granulosus*, is a major health problem among the nomadic communities of Africa, with the highest incidence in the world being reported in Turkana, Kenya. However, the information available on the disease has various gaps which, if filled, would lead to better management of the disease. These studies were carried out in an attempt to bridge some of the gaps. The studies aimed at evaluating ultrasound as a diagnostic method for cystic echinococcosis. The other objective was to evaluate various treatment methods used for the disease in humans using sheep and goat models.

The first study was carried out in two parts. The first part was to determine the sensitivity, specificity and kappa statistic using postmortem examination as the gold standard. Ultrasound examination, followed by postmortem examination was performed in 300 animals (16 sheep and 284 goats). Thirty-one animals (10.3%) were positive for echinococcus cysts on ultrasound examination and 46 (15.3%) were positive on postmortem examination. Twenty-one animals positive on postmortem were falsely identified as negative on ultrasound examination. Of the 254 animals identified as negative on postmortem, six (2.4%) were falsely identified as positive on ultrasound examination. The sensitivity and specificity of ultrasonography was 54.36% and 97.64% respectively. Positive predictive value and negative predictive value was 80.64% and 92.19% respectively. The degree of clinical agreement between ultrasound and postmortem examinations (kappa) was 0.59.

The second part was to document pertinent ultrasonographic features in diagnosis of *Echinococcus* cysts and the costs of performing ultrasonography in sheep and goats. In this study, ultrasonographic examination of 15 animals with *Echinococcus* cysts was performed. Normal ultrasonographic findings of the abdominal organs are presented and illustrated. Ultrasound findings of *Echinococcus* cysts and its differential diagnosis are also presented. The diagnostic features for *Echinococcus* cysts were double membrane (endocyst and ectocyst), presence of 'hydatid sand' (protoscoleces), and septations (daughter cysts). *Echinococcus* cysts needed to be differentiated from *Taenia hydatigena* cysts, empty rumen and gall bladder in hunger animals. Ultrasonography could be used to detect the location, size and nature of the cysts. The cost of ultrasound examination per animal was \$ 0.714.

In the second study, the applicability of Ultrasonography in prevalence studies of cystic echinococcosis was investigated. A total of 1390 goats were examined, 43.6 % (606/1390) of them from Northwestern Turkana, Kenya, and 56.4 % (784/1390) from Toposaland, Southern Sudan. *Echinococcus* cysts were visualised in 1.82 % (11/606) of the goats from Northwestern Turkana and 4.34 % (34/784) of those from Toposaland. Ultrasonography was found to be limited in detection of *Echinococcus* cysts in the lungs. However, it was found to be an appropriate technique where slaughter was not monitored. Ultrasonography also proved to be

a non-invasive and unbiased technique for prevalence studies of *Echinococcus* cysts because unlike slaughter, whole herds of goats were examined.

The third study was carried out to determine the prevalence of cystic echinococcosis in domestic animals in northern Turkana, Kenya. Animals were examined at slaughter in Lokichogio, Kakuma and Central Divisions of Northern Turkana. A total of 6791 animals were examined at slaughter in the three study areas. These included 5752 goats, 588 sheep, 381 cattle and 70 camels. In cattle, sheep, goats and camels, the prevalence of *Echinococcus* cysts was found to be 19.4%, 3.6%, 4.5%, and 61.4% respectively. The prevalence of cystic echinococcosis in cattle, sheep and goats was higher in Lokichogio than in either Kakuma or Central divisions. On the other hand, the prevalence of the disease in camels was higher in Central (84.6%) than either Lokichogio (70.6%) or Kakuma (50%). The differences in prevalence rates in different study areas were attributed to differences in environmental conditions, livestock stoking intensity, and cross-border migration of livestock.

In the fourth study, the efficacy of oxfendazole in treatment of *Echinococcus* cysts was investigated. Nine goats and four sheep, naturally infected with *Echinococcus* cysts, were given oxfendazole orally at 30mg/kg twice per week for 4 weeks. The animals were monitored by ultrasound examination during the treatment period and 4 weeks after treatment. Ultrasound appearance of the cysts, postmortem

examination of the animals, viability of protoscolices and cyst wall histology were used to determine the efficacy of oxfendazole. In the treatment group, *Echinococcus* cysts showed a decrease in size, mixed echogenicity and complete or partial detachment and calcification on ultrasound examination. There was no visible change of cyst appearance in control animals on ultrasound examination. On post-mortem examination, 53% of cysts from treated animals were found to be grossly degenerated. On microscopic examination, protoscolices were dead or absent in 97% of cysts from oxfendazole treated animals compared to 28% of cysts from untreated control animals. Histological examination of the cysts showed severe disorganisation of the adventitial layer with invasion of inflammatory cells in treatment group. This was absent in control group.

In the fifth study, the efficacies of oxfendazole and albendazole were compared in treatment of cystic echinococcosis. 15 animals were randomly selected into 3 groups of 5 animals each. Two groups were subjected to treatment (with either albendazole or oxfendazole) while the third group served as controls. In the treatment groups, ultrasound examination showed similar findings as oxfendazole group in study 4. Microscopic examination of protoscolices for eosin dye exclusion and flame cell motility showed that 60.9% (14/23) of the cysts from albendazole group had dead protoscolices compared to 93.3% (14/15) and 27.3% (3/11) for oxfendazole and control groups respectively.

In the sixth study, PAIR (Puncture, Aspiration, Introduction of a scolical agent, and Re-aspiration of the agent) technique was evaluated. The objective of this study was to determine the effect of ethyl alcohol in the PAIR technique as compared to puncture alone (without ethyl alcohol). Six animals were used in the study. The animals were sedated with xylazine and, under ultrasound guidance, a total of 9 cysts were subjected to puncture and ethyl alcohol while 7 cysts was subjected to puncture alone. One month after the treatment, the animals were scanned with ultrasound and euthanised for postmortem examination. Postmortem examination showed that both groups (puncture and ethyl alcohol group and puncture alone group) had dead protoscoleces. However, the cysts that had both puncture and introduction of ethyl alcohol were grossly degenerated and were surrounded by fibrosis of liver tissues. In contrast, the cysts where puncture alone was carried out, the cysts appeared intact.

The seventh study investigated the presence of different strains of *Echinococcus granulosus* in domestic intermediate hosts. Endocysts or protoscoleces from human, cattle, sheep camels and pigs were examined by polymerase chain reaction (PCR) technique. Primers for known DNA sequences for *E. granulosus* were used. The target sequence for amplification was part of mitochondrial 12S rRNA gene. The PCR was conducted in two steps, one involving a cestode specific primer and the other involving a primer specific to *E. granulosus* sheep strain. In the first step, the primer pair P60.for. and P375.rev. amplified a 373bp

fragment of the DNA. A total of 250ng of DNA was added to a reaction mixture containing 10mM Tris-HCl buffer, 2.5mM MgCl₂, 200uM dNTPs, 40pmols/ul P60.for., 40pmols/ul P375.rev. and 2.5U Taq polymerase. Thermal cycling of the amplification mixture was performed in an automated Gene Amp PCR System 9700®. A cycle represents denaturation for 30 seconds at 94°C, annealing for 60 seconds at 52°C, and elongation for 40 seconds at 72°C. In all the samples collected from humans, sheep, pigs, 88% of cysts from cattle and 20% of cysts from camel had sheep strain *E. granulosus*. 12% of cysts from cattle and 80% of cysts from camels had camel strain *E. granulosus*.

Based on the findings of these studies ultrasonography is an appropriate technique for diagnosis of cystic echinococcosis in sheep and goats. It is non-invasive, relatively inexpensive to perform and can be used even in remote areas where laboratory facilities are not available and slaughter is not monitored. However, it has a limitation of not detecting cysts in the lungs. Oxfendazole had a higher efficacy against cystic echinococcosis than albendazole. Puncture and introduction of 95% ethyl alcohol had similar efficacy against *Echinococcus* cysts as puncture alone but ethyl alcohol treated cysts were more degenerated 1 month post treatment. Both sheep and camel strains were identified in human and animal intermediate hosts from Kenya. Further research is recommended in oxfendazole therapy and PAIR technique.

The authors have been able to show that the use of the above described method for the determination of the concentration of the active ingredient in the formulation is possible and that the results obtained are in good agreement with those obtained by the use of the standard method.

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CHAPTER 1

General introduction and objectives of various studies on cystic echinococcosis

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1.0 INTRODUCTION

Cystic echinococcosis is a zoonotic disease caused by the larval stages of the parasitic tapeworms of the genus *Echinococcus*. It is distributed in all the continents of the world (Matossian *et al.*, 1977). The disease affects most of the warm - blooded animals. The tapeworm forms cysts in any organ in the body of an animal.

Cystic echinococcosis is one of the important helminth diseases in which there is no method of treatment that is applicable in all cases. Although surgical treatment has been the method of choice in human patients, there are no standard surgical techniques that are universally accepted (Aktan, 1999). Mortality rates after operation ranging from 0.9 to 3.6% have been reported (Little, 1976; Aikat *et al.*, 1978). Recurrence rates following surgery range from 2 to 11% (Little, 1976) but rates as high as 22% have also been reported (O'Leary, 1976). In such recurring cases, surgery carries a poorer prognosis where operative mortality rates for first, second, and third operations are 2.6, 6.0 and 20.0%, respectively (Amir - Jahed *et al.*, 1975).

Use of chemotherapy in human patients has resulted in varied responses. Whereas some authors (Morris *et al.*, 1984; Eckert, 1986; Okello, 1986; Rahemtulla *et al.*, 1987) have reported successful use of albendazole in treatment of cystic echinococcosis, other authors (Puntis and Hughes, 1983) have reported an incontrovertible evidence of failure while using the same drug. In cases where albendazole has been reported to be successful in treatment of cystic echinococcosis, very high dosage rates had to be administered for long periods of time (20mg/Kg/day for 30 - 60 days)(Chen *et al.*, 1994). Whereas hepatotoxicity has been reported in high dosages of albendazole, the long - term side effects need to be investigated. In addition, cases of recurrence have been reported after therapy with albendazole (Gupta *et al.*,

1993). Some hospitals have continued to use mebendazole (Chen *et al.*, 1994) despite its reportedly low efficacy (Okello and Chemtai, 1981) and side effects of severe glomerulonephritis (Kung'u, 1982).

A more recent method of treatment of cystic echinococcosis has been percutaneous drainage and alcohol instillation into the cyst, also known as Puncture, Aspiration, Introduction and Reaspiration (PAIR) (Filice, *et al.*, 1991; Baijal *et al.*, 1995). Although this method of treatment has shown promising results, it is not yet clear whether there are side effects in patients subjected to the treatment. Also, it is not yet established whether leaking of the cyst fluid during aspiration leads to anaphylactic shock as has been reported in other cases of hydatid cyst rupture (Boyano *et al.*, 1994). Implantation of the protoscolices from the cyst fluid that leaks during aspiration would also be expected to cause formation of new cysts in the adjacent tissues.

Due to uncertainty of the various methods of treatment of cystic echinococcosis, clinical trials using new and already existing treatment methods is urgently required. Only well planned prospective randomized trials can make the best type of treatment known. Randomized, prospective, and multicentre studies in humans have always been a problem in developing countries where hydatid disease is endemic (Aktan *et al.*, 1998). It is therefore necessary to conduct such trials in an appropriate animal model. Laboratory animals (mice, gerbils, etc.) which are commonly used for this purpose are unconvincing models due to their small body size which only allows abnormal development of the parasite, while the potentially suitable monkeys are extremely expensive to maintain. In contrast, sheep are natural hosts of *E. granulosus* (the causative agent of cystic echinococcosis), have a body size closer to that of humans and are inexpensive to keep under Kenyan conditions. One of the aims of this project was to evaluate treatment methods of cystic echinococcosis using sheep and goats as models for human cystic

echinococcosis. Such animal models will be useful for drug screening and basic molecular research.

Ultrasonography has proven to be a useful and accepted diagnostic technique for screening for cystic echinococcosis in human populations (Macpherson *et al.*, 1987). More recently, ultrasonography has been used to determine the prevalence of cystic echinococcosis in sheep and goats (Maxson *et al.*, 1996). However, the sensitivity and specificity of ultrasonography in diagnosis of cystic echinococcosis and other space-occupying lesions have not yet been determined. For effective diagnosis of cystic echinococcosis using ultrasonography, both sensitivity and specificity of the technique need to be evaluated. This would also determine whether ultrasound could be used as a diagnostic tool in prevalence studies of hydatid cysts among the animal intermediate hosts. Another aim of this project was to evaluate ultrasonography as a diagnostic technique for cystic echinococcosis.

1.1 OBJECTIVES

The main objectives of this study were:

1. To evaluate ultrasonography as a diagnostic technique for cystic echinococcosis in sheep and goats;
 - 1.1 To determine the sensitivity, specificity and clinical agreement (kappa) of ultrasonography.
 - 1.2 To determine the clinical ultrasonographic features of cystic echinococcosis and the costs of performing ultrasound examination in sheep and goats.
 - 1.3 To determine the applicability of ultrasonography in prevalence studies of cystic echinococcosis.

2. To evaluate treatment methods that are used in human cystic echinococcosis using a sheep and goat model. These methods included:
 - 2.1 Use of albendazole and oxfendazole;
 - 2.2 Puncture, Aspiration, Introduction and Reaspiration (PAIR) using 95% ethyl alcohol.
3. To investigate the existence of variations in the species *Echinococcus granulosus* in various mammalian species, including humans, in Kenya, by use of a polymerase chain reaction (PCR) test.
4. To determine the prevalence of cystic echinococcosis in domestic animal intermediate hosts in Northern Turkana, Kenya.

2.1 DEFINITION OF CYSTIC ECHINOCOCCOSIS

Cystic echinococcosis has several synonyms. These include hydatidosis, hydatid disease, hydatid cyst and echinococcus metacestodiasis (Schantz and Schwabe, 1969; Schantz, 1982; Filice *et al.*, 1991)). In this study, the term cystic echinococcosis will be used to refer to the disease in the intermediate mammalian hosts.

2.2 BACKGROUND INFORMATION ON CYSTIC ECHINOCOCCOSIS

Cystic echinococcosis was recognized from as early as 460 BC by Hipocrates who referred to it as water filled bladders in the liver and lungs of livestock and humans (Grove, 1990). In late 18th century, the parasite was named *Taenia visceralis socialis granulosa* (Grove, 1990). A few years later, Batsch (1786) renamed it *Hydatigera granulosa*. In 1852, von Siebold (1852) experimentally infected a dog with hydatid cysts and therefore demonstrated the life cycle and the association between larval and adult stages. The next major breakthrough was to show that the condition known as alveolar colloid (Zeller, 1854) was related to the hydatid parasite. Virchow (1856) recognised it as such and Leuckart (1886) differentiated the multivesicular nature of these hydatid cysts from the unilocular variety caused by *E. granulosa*, and designated a new species, *E. multilocularis*. Later on, Rudolphi (1801) named the genus *Echinococcus* in which the hydatid parasite was known as *Echinococcus granulosus*. Rudolphi (1808) examined the adult stages in a naturally infected dog but identified them as *Taenia cateniformis*. However, the taxonomic status of *E. multilocularis* remained in doubt for nearly 100 years until Vogel (1957) completed the life cycle in the laboratory and described distinct morphological characteristics of the adult tapeworm, and emphasized the multivesicular nature of the larval stage and its occurrence in rodents.

2.3 AETIOLOGY OF CYSTIC ECHINOCOCCOSIS

Currently, four species of *Echinococcus* parasite are recognised, namely *Echinococcus granulosus*, *E. multilocularis*, *E. oligarthrus* and *E. vogeli* (Schantz, 1982) (Table 2.1). *Echinococcus multilocularis* forms cysts that are poorly margined and are more aggressive in growth and ability to spread (Scherer *et al.*, 1978). *Echinococcus multilocularis* occurs in the Northern Hemisphere. *Echinococcus oligarthrus* and *E. vogeli* occur in southern and Central America (Schantz, 1982). *Echinococcus vogeli* causes polycystic hydatid disease. *Echinococcus granulosus* causes cystic echinococcosis, and is seen throughout the world, particularly in cattle and sheep rearing countries such as Argentina, South Africa, Australia and the middle East (Matossian *et al.*, 1977; Schantz, 1982). Recently, Thompson (1999) questioned classification of *E. granulosus* in a single species and proposes to raise up to 4 strains to species level (Table 2.2).

2.4 WORLD DISTRIBUTION OF ECHINOCOCCUS

2.4.1 ECHINOCOCCUS GRANULOSUS

The highest prevalence of cystic echinococcosis in humans and non-human animals is found in countries of the temperate zones, including South America, the entire Mediterranean littoral, the southern and central parts of the former Soviet Union, Central Asia, China, Australia, and parts of Africa. The highest prevalence is found among populations involved with sheep raising, thus emphasizing the overwhelming public health importance of the sheep strain of *E. granulosus*. In many countries of these regions, national diagnostic incidence rates in humans range from 5-20 per 100,000 population (Schantz *et al.*, 1995).

"National" rates are misleading, however, because most urban populations are at low risk; in rural endemic areas diagnostic incidence is many-fold higher and surveys of local populations using ultrasound and/or serologic techniques often measure cystic infection rates of 2%-6%.

The Life Cycle of *Echinococcus granulosus*

Two mammalian hosts, a definitive host and an intermediate host, are required for the causative agent (*E. granulosus*) to complete its life cycle (Thompson, 1979). The disease is divided into two, the pastoral disease and the sylvatic disease, depending on whether the hosts involved in the life cycle are domesticated or not. In the pastoral hydatid disease, one or both hosts are domesticated, whereas in sylvatic disease both are wild.

The adult worm (Fig. 2.1), a hermaphrodite, 2-7 mm long is found in the domestic dog and a wide range of wild carnivores such as jackal, wolf, fox, wild dog, hyena, lion, leopard and the African wild cat (Neison and Rausch, 1963; Schantz and Schwabe, 1969; Macpherson, 1981). The worm lives in the intestines of these definitive hosts attached to the mucosa by a double row of hooks (Saidi, 1976). The terminal segment, the proglottid, is usually gravid and contains eggs, which are discharged into the animal's intestine and expelled in its feces (Saidi, 1976; Dunn, 1978).

The intermediate hosts for *E. granulosus* consist of domestic livestock and a wide range of wild herbivores. The wild herbivores that act as the intermediate hosts include gazelle, wildebeest, buffaloes, reindeer, and waterbuck (Eugster, 1978; Macpherson, 1981)). These hosts ingest eggs while grazing on contaminated pasture.

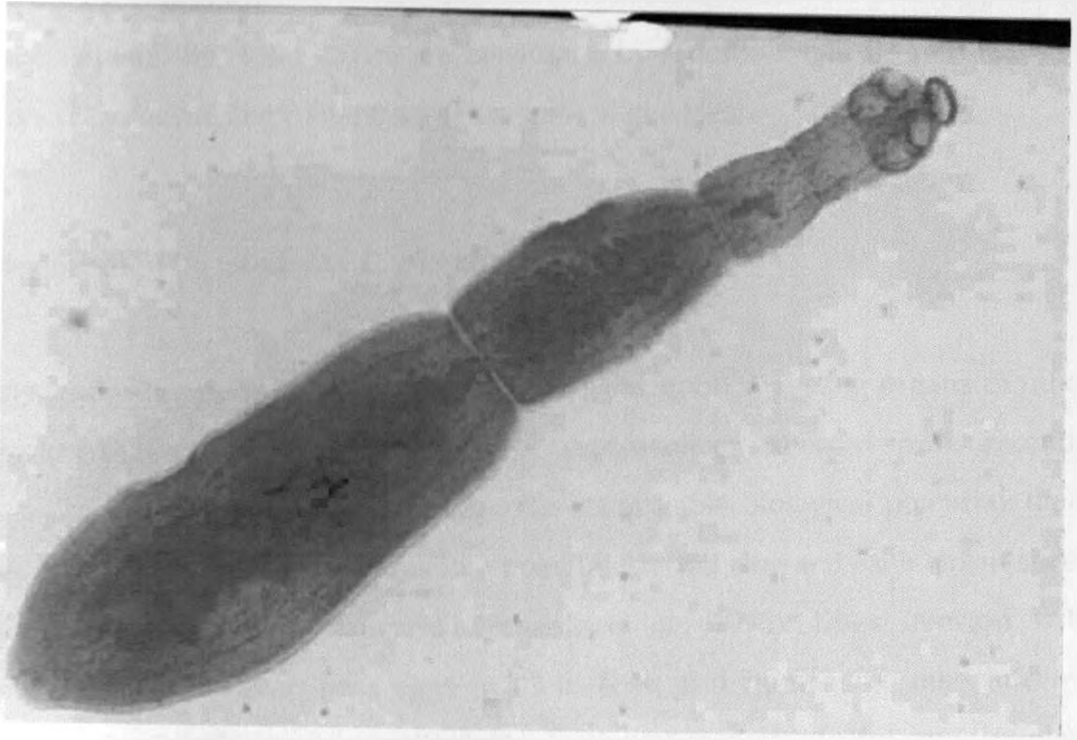


Figure 2.1 Adult tapeworm from the intestines of a dog (*Source: E. Zeyhle, Personal communication*)

Once the eggs are swallowed, the protective chitinous layer is digested in the duodenum (Radford, 1982; Saidi, 1976). This releases the hexacanth embryo, which rapidly passes through the intestinal wall to enter the portal veins. The liver is consequently the organ most frequently involved. Some of the embryos end up in the lungs, kidney, spleen, eye orbit and other organs (Heath, 1973). The embryo develops into a cyst and may reach 5mm or more in diameter after three months. The life cycle is completed when infected intermediate host dies and the viscera, which contain the cystic stages, are consumed by a definitive host. Man becomes infected by consuming contaminated water or vegetables.

Transmission Dynamics of *E. granulosus*

Dogs and other definitive hosts become infected upon ingesting organs of other animals that contain hydatid cysts or protoscolices released from recently ruptured cysts. *Echinococcus granulosus* has remarkable biological potential; there may be as many as 40,000 worms in a heavily infected dog and each worm sheds about 1000 eggs every 2 weeks (Schantz *et al.*, 1995). Dogs infested with *Echinococcus* tapeworms pass eggs in their feces, and humans become infected through fecal-oral contact, particularly in the course of playful and intimate contact between children and dogs. Each egg adheres to hairs around the infected dog's anus and is also found on the muzzle and paws. Indirect means of contact, via water and contaminated vegetables, or through the intermediary of flies and other arthropods, may also result in human infections. Eggs of *E. granulosus* are capable of surviving snow and freezing conditions, remaining viable for at least one year on pasture, but they are susceptible to desiccation and will become incapable of hatching after only a few hours when exposed to sunshine (Wachira *et al.*, 1991). People of both sexes and all ages appear susceptible and opportunities for exposure are mainly related to the contact with

an infested dog. When infested dogs are maintained close to the family home, all members of the family may be exposed. In certain cultural situations, one sex or another may take responsibility for feeding and handling the dogs, thus favoring exposure of persons of that gender (Schantz *et al.*, 1995).

2.4.2 *ECHINOCOCCUS MULTILOCULARIS*

By the end of 1980s, endemic areas of *E. multilocularis* were known to exist in only four countries of central Europe, namely in Austria, France, Germany and Switzerland. Since 1989, the area of geographical distribution has significantly increased. The parasite has been identified in red foxes (*Vulpes vulpes*) in additional regions of previously known endemic countries and in six further countries including the Netherlands, Belgium, Luxembourg, Liechtenstein, Poland, and the Czech Republic (Eckert, 1998; Eckert and Deplazes, 1999). *Echinococcus multilocularis* is also endemic in Russia, Turkey and Japan. Cases of alveolar echinococcosis in humans and animals have also been reported from other countries such as Slovenia, Hungary, Bosnia, Bulgaria, Romania and Greece (Siko Barabasi *et al.*, 1995; Schantz *et al.*, 1995).

Life cycle of *Echinococcus multilocularis*

In Central Europe, the life cycle of *E. multilocularis* is predominantly sylvatic, involving the red foxes (*Vulpes vulpes*) as definitive hosts and a relatively small number of rodent species (e.g. the common vole (*Microtus arvalis*)), the water vole (*Arvicola terrestris*), and the muskrat (*Ondatra zibethica*)) as intermediate hosts. However, neither the range of potential intermediate hosts, nor their significance in disease transmission has been adequately studied. In most parts of Europe, the sylvatic cycle of *E. multilocularis* is not restricted to rural regions but also occurs

in densely populated areas close to or even within villages and urban areas. This means that the ecological barrier between foxes infected with *E. multilocularis* and human populations is low or does not exist. Furthermore, domestic dogs and cats have been identified as final hosts of *E. multilocularis* in at least three Central European countries (France, Germany and Switzerland), suggesting an existence of a synanthropic cycle. There is evidence that domestic carnivores acquire the infection from the sylvatic cycle by ingesting rodents infected with metacestodes. On the other hand, it is unknown to which extent *E. multilocularis* eggs excreted by the domestic carnivores contribute to the infection of the rodent population. Both foxes and domestic carnivores have to be considered as potential sources of human infection.

In the Japanese islands, *E. multilocularis* is transmitted between the red fox (*Vulpes vulpes*) populations as the definitive hosts and the gray-sided vole (*Clethrionomys rufocanus*) as the intermediate hosts (Takahashi *et al.*, 1999). Infection rates in foxes depend on the abundance of voles.

Transmission Dynamics of *E. multilocularis*

E. multilocularis in foxes

Echinococcus multilocularis in foxes has a negative binomial distribution, with only a small number of animals harboring a large number of parasites (Eckert, 1999). In a recent quantitative study (Eckert, 1999), 75% (n=36) foxes had worm burdens below 1000 while 25% had over 1000 with a maximum of approximately 60,000. Only two foxes in this population harboured 78% of the total parasite biomass. According to studies in Japan, egg excretion in foxes persists for about 1 to 4 months after the end of the short prepatent period (26 to 29 days); it is highly

variable and may reach egg counts as high as 100,000 per gram. It has been estimated that a fox infected with 10,000 mature *E. multilocularis* could theoretically excrete 240,000 to 420,000 eggs per day (Eckert, 1999). The increasing fox populations in Europe and the apparent lack of an efficient immunological downregulation of the parasite burdens in foxes are factors enhancing their important epidemiological role.

E. multilocularis in dogs and cats

Little information exists in Europe on the prevalence of *E. multilocularis* in populations of dogs and cats. However, new diagnostic tools such as the coproantigen ELISA and PCR can now be employed for screening larger populations of living domestic carnivores. Using these techniques, 660 dogs and 263 cats originating from the endemic areas of eastern Switzerland have been examined, of which 0.3% and 0.4% were infected with *E. multilocularis*, respectively (Deplazes *et al.*, 1999). Higher prevalence levels have to be expected in groups of dogs and cats that have regular access to infected rodents, especially in microfoci of disease transmission. Indeed, in a highly endemic small focus in western Switzerland, ELISA and PCR identified 12% of 41 dogs as carriers of *E. multilocularis* (Gottstein *et al.*, 1997).

2.4.3 *ECHINOCOCCUS OLOGARTHRUS*

Echinococcus oligarthrus is the only currently recognized species of *Echinococcus* that has wild felids as definitive hosts. Naturally acquired infections have been demonstrated in the puma, the jaguarondi, the jaguar, the ocelot, the pampas cat and the Geoffroy's cat (Thakur, 1999). The larval form of *E. oligarthrus* has been described in agoutis, pacas, spiny rats and rabbits (*Sylvilagus floridianus*). The

cestode has been reported in naturally infected intermediate or definitive hosts throughout a very wide range of Central and South America, as far north as Costa Rica and as far south as La Pampas Province, Argentina (Thakur, 1999).

2.4.4 ECHINOCOCCUS VOGELI

Echinococcus vogeli was first identified in the specimens recovered from a bush dog, *Speothos venaticus* (Rausch and Bernstein, 1972). Subsequently, the cestode has been reported in Central and South America where it is an important cause of human disease (d'Allessandro, 1996). *Echinococcus vogeli* has been reported in both humans and lower animal intermediate hosts in Costa Rica, Panama, Colombia, Ecuador, Venezuela, Brazil and Bolivia (Rausch, 1986). Polycystic hydatid disease due to *E. vogeli* infection has been reported in these countries.

The life cycle of *E. vogeli* involves the bush dog (*Speothos venaticus*) as the definitive host, and the paca (*Cuniculus paca*) as the intermediate host. Little is known about the circumstances associated with infection in humans. Bush dogs are rare and avoid human beings, and therefore, probably play little role in direct exposure of humans. In endemic areas, dogs are commonly fed viscera of paca and human infections are probably acquired from the feces of dogs that become infected when they ingest viscera of infected pacas; this practice has been reported commonly by patients (Meneghilli *et al.*, 1992). The disease caused by *E. oligarthrus* and *E. vogeli* has been named polycystic echinococcosis and has been reviewed by d'Allessandro (1996).

Table 2.1 The Four currently recognized species of *Echinococcus* (Source: R.C.A. Thompson, 1999).

	<i>E. granulosus</i>	<i>E. multilocularis</i>	<i>E. oligathrus</i>	<i>E. vogeli</i>
Geographic distribution	Cosmopolitan	Central and Northern Eurasia; North America	Central and South America	Central and South America
Host Range				
Definitive hosts	Domestic and wild Canids	Foxes, domestic dogs, cats	Wild felids	Bush dog
Intermediate hosts	Ungulates, marsupials and primates (including man)	Arvicolid rodents, man	Rodents (agoutis, paca, spiny rats), humans	Agoutis, other rodents and humans
Metacestode				
Nature of cyst	Unilocular, endogenous proliferation, infiltration and metastasis	Multivesicular, endogenous proliferation, infiltration and metastasis	Polycystic, endogenous and exogenous proliferation, no infiltration or metastasis	Polycystic, endogenous and exogenous proliferation, no infiltration or metastasis
Location of cyst	Visceral, primarily liver and lungs	Visceral, primarily liver with subsequent metastatic spread particularly in humans	Peripheral, primarily muscles	Visceral, primarily liver
Adult				
Mean number of segments (range)	3 (2-7)	5 (2-6)	3	3
Total length of strobilar (mm)	2.0-11.0	1.2-4.5	2.2-2.9	3.9-5.5

Table 2.2 Proposed “new” species in the genus *Echinococcus* (Source: R.C.A. Thompson, 1999)

Suggested taxonomic designation	Known definitive hosts	Known intermediate hosts	Geographic distribution	Synonyms	Strains
<i>E. granulosus</i>	Dog, fox, dingo, jackal, hyena	Sheep, cattle, goats, buffalo, camels, macropods, humans	Australia, Europe, USA, New Zealand, Africa, China, Middle East, Asia, South America, Russia	<i>E. patagonicus</i> , <i>E. cepanazo</i> , <i>E. granulosus</i> <i>E. newzealandensis</i>	Common sheep strain, Tasmanian sheep strain, Buffalo strain
<i>E. equinus</i>	Dog	Horses and other equines	Europe, Middle East, South Africa, New Zealand USA	<i>E. granulosus equinus</i> , <i>E. granulosus</i> horse strain	
<i>E. ortleppi</i>	Dog	Cattle buffalo, humans	Europe, Africa, India, Sri Lanka, Russia	<i>E. granulosus ortleppi</i> , <i>E. granulosus</i> cattle strain	
<i>E. intermedius</i>	Dog	Pigs, humans, camel	Europe, Russia, South America	<i>E. granulosus</i> pig strain	Pig strain, camel strain
<i>E. multilocularis</i>	Fox, dog, cat	Rodents, pigs, horses, humans	Europe, North America, Canada, Japan, China	<i>E. sibiricensis</i>	European strain, North American strain, Alaskan strain, Hokkaido strain
<i>E. vogeli</i>	Bush dog	Rodents, humans	South America		
<i>E. oligarthrus</i>	Felines	Rodents, humans	South America	<i>E. pampeanus</i> , <i>E. cruzi</i>	

2.5 THE ECHINOCOCCUS CYST

An *Echinococcus* cyst has three layers (Saidi, 1976; Hadidi, 1982). The outer pericyst is composed of granulation tissue and fibrous tissue derived from the host. This forms a rigid protective layer only a few millimetres thick (Saidi, 1976). The middle-laminated (endocyst) (Fig. 2.2) membrane is composed of a chitin-like substance and is easily ruptured. It is acellular and permits the passage of nutrients but is impervious to bacteria. Disruption of the laminated membrane predisposes to infection. The inner germinal layer is thin and translucent. It produces the laminated membrane and the protoscoleces that represent the larval stage. Protoscoleces (Fig. 2.3) are also produced by brood capsules, which are small spheres of disrupted germinal layer. These may remain attached to the germinal layer, but free-floating brood capsules and protoscolices form white sediment known as hydatid sand. During surgical treatment, hydatid sand may leak into the adjacent tissues and organs and is postulated to cause recurrence of the disease in surgical patients (Aaron, 1979). The cyst fluid that leaks may also cause anaphylactic shock.

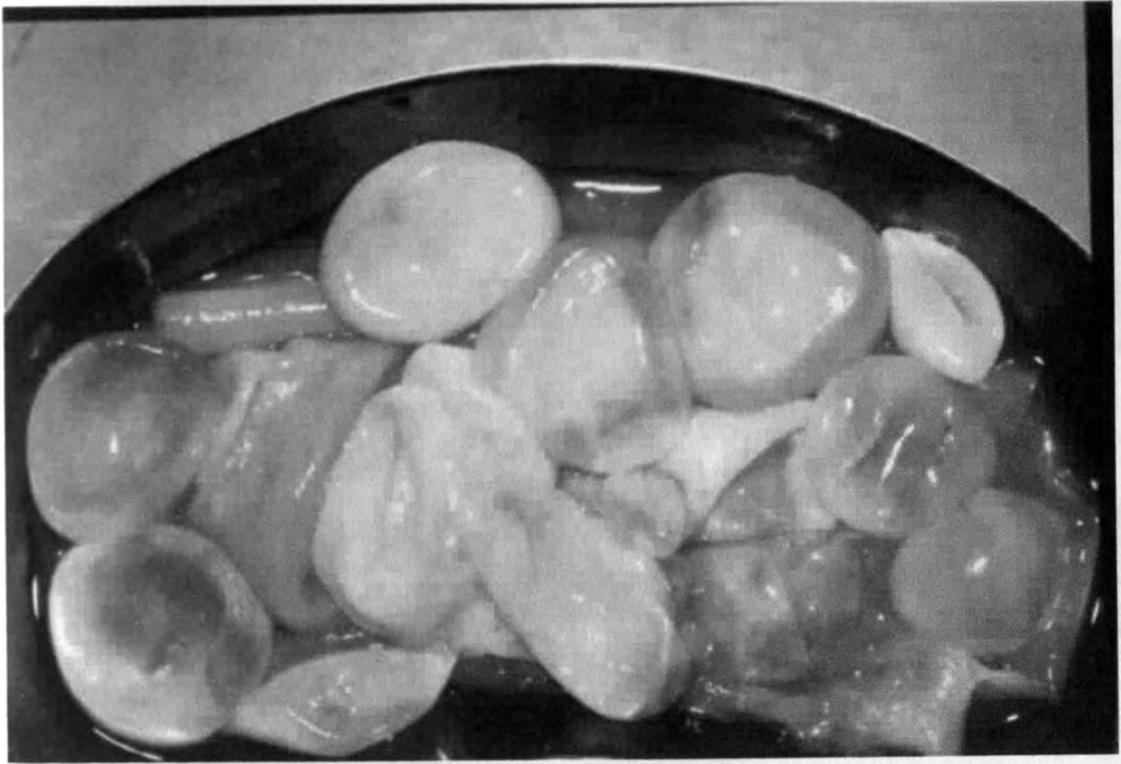


Figure 2.2 Endocysts from a cystic echinococcosis in a human patient after abdominal surgery. *Kakuma Mission Hospital, November 1999*

various countries in the temperate zone of North America and Europe. In the United States, the prevalence of hydatid cysts in sheep is about 10% (Henderson, 1960). In New Zealand, the prevalence of hydatid cysts in sheep is about 10% (Henderson, 1960). In the United Kingdom, the prevalence of hydatid cysts in sheep is about 10% (Henderson, 1960). In the United States, the prevalence of hydatid cysts in sheep is about 10% (Henderson, 1960).

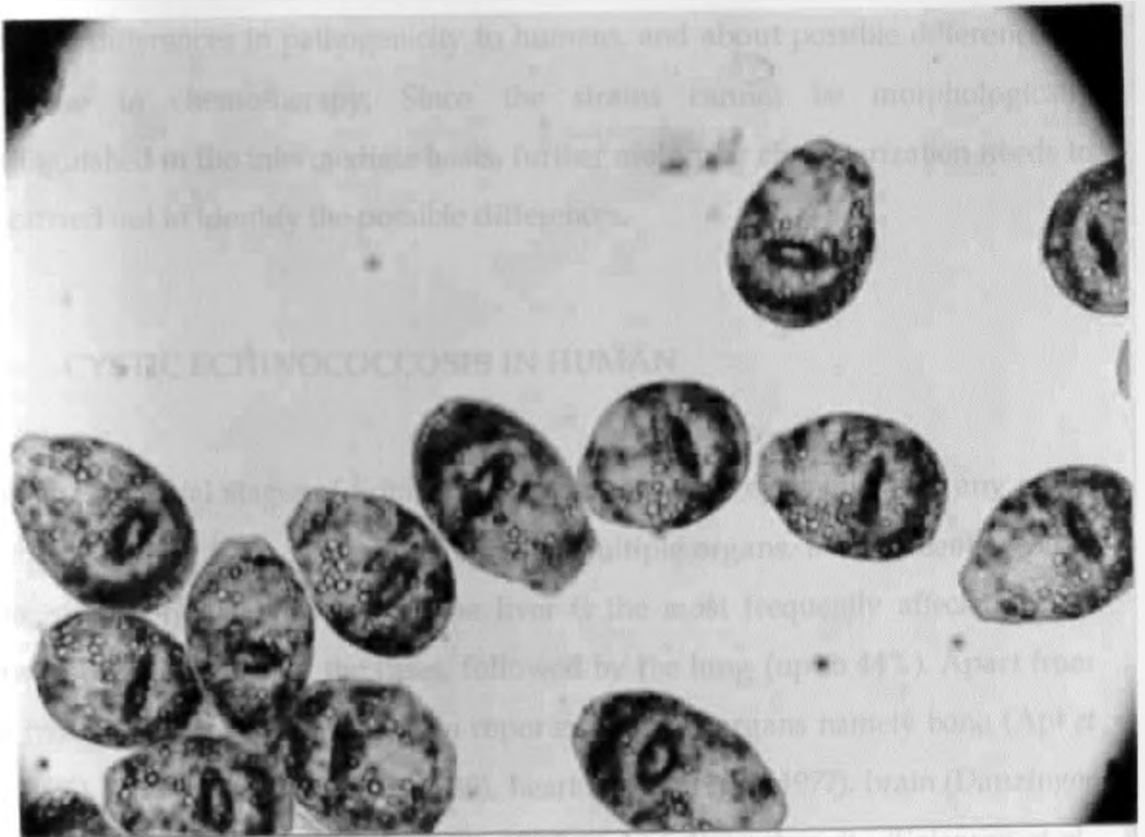


Figure 2.3 Protoscoleces of Echinococcus granulosus harvested from hydatid cysts from a sheep

2.6 AETIOLOGY OF CYSTIC ECHINOCOCCOSIS IN KENYA

Echinococcus granulosus is the causative agent of cystic echinococcosis in Kenya (Nelson and Rausch, 1963). It has been previously shown that at least two genetically distinct strains of *E. Granulosus* exist in Kenya (Wachira *et al.*, 1993). Both are known to be present in sheep. However, nothing is known about their possible differences in pathogenicity to humans, and about possible differences in response to chemotherapy. Since the strains cannot be morphologically distinguished in the intermediate hosts, further molecular characterization needs to be carried out to identify the possible differences.

2.6 CYSTIC ECHINOCOCCOSIS IN HUMAN

In man, the larval stages of *Echinococcus granulosus* form cysts in almost any organ in the body. The cysts may affect single or multiple organs. Most patients (>80%) have single organ involvement. The liver is the most frequently affected organ constituting 52% -77% of the cases, followed by the lung (up to 44%). Apart from the two organs, the cysts have been reported in other organs namely bone (Apt *et al.*, 1976), kidneys (Gilsanz *et al.*, 1980), heart (Gilsanz *et al.*, 1977), brain (Danzinger and Bloch, 1975), orbit (Hamza *et al.*, 1982) and peritoneal cavity (Solomon *et al.*, 1982). The cysts have a varied course of infection. Some cysts may grow to a certain size and then persist without a noticeable change for many years. Other cysts may either rupture or collapse and may completely disappear. Those that rupture may drain the cyst fluid into the pericardium, the bile duct, gastrointestinal tract, the bronchioles and bronchi of the lungs or the blood vessels. Still, other cysts cure spontaneously (Romig *et al.*, 1986). The cysts cause a clinical disease when they drain into vital organs after rupture, in which case the patient develops various complications depending on the organ involved; they also cause disease when they

are active, growing and start exerting pressure on adjacent tissues and vital organs. The cysts may lead to pain, discomfort, abdominal swelling (Fig. 2.4), jaundice, hemoptysis, pathologic bone fracture, spinal cord compression, syncope and various nervous signs. The largest reported *Echinococcus* cyst contained 24 liters of fluid in a 27-year-old Turkana woman (Zeyhle *et al.*, 1999) (Fig. 2.5).



Fig. 2.5 A 27-year-old Turkana woman with a large abdominal swelling due to a large *Echinococcus* cyst. (Zeyhle *et al.*, 1999)



Figure 2.4 A 12 year old Turkana girl with abdominal cystic echinococcosis. Note the distended abdomen. *AMREF-Camp Lokichoggio, July 1998.*

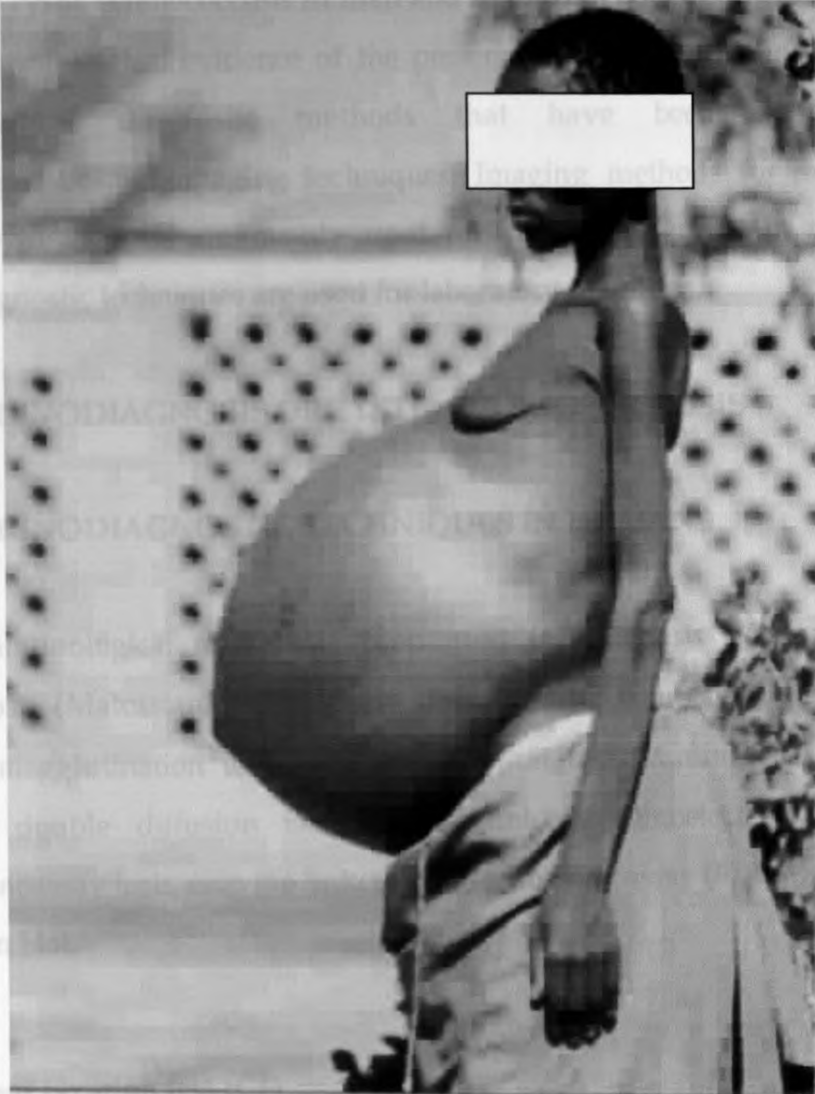


Figure 2.5 A 27 year old Turkana woman with abdominal cystic echinococcosis. After surgery, 24 Liters of fluid was drained from the cyst. Note the distended abdomen. *Kakuma Mission Hospital, November 1998.*

2.7 DIAGNOSIS OF CYSTIC ECHINOCOCCOSIS

Diagnosis of cystic echinococcosis in man and livestock is difficult because there is no direct parasitological evidence of the presence of the cysts in host organs or tissues. Indirect diagnostic methods that have been used include immunodiagnostic and imaging techniques. Imaging methods for detection of space-occupying lesions are mainly used for clinical diagnosis in man while immunodiagnostic techniques are used for laboratory diagnosis.

2.7.1 IMMUNODIAGNOSIS OF CYSTIC ECHINOCOCCOSIS

2.7.1.1 IMMUNODIAGNOSTIC TECHNIQUES IN HUMANS

Various immunological tests have been used in diagnosis of human cystic echinococcosis (Matossian, 1977). These tests include: complement-fixation test, indirect hemagglutination test, latex-agglutination test, immuno-electrophoresis test, arc-5 double diffusion test, arc-5 counter-immunoelectrophoresis test, radioimmunoassay tests, enzyme-linked immunosorbent assay (ELISA), dot-ELISA and western blot.

Complement Fixation test (CF)

The sensitivity of this test ranges from 36-93% (Matossian, 1977). Other authors recorded a sensitivity of 63.3% with 10% false positive cases (Chemtai *et al.*, 1981). It is therefore not a very useful test for the screening of this disease.

Indirect Hemagglutination Test (IHA)

This test has a lot of variations which may be mainly due to the use of different antigens, use of erythrocytes from various species, treatment of erythrocytes with various chemicals to facilitate antigen binding or preservation, use of different criteria for determining test positivity, use of tube, microtiter and slide agglutination techniques (Varela-Diaz *et al.*, 1975). Erythrocytes are fixed so as to facilitate antigen binding and four techniques are used to do this including tannic acid, glutaraldehyde, benzidine and formaline (Varela-Diaz *et al.*, 1975; Schantz *et al.*, 1986). All these variations may cause large differences in sensitivity and specificity. The IHA test has an overall sensitivity of 86.7% (Chemtai *et al.*, 1981).

Latex-Agglutination Test (LA)

In latex agglutination test, the test serum is considered positive when clumps of latex particles are detected with sensitised latex particles (Varela-Diaz *et al.*, 1975). Varela-Diaz *et al.* (1975) achieved an almost 100% specificity. However, Rickard (1984) obtained a false positive rate of 17.6% and a sensitivity of 70.7%. Discrepancies in sensitivity and specificity have been reported by different authors and may be due in part to differences in latex particle size and composition (Rickard, 1984; Schantz *et al.*, 1986). In addition, serum is also known to agglutinate unsensitized latex particles (Schantz *et al.*, 1986).

Immuno-electrophoresis Test (IEP)

The diagnostic criterion for the test is qualitative, that is the demonstration of a particular antigen, for example arc 5 antigen, which should be one of the several

antigen-antibody precipitin bands demonstrable in the serum of patients with hydatidosis (Schantz, *et al.*, 1986). The test has been reported to achieve a sensitivity of 78.8% (Rickard, 1984). In a survey done in Turkana, IEP was unable to detect anti-echinococcus antibodies in 42.5% of the cases, resulting in an overall sensitivity of 55% (Chemtai *et al.*, 1981). Immuno-electrophoresis is not suitable for mass screening of serum samples because of the large amount of serum and antigen required, in addition to the technical intricacy of the test (Varela-Diaz *et al.*, 1975; Rickard, 1984).

Arc-5 Double Diffusion Test (DD5)

This test uses a control anti-serum against *E.granulosus* arc-5 antigens to recognize arc-5 positive sera by a reaction of identity (Schantz *et al.*, 1986; Thompson, 1986). Sensitivity figures given for the test are 54% (Kagan, 1976) and 55% (Chemtai *et al.*, 1981). Some studies have shown that when DD5 and IEP are used for testing against sera from patients with surgically confirmed hydatid disease, DD5 test is more sensitive than the IEP test and is equally specific (Coltorti and Varela-Diaz, 1978; Schantz *et al.*, 1986).

Though this test is simple to perform and highly specific, it is not practical for population studies due to the time lapse between testing and receiving results (Coltorti *et al.*, 1988).

Arc-5 Counter-immunoelectrophoresis Test (CEP5)

Hira *et al.* (1990) reported both the sensitivity and specificity of CEP5 to be 98% respectively. However, these findings should be taken with caution as the antigen preparation was not purified antigen 5, but a commercial hydatid cyst fluid

preparation capable of eliciting the arc 5 reaction in an IEP test. Furthermore, specificity was not assessed with sera from other larval cestode infections (Hira *et al.*, 1990). Also, the entire procedure of this test takes 90 minutes and therefore it would take a long time to perform the procedure in a large population.

Casoni Test

The appearance of an immediate hypersensitivity reaction following the intradermal injection of hydatid cyst fluid antigen has been widely used in the immunodiagnosis of human hydatidosis since its demonstration by Casoni in 1912 (Thompson, 1986). In a positive casoni test reaction, a wheal develops within 15 minutes after injection in 75% of hydatidosis cases, making the test a very easy one to perform with results obtained in a very short time. The test is reported to have a high sensitivity of 90%. The test has, however, a poor specificity due to the use of hydatid cyst fluid as an antigen and so resulting in non-specific reactions (Thompson, 1986). False positivity of 2-45% has been reported in people with some other parasitic diseases (Schantz *et al.*, 1986).

Radioimmunoassay Tests (RIA)

Radioimmunoassay test uses tracer amounts of labeled antigen to determine the percentage of unlabelled antigen bound by specific antibodies (Matossian, 1977). The antigen may or may not be bound to a solid phase and the percentage binding of labelled antigen by the serum is compared with that of the standard serum (Matossian, 1977).

A simplified RIA test was developed by Matossian (1981) for the diagnosis of human hydatidosis and trichinosis and reported a sensitivity of 93%. Although the

test has high sensitivity, it requires skilled personnel and specialized equipment to perform (Matossian, 1981).

Enzyme-linked Immunosorbent Assay (ELISA)

This test system is based on the reaction of *Echinococcus* antigens or anti-*Echinococcus* antibodies bound to a solid matrix with the counterpart antibodies or circulating antigens in the patient test sera (Schantz *et al.*, 1986). The test serum is then incubated with this bound antigen or antibody to form an antigen-antibody complex (Matossian, 1977). An enzyme linked antiglobulin when added combines with the complex. The substrate is then added, optical density readings are taken using a reader (Rickard *et al.*, 1984). Using antigen "880", a sensitivity of 82.7% and a specificity of 73.3% has been reported (Njeruh *et al.*, 1986). However, Gathura (1991), using antigen "346", obtained a sensitivity of 66.6% and a specificity of 84.3%.

Dot-Elisa

A dot enzyme-linked Immunosorbent assay (Dot-ELISA) was developed as a field-test for the diagnosis of cystic hydatid disease (Rogan *et al.*, 1991). In this type of ELISA, the antigen is applied as a spot or dot on nitrocellulose paper (Gathura, 1991). In a study carried out in Turkana, the test recorded a sensitivity of 76.6% and specificity of 90% (Gathura, 1991) while in another study carried out in the same place the test showed a sensitivity of 94% and specificity of 90.3% (Rogan *et al.*, 1991). In a different study done using 18 hydatid patient sera and 32 control sera from healthy individuals, the test showed a sensitivity of 88.9% and a specificity of 96.9% (Romia *et al.*, 1992). In another study carried out with 17 hydatid patient sera

and 36 controls, all the patient sera were positive while none of the controls were positive (Mistrello *et al.*, 1995). The advantages of this test are that it is rapid, inexpensive, and simple to perform (Rogan *et al.*, 1991). However, the sample sizes used in this test are too small to make accurate interpretation of the results.

Western blot

In a study carried out using 105 cystic echinococcosis patient sera and 86 control sera, the test sera showed that 80% of the patient sera recognized the 8kDa antigen while none of the control sera did (Ayadi *et al.*, 1995). Out of the 105 patients, 103 were able to recognize at least one of the 8, 21, 30, or 92 kDa specific antigens. In another study using the 30kDa antigen this test was shown to have a sensitivity of 80% and a specificity of 100% (Planchart *et al.*, 1994).

2.7.1.2 IMMUNODIAGNOSTIC TECHNIQUES IN LIVESTOCK

There is currently no suitable serologic test available for cystic echinococcosis in any livestock species (Lightowlers, 1990). When the same antibody detection methods (within the same antigen preparations as used in human cystic echinococcosis) were used in ovine cystic echinococcosis, lower levels of sensitivity and specificity were recorded for various immunological tests. The tests include indirect haemagglutination (IHA) (Yong *et al.*, 1978), arc-5 IEP and DD5 (Yong and Heath, 1979; Conder *et al.*, 1980), and ELISA (Yong and Heath, 1979; Craig and Rickard, 1981; Yong *et al.*, 1984). Even when harbouring large mature hydatid cysts, sheep frequently exhibit no or weak antibody response (Lightowlers, 1990). Positive reactions have, in most cases, turned out to be due to other larval taenids namely *Taenia hydatigena*, *T. ovis*, *T. multiceps* (Craig and Rickard, 1981; Yong *et al.*, 1984) and due to *Fasciola hepatica* (Craig *et al.*, 1980; Craig and Rickard, 1981).

Njeruh *et al.* (1986) reported a sensitivity and specificity of 91% and 100% respectively using a semi-purified heat-stable hydatid cyst fluid antigen designated 'antigen 880' to detect serum antibody by ELISA in sheep and goat. Unfortunately, no details are provided concerning the sheep and goats used in the study which would allow an estimation of the relative exposure of the infected and the control groups to other taeniid cestodes which may evoke the production of cross-reactive antibodies (Lightowers and Gottstein, 1995).

2.7.2 DIAGNOSTIC IMAGING OF CYSTIC ECHINOCOCCOSIS

Various imaging techniques have been used in diagnosis of cystic echinococcosis. These include X-ray, ultrasonography, computerised tomographic (CT) scan, and magnetic resonance imaging (MRI) (Schantz and Gottstein, 1986). These techniques are used in the diagnosis of cystic echinococcosis in humans due to their ability to detect space - occupying masses.

2.7.2.1 RADIOGRAPHY (X-rays)

X-rays form one part of the electromagnetic radiation, which includes radiowaves, microwaves, infrared, visible light, ultraviolet light, X-rays and gamma rays (Douglas *et al.*, 1987). They are produced when fast moving electrons are slowed down or stopped. There are three basic requirements for X-ray production namely a source of electrons (cathode), a target to stop the electrons (anode), and a method of accelerating the electrons from the source to the target.

X-rays are of short wavelength, high frequency, and high energy (Douglas *et al.*, 1987). In addition, they have the ability to produce a latent image on a photographic film that can be made visible by processing the film. They also have

the ability to penetrate tissues that are opaque to visible light, but are gradually absorbed the further they pass through an object (Gillette *et al.*, 1977; Douglas *et al.*, 1987). The amount of absorption depends on the atomic number and the density of the tissue and on the energy of the X-rays. These properties make X-rays useful in diagnostic imaging. However, prolonged exposure to X-rays leads to various radiation injuries. These include:

1. Immediate tissue damage (Acute tissue intoxication). Depending on how much area of the body is exposed and the area exposed, this can lead to sudden death,
2. Long term tissue injury due to prolonged exposure to sub-lethal doses. This usually leads to cancer and the tissue most susceptible is the bone marrow.

Tissue and subject susceptibility:

1. Young cells - bone marrow, lymph nodes, gonads, embryos and tumours. These are more susceptible to radiation than other tissues,
2. Age - Anybody who is below 16 years old should not be allowed to assist in radiography,
3. Pregnancy - Pregnant women should not assist in radiography to protect the foetus.

Use of X-ray in Diagnosis of *Echinococcus* Cysts

X-ray examination retains its diagnostic applicability in *Echinococcus* cysts of the lungs and sporadically in finding calcified lesions elsewhere (Pawlowski, 1997). In many cases, however, characteristic cyst structures do not present as a clear image or are absent (Rogan *et al.*, 1990).

2.7.2.2 ULTRASOUND

Ultrasound is a mechanical, vibrational energy generated at a frequency above the range of the human ear (ie. above approximately 20 KHZ). For most diagnostic purposes, the frequencies used are in the 0.5 MHZ to 20 MHZ range (Barnett and Morley, 1974). Ultrasound is reflected by tissue interfaces within the body of an animal and the resulting image is displayed electronically on an oscilloscope screen. There are five modes of Ultrasound that are used in diagnosis (Donald and Levi, 1975; McDickens, 1976; Wells, 1977) including

1. A scan

This produces a one dimensional image. The amplitude of the vertical deflection is proportional to the strength of the echo. A scan is commonly used in fetal cephalometry,

2. B Scan

This produces a two dimensional image. Series of bright dots on the Oscilloscope builds up an anatomical image. This mode is used in general abdominal and obstetric work,

3. Real-time scan

This is similar to B scan but allows movement to be detected. It is used in obstetrics and general abdominal imaging,

4. Time-motion scan

This mode traces an echo pattern against a time base. It has been used in tracing the movement of cardiac valves,

5. Doppler

This mode reflects a change in ultrasonic frequency due to movement. It is used in fetal heart monitoring and bloods flow studies,

The mode of ultrasound best suited for, and most commonly used in human cystic echinococcosis, is real-time scan because most of the human cases are in the liver.

Advantages of ultrasound

Mann (1982) summarized the advantages of using ultrasound in cystic echinococcosis as follows:

- (a) It is non-invasive, easily repeatable and relatively cheap;
- (b) It does not require the taking of blood for laboratory tests -a procedure often abhorred by nomads amongst whom cystic echinococcosis is often rampant;
- (c) It avoids the preservation and transportation of samples from remote areas to costly laboratories served by highly skilled staff in towns;
- (d) It avoids sophisticated laparotomy to view abdominal masses, take biopsy or aspiration;
- (e) It allows the monitoring of progress of any chemotherapeutic drug used for treatment.

In human medicine in Kenya, ultrasound has been used for mass survey of hydatidosis and other intra-abdominal diseases such as hepatocellular carcinoma, gastric carcinoma, hepatic metastases, hepatic and renal polycystic disease, amoebic liver abscesses, bile duct strictures, gallstones, extrahepatic obstructive jaundice, and tumours of the kidneys, pancreas and heart (Mann, 1982).

The most expensive technological diagnostic advances have been computerised tomographic scanning (CT) and magnetic resonance imaging (MRI). The ultrasound machine, on the other hand is small, portable and relatively less expensive and can be used in both diagnostic and therapeutic assessment of tropical diseases (MacKenzie, 1985). It is about one-tenth the price of a CT scanner. Unlike CT and MRI, during ultrasound examination, the probes are hand-held and

a lesion can be viewed or searched from a varied number of angles and positions. Unlike X-rays, ultrasound is non-invasive and has no risk of personnel exposure to radiations.

Use of Ultrasound in Diagnosis of *Echinococcus* Cysts

Most ultrasound examinations of *Echinococcus* cysts have been carried out in humans. Ultrasound will demonstrate well-defined cysts, which may be single or multiple, uni-or multiloculated, thin or thick walled (Beggs, 1983; Gonzalez *et al.*, 1979; Itzhak *et al.*, 1980; Gharbi *et al.*, 1981; Niron and Ozer, 1981; Hadidi, 1982). Calcification may be present in the cyst wall or internally (Itzhak *et al.*, 1980; Hadidi, 1982). Daughter cysts initially produce localized thickening of the cyst wall (Niron and Ozer, 1981) or small, solid echogenic areas (Itzhak *et al.*, 1980). As they mature and become cystic, the pressure within the parent cyst increases and this may distort their shape (Itzhak *et al.*, 1980). They may be single or multiple and produce a multiloculated appearance indistinguishable from polycystic disease (Hadidi, 1982). Infolding of the collapsed parent endocyst may contribute to this honey comb appearance (Gharbi *et al.*, 1981). Multiple univesicular cysts are usually separated by normal liver parenchyma.

Separation of the laminated membrane from the pericyst produces a split wall or floating membrane appearance (Niron and Ozer, 1981). Complete collapse results in a sonographic water-lily sign when the parasite lies in the most dependent part of the cyst (Niron and Ozer, 1981) or produces an irregular, solid echo pattern (Gharbi *et al.*, 1981).

Infected cysts are usually poorly defined by other imaging techniques but remain well defined on ultrasonography (Hadidi, 1982). They contain internal echoes, air and an air-fluid level or layering, but these appearances may also occur with

uninfected ruptured cysts. Daughter cysts impacted in the biliary tree produce bile duct dilatation.

Classification of Hydatid Cysts

Hydatid cysts have been classified into various types based on their ultrasound appearance. Two main classifications exist namely the Gharbi classification and the WHO standardised classification.

Gharbi classification

In this classification, hydatid cysts have been classified in five types (Figure 2.6) (Gharbi *et al.*, 1981).

Type I

This type of cyst appears as anechoic space with marked enhancement of back-wall echoes. The fluid collection is rounded with well-defined borders but the walls vary in thickness. This cyst is usually young and in the initial stages of development.

Type II

This cyst has a well-defined contour but is less rounded. It appears to be “sagging” in some places. The split wall appearance may be visualized outside or inside the cyst. Inside the cyst, the split wall appears as a “floating membrane” (water-lily sign). This is a mature cyst that for some reason may have stopped growing.

Type III

This cyst has a well-defined contour. Additionally, it has septa divisions inside forming oval or rounded structures, where it appears as a honeycomb image. The

echoes within the cysts show images of simple or multiple secondary vesicles. This cyst is in most cases fertile with secondary vesicles that have viable protoscoleces.

Type IV

This cyst is usually infected and degenerating. The cyst has irregular contour and echo patterns. Three types of echo patterns are found in this type of cyst:

1. Hypoechoic appearance with few regular echoes;
2. Hyperechoic solid pattern without back-wall shadow;
3. Intermediate pattern, including both hypoechoic and hyperechoic structures.

Type V

This type of cyst appears as a formation with a hyperechoic contour, with a cone-shaped shadow that is usually outlined. This cyst is old and usually degenerate, non-viable and calcified.

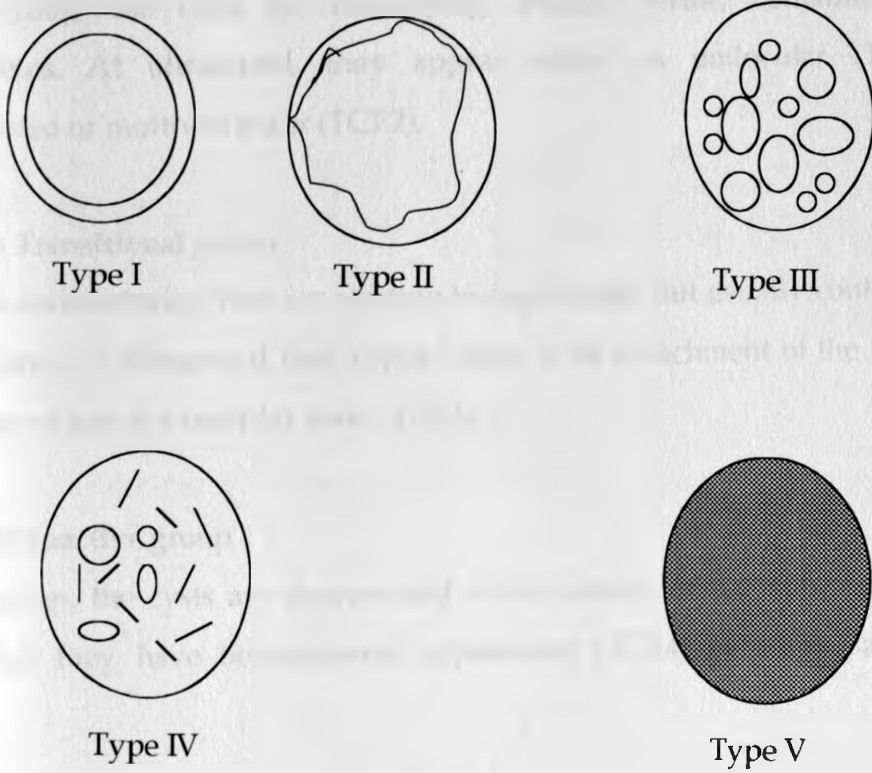


Figure 2.6 Ultrasound classification of Echinococcus cysts according to Gharbi et al. (1981)

World Health Organization (WHO) standardised Classification

In WHO classification (Macpherson, 2001a) both ultrasound appearance and clinical nature of the cysts are used. Three groups exist (Fig. 2 7):

Group I: Active group

In this group, the cysts are developing, usually fertile, containing viable protoscoleces. At ultrasound, they appear either as unilocular (ICE1) or multiseptated or multivescicular (ICE2).

Group II: Transitional group

The cysts are involuting, they are starting to degenerate, but usually contain viable protoscoleces. At ultrasound they appear either with detachment of the laminated membrane or just as a complex mass (ICE3).

Group III: Inactive group

In this group, the cysts are degenerated or are totally or partially calcified. At ultrasound, they have heterogenous appearance (ICE4) or show calcification (ICE5).

This classification has several clinical implications (Caremani and Lapini, 2001):

1. It permits to identify the group a cyst belongs to and therefore helps the clinician decide on whether to treat or not;
2. It allows a choice of treatment (surgery, chemotherapy or PAIR therapy);
3. It allows the degree of response to be determined after treatment;
4. In case of non-response, it permits the choice of an alternative therapy;
5. It allows identification of eventual complications natural or induced;

WHO Standardised classification of CE

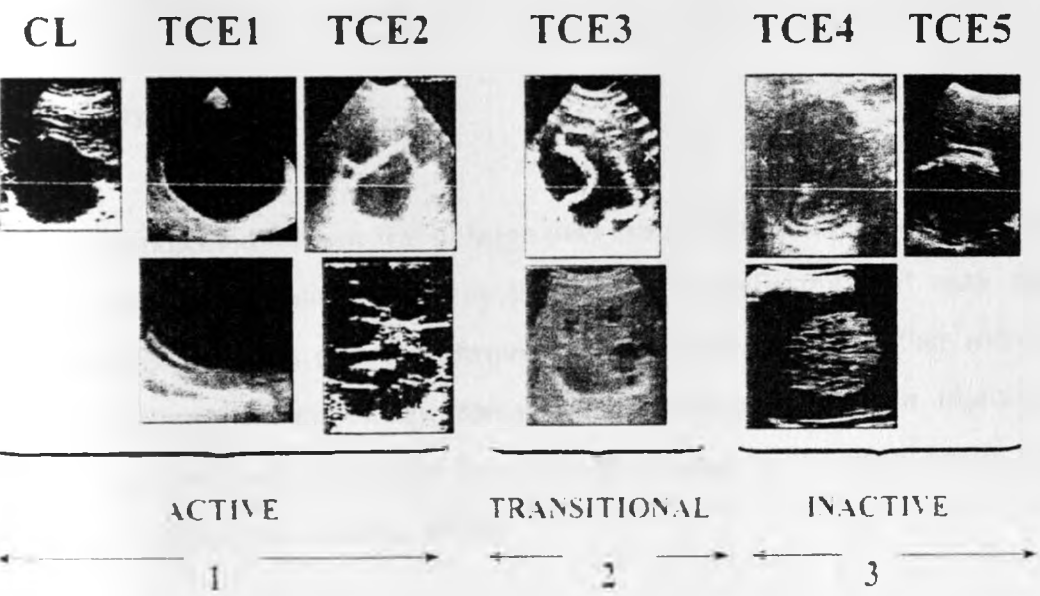


Figure 2.7 WHO classification of Echinococcus cysts (Macpherson, 2001a)

2.8 TREATMENT OF CYSTIC ECHINOCOCCOSIS

2.8.1 SURGERY

To date, surgical removal of the *Echinococcus* cyst has been the method of choice in man. Surgery is usually done in combination with chemotherapy in which massive doses of drugs are administered to the patient (Aaron, 1979; Chen *et al.*, 1994).

Indications

Surgery is indicated for removal of large liver cysts with multiple daughter cysts. It is also indicated in single liver cysts situated superficially that may rupture spontaneously or as a result of trauma, and in cysts that are either infected or communicating with the biliary tree and are exerting pressure on adjacent vital organs. Surgery is also indicated for removal of cysts in the lung, brain, kidney, bones, and other organs (WHO, 1996)

Contraindications

Surgery is contraindicated in patients with concomitant severe diseases (cardiac, renal or hepatic diseases, diabetes, or hypertension), patients at the extremes of age (too young or too old), pregnant women, or patients refusing surgery. In addition, surgery is contraindicated in patients with multiple cysts, cysts that are difficult to access, dead cysts, either partly or totally calcified cysts, and in patients with very small cysts (WHO, 1996).

Surgical Procedure

Three types of surgical procedures exist. These include palliative surgery that involves simple tube drainage of infected cysts or communicating cysts, conservative surgery that requires open cystectomy with or without omentoplasty, or radical surgery that involves total pericystectomy or partial hepatectomy (WHO, 1996).

Conservative surgery, the commonly used approach, involves making an incision to allow adequate access to as many of the cysts as possible (Aaron, 1979). In some cases it may be necessary to make several incisions at different sites. The common incision sites are the paramedian abdominal incision and either left or right thoracotomy. Other sites include thoracoabdominal and the trans-thoracic trans-diaphragmatic approach.

After the incision is made, the cyst is exposed and a suction device is placed on the exposed area of the cyst. A trochar and cannula are then inserted into the cyst and as much of the contents as possible aspirated. Once the tension has been released by removal of most of the cyst contents, the danger of spillage is considerably reduced. Stay sutures are applied to the exposed wall of the cyst, and the hole made by the trochar opened more widely. The rest of the cyst contents are then removed from the host capsule using sponge forceps, suction, hydatid spoons or any other device necessary to lift the solid material out (Fig. 2.8).



Figure 2.8 Surgical removal of Echinococcus cyst from a Turkana patient
(Kakuma Mission Hospital, November 1999)

For intraoperative killing of protoscolices, there is no ideal protoscolicidal agent that is both effective and safe. The lethal action observed *in vitro* may be hampered *in vivo* by the instability of the substance used (e.g. hydrogen peroxide), or by an unpredictable dilution by hydatid fluid. Potential communication between the *Echinococcus* cyst and the biliary tree eliminates protoscolicides (such as formalin) that can cause chemical cholangitis leading to sclerosing cholangitis. At present, three types of protoscolicides are recommended for use due to their low risk of toxicity and any of them may be used alone. These include 70-95% ethanol, 15-20% hypertonic saline or 0.5% cetrimide (WHO, 1996). For optimal efficacy, the substances have to be left in contact with the cysts for at least 15 minutes.

The surgery is usually accompanied by a routine intravenous drip of corticosteroids to alleviate the possible occurrence of an allergic reaction during the operation and postoperative pyrexia (Chen *et al.*, 1994). Very high doses of albendazole or mebendazole are also administered for long periods of time (20mg/Kg/day for 30 - 60 days), to destroy the protoscolices left in the body during the operation.

Despite all the precautions taken, radical surgery of hydatid cysts is always risky. The risks include those associated with any surgical intervention (anaesthesia, stress, and infections including those transmitted by blood transfusion (e.g. hepatitis, human immunodeficiency virus (HIV)). In addition, there may be anaphylactic reactions, secondary echinococcosis owing to spillage of viable parasite material (2-25% of the cases) and possible recurrences if other cysts are present. Reported recurrence rates following surgery range from 10 to 20% (Morris *et al.*, 1992). Operative mortality varies from 0.5% to 4%, but may be higher if surgical and medical facilities are inadequate.

28.2 PUNCTURE, ASPIRATION, INTRODUCTION, REASPIRATION (PAIR)

A more recent method of treatment of hydatidosis has been percutaneous ultrasound-guided cyst puncture, also known as PAIR (Puncture, Aspiration, Introduction and Reaspiration) (Filice *et al.*, 1991; Baijal *et al.*, 1995). The technique has both therapeutic and diagnostic potential. However, diagnostic puncture is recommended only if other diagnostic methods have failed (WHO, 1996).

Indications

The PAIR technique is indicated for inoperable patients and those who refuse surgery. It has been used in the treatment of hydatid cysts in the liver, abdominal cavity, spleen, kidney and bones, but should not be used for lung cysts (Gargouri *et al.*, 1990). PAIR is also recommended in cases of relapse after surgery or in failure to respond to chemotherapy. It might also be indicated for pregnant women with symptomatic cysts but the risk associated with peri-intervention benzimidazole treatment has to be carefully assessed since benzimidazoles are contraindicated during the first 3 months of pregnancy. PAIR is also indicated for liver cysts that are anechoic and >5cm in diameter, and Gharbi types I and II (Gharbi *et al.*, 1981). It is also indicated for cysts with a regular double laminated membrane, cysts of >5cm in diameter with multiple septal divisions (Gharbi type III) except honeycomb like cysts, and multiple cysts of <5cm in diameter in different liver segments (WHO, 1996).

Contraindications

The PAIR technique is contraindicated for inaccessible cysts. It is also contraindicated for cysts that are located superficially due to risk of spillage of cyst contents into the abdominal cavity. The technique is also contraindicated for honeycomb-like cysts, cysts with echogenic lesions, inactive or calcified cysts, communicating cysts and lung cysts.

PAIR Procedure

Before the procedure for PAIR is performed, the patient should be treated with benzimidazoles for a minimum of 4 days. The treatment should be continued for 30 days (albendazole) or 90 days (mebendazole) after the procedure (WHO, 1996).

In this technique, the cyst fluid is first aspirated by puncturing the cyst percutaneously under ultrasonic guidance without surgically opening up the patient. A protoscolicidal agent (95% ethyl alcohol or 20% sodium chloride) is then introduced into the cyst to cauterize the protoscoleces. Although this method of treatment has shown promising results, the efficacy and potential risks have not yet been fully evaluated and require further properly controlled long-term studies (WHO, 1996).

2.8.3 CHEMOTHERAPY

Hydatid disease is among the few important helminth diseases for which there is no effective chemotherapy. Mebendazole was shown to be the first drug able to destroy larval cestodes (Campbell and Blair, 1974; Heath and Chevis, 1974; Kammerer and Judge, 1976; Bekhti *et al.*, 1977; Okello and Chemtai, 1981). The drug, however, has a very low efficacy (Anon, 1984). Adverse side effects such as

severe glomerulonephritis have also been reported (Bekhti *et al.*, 1977; Beard *et al.*, 1978; Kungu, 1982). Albendazole is also used for the treatment of hydatidosis and has shown very promising results, although the long-term side effects are yet to be determined (Morris *et al.*, 1983; Okello, 1986).

28.3.1 ALBENDAZOLE

Albendazole (methyl [5-(propylthio)-1H-benzimidazol - 2 - yl] carbonate) is a very stable, white, odourless powder that is insoluble in water and only slightly soluble in most organic solvents. It is a broad-spectrum anthelmintic, effective against most helminthes of animals and man.

Mode of action

Albendazole is metabolized via sulphur oxidation, followed by carbonate hydrolysis. Three metabolites are formed, namely sulfoxide, sulfone, and 2-aminosulfone. It is thought that the albendazole sulfoxide is the active substance in the blood and tissues of the treated animals. *In vitro* studies of albendazole on hydatid protoscolices have also shown that albendazole sulphoxide significantly reduces the viability of protoscolices (Chinnery and Morris, 1986). Albendazole sulfoxide kills worms by inhibiting polymerization of tubulin, a ubiquitous cellular protein, into microtubules. This action disrupts the parasite's cell nutrition, division, and development processes.

Spectrum of action

In man, albendazole (Zentel[®], Smithkline & Beecham) has been used for the treatment of intestinal helminth infections, hydatid disease, and cysticercoses

(Anon, 1984). In adult, a single dose of 400mg is effective against roundworms, hookworms and most cases of whipworms. The same dose repeated for three consecutive days is effective against strongyles and cestodes.

In sheep, albendazole (Valbazen®) at dosage rate of 3.8 mg/kg body weight had been found to be effective against *Haemonchus*, *Ostertagia*, and *Trichostrongylus* in the abomasum, *Nematodirus*, *Cooperia*, *Marshallagia*, *Bunostomum*, *Gaigeria*, *Charbetia*, *Oesophagostomum*, *Moniezia*, and *Avitellina* in the intestines, and *Dictyocaulus* in the lungs (Lukovich *et al.*, 1979). For the control of *Fasciola hepatica* in sheep, albendazole is administered at the rate of 4.75 to 7.5mg/kg body weight. Outstanding efficacy was observed against *Fascioloides magna* in sheep at a dose of 7.5mg/kg body weight. At 15mg/kg body weight, albendazole is also effective against *Dicrocoelium dendriticum*.

Toxicity

Albendazole has a broad safety margin in various animals. It was well tolerated by mice at 30mg/Kg/day from day 6 to day 15 of gestation with no teratogenesis (Theodorides, 1990). In cattle, a single dose of 75mg/kg body weight albendazole was well tolerated. Albendazole was, however, embryotoxic when administered to cows at a dosage rate of 25mg/kg body weight during the first 7 to 17 days of gestation. In ewes too, under experimental conditions, albendazole was found to induce fetal skeletal abnormalities when administered at a dosage rate of 11mg/kg body weight during the first 10 to 17 days of gestation. This has, however, not been reported in clinical use of the drug. The maximum tolerated dose in sheep is reported to be 37.5mg/kg body weight. Doses above 37.5mg/kg body weight lead to hepatotoxicity and nephrotoxicity (Theodorides, 1990).

In man, Morris *et al.* (1985) have also reported hepatotoxicity while using albendazole at 20mg/kg body weight for 6 weeks. The abnormalities of liver function tests were, however, reversible.

Use of albendazole in the treatment of cystic echinococcosis

In humans, several trials of treatment of cystic echinococcosis using albendazole have been conducted (Morris *et al.*, 1983; Puntis and Hughes, 1983; Okello, 1986). In one trial (Morris *et al.*, 1983), the authors reported a high rate of efficacy. After 6 weeks of treatment with 10mg/kg body weight or 20mg/kg body weight the cysts scanned with ultrasound were found to be flaccid, with only a little yellowish fluid, rather than being tense and full of crystal - clear fluid as before treatment. However, Puntis and Hughes (1983) reported failure of albendazole in the treatment of cystic echinococcosis while using the same dosage as well as an additional 30mg/kg daily for 4 weeks. In another study (De Rosa and Teggi, 1990), albendazole was reported to cause cure in only 8.7% of the patients while 67.4% had slightly improved. In the same study, 23.9% showed no improvement. While the above authors could assess the results of chemotherapy, Rahemtulla *et al.* (1987) could not adequately assess the results after using recommended dosages of albendazole. Due to conflicting reports on use of albendazole in treatment of cystic echinococcosis, it is necessary to evaluate the efficacy of the drug using an animal model. Evaluation of the clinical response and viability of protoscoleces after treatment with albendazole would provide information that would enhance the treatment of humans.

New formulations of albendazole

In recent years, many scientists have made efforts to improve the formulation of albendazole from the parent drug in order to enhance its bioavailability and efficacy.

Injectable formulation of albendazole

A parenteral formulation of albendazole (PLA-Alb) for intravenous administration has been developed, using poly (D,L-lactide, PLA) nanoparticles as a biodegradable carrier (Rodrigues *et al.*, 1995). The formulation has been used in mice with *E. multilocularis* infection. Less of the intravenous formulation was required compared to the oral formulation (6mg/kg of PLA-Alb had similar effect to 1500mg/kg of oral albendazole). The formulation was, however, not found to have dose-dependent efficacy, even when higher daily and total doses were used.

Albendazole-liposome (Alb-L)

Wen *et al.* (1993, 1996) found that when cotton rats infected with *E. multilocularis* for 3 months were treated orally with a daily dose of 35mg/kg body weight Alb-L or 50 mg/kg body weight albendazole for 5 days, followed by a repeated regime every week for 8 weeks, the drug contents in blood, liver and cyst tissues were 2 to 4-fold higher in Alb-L group than in albendazole group 7 hours after the last medication. However, no apparent improvement in therapeutic efficacy was observed. In a similar study, when mice infected with *E. granulosus* for 3 months were treated orally with 200mg/kg body weight of either Alb-L or albendazole daily for 3 months, the inhibition rate of cyst weight was higher in Alb-L group (87%) than in albendazole (68%) (Shao and Wen, 1998).

In order to improve the targeting of the drug, Wang *et al.* (1997) have developed an immuno-liposome linked covalently with monoclonal antibody and prepared an immunoliposome-carried albendazole compound. When mice infected with *E. multilocularis* for 3 months were treated with albendazole, Alb-L or immunoliposome-carried albendazole at a daily dosage rate of 50mg/kg for 20 days, the inhibition rate of cyst weight was 15.5%, 42.8% and 79.8% respectively. This indicated that the immunoliposome-carried formulation could apparently increase the effect of albendazole on *E. multilocularis*

Combination of Albendazole with other drugs

Albendazole combined with cimetidine

Luder *et al.* (1986) found that cimetidine could alter the metabolism of mebendazole, resulting in an increase of the drug concentration in the blood. Due to these encouraging results cimetidine was combined with albendazole in treatment of cotton rats infected with *E. multilocularis* (Prez-serrano *et al.*, 1994). This resulted in an increased albendazole sulfoxide concentration in cyst tissues of the combined treatment group than that in the group treated with albendazole alone. The efficacy of the combined treatment was, however, similar to that of albendazole alone.

Albendazole combined with albendazole sulfoxide

In an *in vitro* set up (Casado *et al.*, 1996), protoscolices exposed to a combination of 5µg/ml albendazole and 5µg/ml albendazole sulfoxide died after 12 days of incubation. Two days after the protoscolices were exposed to albendazole

combined with albendazole sulphoxide, vesiculation was seen in their tegument and the internal tissue was severely damaged. However, when protoscolices were exposed to 10µg/ml albendazole alone or 10µg/ml-albendazole sulphoxide alone, about 80% of them died after 27 days of incubation. In another study (Prez-serrano *et al.*, 1997), mice infected with *E. granulosus* protoscolices for 3 days were treated with 25mg/kg body weight albendazole in combination with 25mg/kg body weight albendazole sulphoxide daily for 5 days, followed by a similar course for 12 weeks. This combined treatment exhibited a potential preventive effect which resulted in a much lower cyst number and a higher inhibition rate of cyst weight compared to groups treated with either 50mg/kg body weight albendazole or 50 mg/kg albendazole sulphoxide alone.

Albendazole combined with dipeptide methyl ether

In metacestodes of *E. multilocularis*, the laminated layer is the barrier for the penetration of the drugs. Walchshofer *et al.* (1993) developed a dipeptide methyl ester (Phe-Phe-Ome) which exhibited significant damage to the parasite laminated layer. After gerbils infected with *E. multilocularis* for three months were treated orally with 50mg/kg body weight Phe-Phe-Ome for 18 days, histological examination showed a nearly complete destruction of the germinal layer with cellular infiltration of metacestodes by host cells. In the same study, combination of 50mg/kg albendazole with 50mg/kg body weight Phe-Phe-Ome showed that Phe-Phe-Ome increased the effect of albendazole so much that no cysts were seen in gerbils treated with two courses of the combined treatment.

Albendazole combined with levamisole

Albendazole, when combined with levamisole, showed an inhibitory effect on the proliferation of *E. granulosus* protoscolices and *E. multilocularis* tissues in mice

(Li, 1993). When used on *E. granulosus* developed cysts, the cysts had severe necrosis and desquamation of the germinal layer. However, when *E. multilocularis* tissues (from mice treated with the combination) were transplanted to the peritoneal cavity of another group of mice, they still showed infectivity.

Albendazole combined with praziquantel

In vitro and *in vivo* tests carried out in different laboratories demonstrated that praziquantel exhibited a potential effect against echinococcus protoscolices and germinal layer of hydatid cysts (Thompson *et al.*, 1986). Further studies indicated that a higher concentration of praziquantel (4µg/g-cyst tissue) was necessary to induce severe damage of the germinal layer. However, such higher concentrations were not reached in the cyst when a normal therapeutic dose was given to the host (Chen *et al.*, 1989; Sheng *et al.*, 1994; Xiao *et al.*, 1994).

In cystic echinococcosis, studies have shown that praziquantel exhibits rapid *in vitro* action on the protoscolices compared to albendazole. The protoscolices exposed to albendazole or albendazole sulfoxide combined with praziquantel resulted in an increase in protoscolicidal effect. Similar results have been obtained in experimental therapy of alveolar hydatid disease (Taylor *et al.*, 1988). In another study (Xiong *et al.*, 1996), when mice infected with *E. granulosus* protoscolices were treated with a combination of albendazole and praziquantel on the day of infection (d 0), 4 weeks or 5 months, the efficacy was increased compared to either drug used alone.

Among human subjects, 21 patients with cystic hydatid disease were treated orally with a combination of 10mg/kg body weight albendazole and 25mg/kg body weight praziquantel daily for one month before surgical operation. In the same study, another group of 26 patients was treated with albendazole alone at a

daily dose of 20 mg/kg (Cobo *et al.*, 1998). Albendazole sulfoxide concentrations in both blood and cyst fluid collected after the final medication and during the operation were higher in the combined treatment group than in the group treated with albendazole alone. Furthermore, live protoscolices were present in 2 out of 21 patients in the combined treatment compared to 13 out of 26 cases treated with albendazole alone (Cobo *et al.*, 1998). In another study, (Yasway *et al.*, 1993) 4 patients with cystic hydatid disease were treated with 400mg of albendazole for 2 – 5 courses combined with 50mg praziquantel given once daily every 1 – 2 weeks. The cysts in 3 patients disappeared completely 3 months after combined treatment.

28.3.2 OXFENDAZOLE

Oxfendazole has not been used in treatment of cystic echinococcosis and no explanation has been given for lack of use. Oxfendazole is a benzimidazole related to albendazole with the sulfur molecule in the albendazole substituted by an oxygen molecule. It has a slower rate of elimination and has greater potency at lower dose levels than the parent benzimidazole (thiabendazole). It is similar in action to albendazole (Table 2.3).

Whereas albendazole is only detected in serum as its metabolites (Sulfoxide and sulfone) for up to 48 hours, oxfendazole is detectable in serum for up to 11 days after administration in sheep (Bogan and Marriner, 1980). Oxfendazole may, therefore, have higher efficacy because it is detected in serum for longer periods of time than the metabolites of albendazole. Oxfendazole reaches a mean peak plasma concentration of 0.8mg/ml (Bogan and Marriner, 1980) while albendazole sulfoxide has a peak plasma concentration of 0.54mg/ml (Morris *et al.*, 1985). Since oxfendazole has higher serum concentration levels than the metabolites of albendazole, it may prove to have higher efficacy in treatment of cystic

echinococcosis. Trials of oxfendazole in an animal model would provide useful information that could lead to an effective chemotherapy in human hydatid infections. The drug has been tested for use in pigs against cysticercosis and a single dose was found to be effective in the treatment of the disease (Gonzales *et al*, 1996).

Table 2.3 Comparison of the efficacy of Oxfendazole with Albendazole
(Adapted from Brander *et al.*, 1982; Bogan and Marriner, 1980)

	OXFENDAZOLE	ALBENDAZOLE
Parasite	Minimum effective Dose	
	5mg/kg	5mg/kg
Haemonchus	4	4
Ostertagia	4	4
Trichuris	4	4
Nematodirus	4	4
Cooperia	4	4
Strongyloides	3	3
Trichuris	2	1
	-	Teratogenic (sheep)
	-	Hepatotoxic (Man)

Key: 4 = 95-100% Efficacy
3 = 85-95% Efficacy
2 = 60-85% Efficacy
1 = < 60% Efficacy

2.9 POLYMERASE CHAIN REACTION (PCR)

Polymerase chain reaction is an *in vitro* method of nucleic acid synthesis by which a specific target nucleic acid sequence can be replicated (Mullis and Faloona, 1987; Saiki *et al.*, 1988). It involves two oligonucleotide primers that flank the DNA fragment to be amplified and a repeated cycle of heat denaturation of the DNA at 94°C, annealing of the primers to their complementary sequences at 40°C, and extension of the strands by DNA polymerase at 72°C. The DNA synthesis by polymerase proceeds across the region between the primers and stops at the 5' end of the second primer.

Since the extension products are also complementary to and capable of binding the primers, they also act as target DNA in the subsequent cycles of amplification. Due to this, there is an exponential accumulation of the specific target fragments, approximately 2^n where n is the number of cycles of amplification performed. Availability of modern thermocyclers and ability to manufacture synthetic oligonucleotide primers enabled the semi - automation of the PCR procedure. The template DNA is mixed with oligonucleotide primers, deoxynucleotide triphosphates (dNTPs) and thermostable Taq DNA polymerase enzyme (obtained from the bacteria *Thermus aquaticus*) in a suitable PCR buffer and run through the 3 cycles until the desired amount of amplification is achieved. A layer of mineral oil above the PCR mixture prevents the evaporation in the thermal cyclers. The oil also prevents sample to sample contamination.

The PCR technique has been used for amplification of unknown sequences, genetic examinations, DNA amplification fingerprinting, and detection of infectious agents of rare sequences (Vodkin *et al.*, 1992; Dirie *et al.*, 1993).

29.1 USE OF PCR IN DIFFERENTIATION OF *ECHINOCOCCUS* SPECIES

The PCR technique has been used to isolate two strains of *Echinococcus granulosus* (camel and sheep strains) and to determine the intermediate host range in Kenya (Wachira *et al.*, 1993). In the same study (Wachira *et al.*, 1993), it was postulated that humans appear refractory to infection with the camel strain. A PCR system which does not require prior sequence information but instead uses primers of arbitrary sequence, arbitrary primer PCR (AP-PCR) was used in the study. Primers specific to cestodes as well as to strains of *Echinococcus* have been developed (Dinkel, 1998; Dinkel *et al.*, 1998). These primers could be applied to further screen and differentiate *E. granulosus* strains present in man and livestock in Turkana.

INTRODUCTION

CHAPTER 3

Evaluation of ultrasonography as a diagnostic technique for cystic echinococcosis in sheep and goats

3.0 INTRODUCTION

The study of cystic echinococcosis in the intermediate hosts has been hampered by lack of a suitable diagnostic technique. Serological tests that have been developed for use in the intermediate hosts have low sensitivity and specificity due to cross-reactions with other helminth parasites and therefore cannot be relied upon. Slaughter data have previously been used to study the prevalence of the disease. This study evaluated the suitability of ultrasonography in diagnosis of cystic echinococcosis in sheep and goats. The study was conducted in three parts. The first part was to determine the sensitivity, specificity and kappa using post mortem examination as the gold standard. The second part was to document pertinent ultrasonographic features in diagnosis of *Echinococcus* cysts and the costs of performing ultrasonography in sheep and goats. The third part was to determine the applicability of ultrasonography in prevalence studies of hydatid cysts in goats.

3.1 MATERIALS AND METHODS

3.1.1 Determination of sensitivity, specificity and kappa of ultrasonography

The study was carried out at Kiserian slaughterhouse in Ngong Division of Kajiado District, Kenya. All the Sheep and goats presented at the slaughterhouse were used for this experiment. The animal was restrained manually in standing position. Hair was shaved from the right side of the animal using an electric clipper (Sunbeam Stewart clipmaster, model 510A Head; Oster Golden A5(R) model 5-55K, USA). Shaving was from ventral to dorsal, against the direction of the hair grain, and a coupling gel was applied to the shaved area. Ultrasound examination was performed with a real time B-mode scanner with a 3.5 mHZ linear array transducer with electronically variable focus (Concept 2, Dynamic Imaging, West Lothian, Scotland) powered by a 1.9kW portable electricity generator (Honda Generator JB 2200, Honda Inc, New York, NY, USA). Static images were recorded on a graphic printer (Sony Graphic Printer, model UP-850 Sony Corp., Japan). The number, size, and location of the cysts were noted. The animals were then tagged before slaughter. After stunning, carcasses of these animals were tagged with numbers corresponding to those of the respective animals. A postmortem examination of the carcasses was carried out blindly to determine the number, size, and location of cysts. The sensitivity and specificity of ultrasonography was calculated using the following formulae in table Table 3.1 (Sacket *et al*, 1985).

Table 3.1 Determination of sensitivity, specificity and kappa statistic of ultrasound using postmortem as the standard.

		True diagnosis (Postmortem)		
		+	–	Total
Test results (Ultrasound)	+	a	b	a+b
	–	c	d	c+d
	Total	a+c	b+d	a+b+c+d=n

Sensitivity = (a x 100)/a+c

Specificity = (d x 100) / b+d

Key:

- a Number of animals in which cysts were found at both ultrasound scanning and postmortem examination.
- b Number of animals in which cysts were detected by ultrasound scanning but absent at postmortem examination.
- c Number of animals in which cysts were found at postmortem but could not be detected by ultrasound scanning.
- d Number of animals in which cysts were absent at both postmortem examination and ultrasound scanning.

3.1.2 Determination of pertinent ultrasonographic features and the costs of performing ultrasonography

This study was carried out in Lokichogio, Kenya. A total of 15 goats identified to have cystic structures were used in this study. Ultrasound examination was performed as described in Section 3.1.1 (Figures 3.1 & 3.2). A 3.5 MHz linear array or a 3.5MHz microconvex transducer was used depending on the width of the intercostal space. The liver, kidneys, rumen, and entire right side of the abdomen were examined in detail. The ultrasound features of various organs detected were recorded. Ultrasound findings were validated by postmortem examination of the animals.

The cost of ultrasound examination was determined by calculating how much money was required to perform a scan per goat. This was based on purchase price of portable ultrasound equipment, clippers/shavers, electric cables, and electric generator. It was also based on recurrent expenditure on items that are used during ultrasound examination. These items include ultrasound gel, ultrasound film, power/fuel, and labour. Information on the purchase price of various items was obtained from various sources. These included Nairobi X-ray center, Kenya Electronics, and Caltex petrol station in Lokichogio.

The following formulae were used to determine the cost of ultrasound examination per goat:

$$\text{Cost of examination / goat} = a + b$$

Where a = direct costs

b = indirect (equipment) costs

$$a = c_1/n_2.n_3 + c_2/n_1 + c_4/n_1 + n_4/n_1.C_3$$

$$b = d/n_1$$

Key:

- c₁ Cost of ultrasound gel (5Lt)
- c₂ Cost of film (1 roll)
- c₃ Cost of fuel/litre
- c₄ Labour costs
- n₁ Number of goats scanned / day
- n₂ Number of litres of ultrasound gel
- n₃ Amount of ultrasound gel used / goat
- n₄ Amount of fuel used per day
- d depreciation costs / day



Figure 3.1 Preparation of a goat for ultrasound scanning. AMREF camp, Lopiding, August 2000



Figure 3.2 Ultrasound Scanning of a goat. AMREF camp, Lopiding, August 2000.

3.1.3 Application Ultrasonography in prevalence studies of *cystic echinococcosis*

The study was carried out in the villages of Lopiding, Aposta, Lokichoggio and Nanam of Northwestern Turkana (Fig. 3.3) during the months of May to December 1998. Goats from Toposaland, Southern Sudan were examined at the Kenya-Sudan border on their way to be traded at the Lokichoggio market, Kenya. Cluster sampling was used in this study. Ultrasound examination was performed in different villages that were randomly selected and on whole flocks of goats. Ultrasound examinations were carried out in the mornings between 07:00 – 10:00 and early evenings between 17:00 – 19:00. The animals were allowed to graze between these two periods.

Ultrasound examination of the liver and the lung was performed as described in section 3.1.1. A 3.5 MHz linear array or a 3.5MHz microconvex transducer was used, depending on the width of the intercostal space. The diagnostic features used to detect hydatid cysts included multiple or single unilocular cysts with a distinct host-parasite wall (Fig.3.4), the presence of daughter cysts, multiloculated cysts and separation of the laminated membrane from the cyst wall (Pant and Gupta 1987). The location of each cyst was noted.

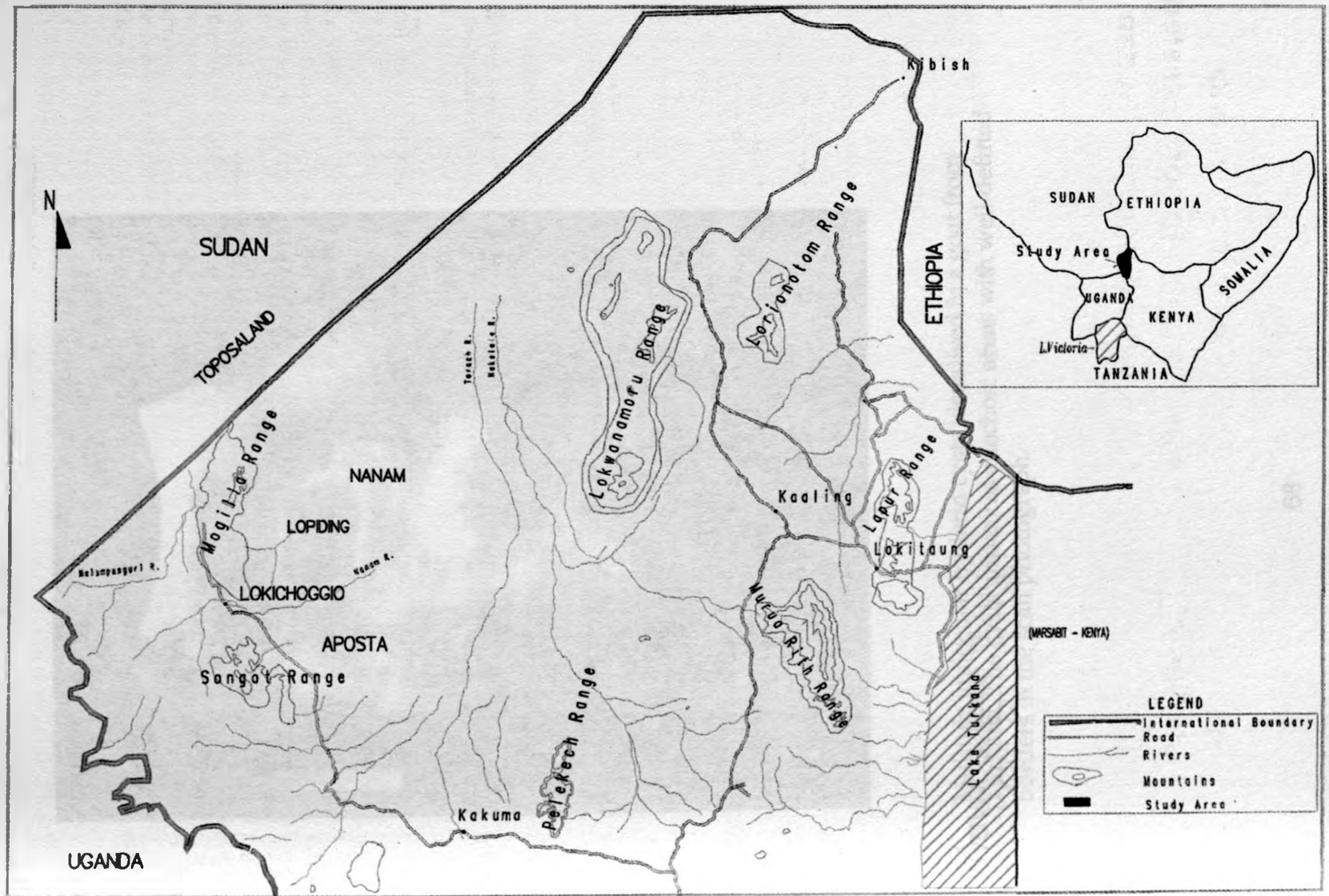


Figure 3.3 Map of Northwestern Turkana, Kenya and Toposaland Southern Sudan, showing the areas from which the goats used in the study originated

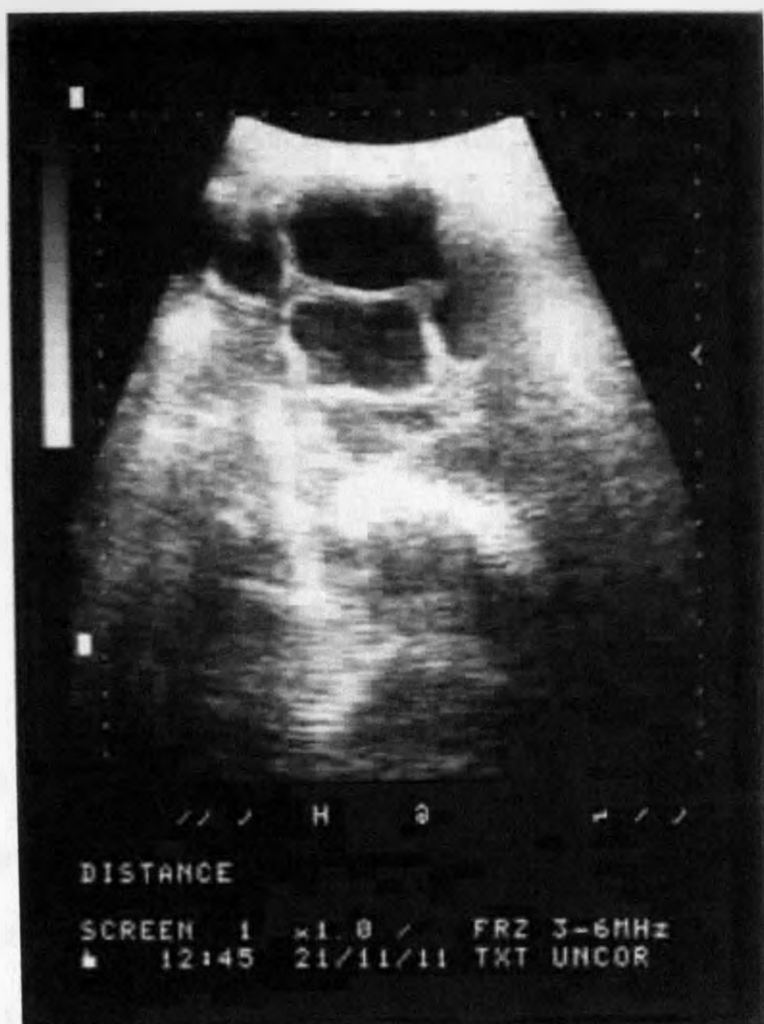


Figure 3.4 Multiple unilocular hydatid cysts visualised in a goat from Turkana. The cysts appear as anechoic areas with well-defined borders in the scan photograph.

3.2 RESULTS

3.2.1 Determination of sensitivity, specificity and kappa of ultrasonography

Approximately 40 animals were examined per hour. A total of 300 animals (284 goats and 16 sheep) were examined. Most of the animals (88%) were more than 1 year old (based on their dentition). Of the 300 animals examined, 31 (10.3%) were positive on ultrasound examination and 46 (15.3%) were positive on postmortem examination (Table 3.2). All goats under 1 year of age were negative on both ultrasound and postmortem examination. Twenty-five animals were positive on both postmortem and ultrasound examination (Appendix III). Twenty-one animals positive on postmortem examination were falsely identified as negative on ultrasound examination (Appendix IV). Of the 254 animals negative on postmortem examination, 6 (2.4%) were falsely identified as positive on ultrasound examination (Appendix V). Using the postmortem findings as the gold standard the sensitivity and specificity of ultrasound examination for the detection of *Echinococcus* cysts was found to be 54.3% and 97.6%, respectively. Infection rate of cystic echinococcosis in this study was found to be 15.3%. The positive predictive value of ultrasound test at the current level of prevalence was 80.6%. The negative predictive value was 92.2%.

Table 3.2 Findings of ultrasound and postmortem examination of 300 animals under study

		Postmortem Results		
		+	–	Total
Ultrasound results	+	25	6	31
	–	21	248	269
	Total	46	254	300

Sensitivity = (25x100)/ 46 = 54.3%

Specificity = (248x100)/ 254 = 97.6%

Positive predictive value = (25 x 100)/ 31 = 80.6%

Negative predictive value = (248 x 100)/ 269 = 92.2%

Clinical agreement between ultrasound and postmortem findings

Observed agreement = (25+248)/ 300 = 0.91

Expected agreement due to chance = {[(46/300)x31] + [(254/300)x269]}/ 300 = 0.78

Actual agreement beyond chance = 0.91 - 0.78 = 0.13

Potential agreement beyond chance = 1.00 - 0.78 = 0.22

kappa = 0.13/0.22 = 0.59

Of the animals positive on postmortem examination, 56.5% had hydatid cysts distributed in both the liver and the lungs. Hydatid cysts were present in the right lung only, left lung only and liver only in 15.2%, 6.5%, and 21.7% of the cases, respectively. Of the 6 animals falsely identified as positive on ultrasound examination, 4 had no abnormalities adjacent to the lung or liver and 2 had *Taenia hydatigena* cysts adjacent to or in the liver on postmortem examination. All the animals falsely identified as negative on ultrasound examination had hydatid cysts within the pulmonary parenchyma or within the cranial lobe of the liver that is located under the aerated lung tissue.

3.2.2 Ultrasonographic features of the abdominal organs and *Echinococcus* cyst

Liver

The liver was imaged from the 7th to 10th intercostal spaces. It was ventral to the lung margins and medial to the body wall and diaphragm. The liver had mid-level echogenicity. It was more echogenic than the kidney. Hepatic and portal vessels appeared as round or oval anechoic structures but longitudinal section of the vessels could also be viewed on rotation or tilting of the transducer (Figure 3.5). The bile duct was visualized as anechoic longitudinal structure. The gall bladder was observed on the ventral part of the liver. It was anechoic fluid-filled organ (Figure 3.6) that was either round or oval depending on the placement of the transducer.

Rumen

The rumen was visualised just behind the 13th rib. It appeared as anechoic structure with echogenic specks in a hungry animal and hypoechoic structure when the animal was examined in the evening. Ruminal movements were visualised when the transducer was held motionless for 2-3 minutes (Fig.3.7).

The Kidneys

The right kidney was visualized from the 12th intercostal space to just behind the last rib. The left kidney could not be visualized. The right kidney was imaged in both transverse and longitudinal planes. The size of the kidney was determined by measuring the length of both planes. The kidney was less echogenic than the liver but more echogenic than the rumen. The cortex, medulla, renal pelvis, renal artery, and ureter were visualized ultrasonographically. The cortex was slightly more echogenic than the medulla (Figure 3.8) and at the corticomedullary junction, arcuate vessels were visualized as pinpoint echogenic foci with acoustic shadows.



Figure 3.5 Sonogram of a goat shows the liver parenchyma. Note the hepatic vessels (Arrow)

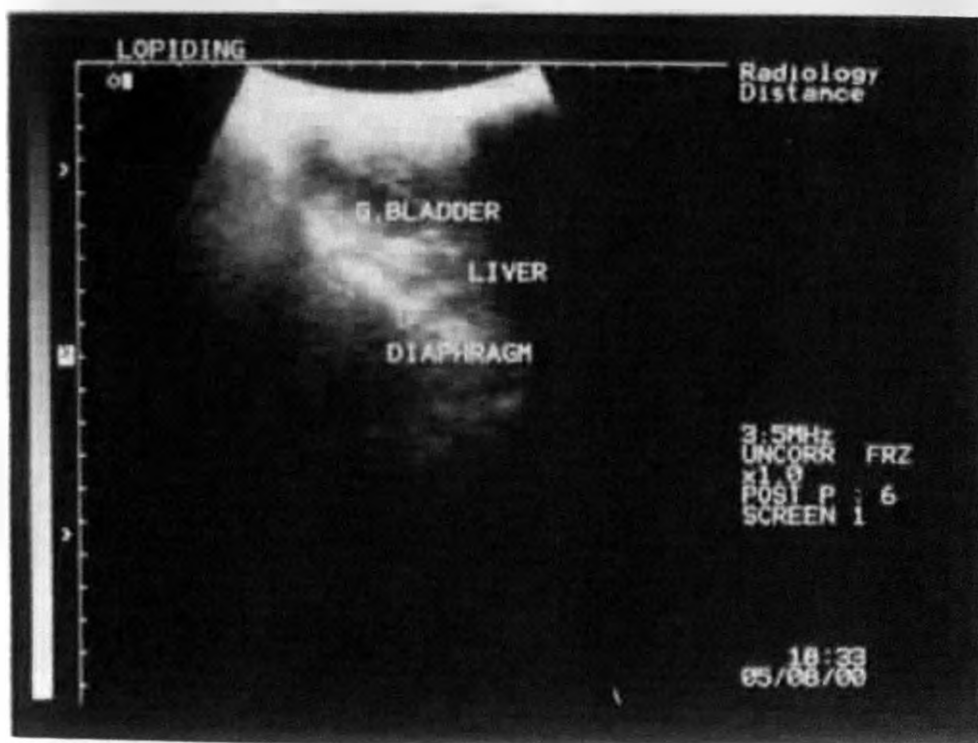


Figure 3.6 Sonogram of a goat showing the gall bladder, liver parenchyma and diaphragm

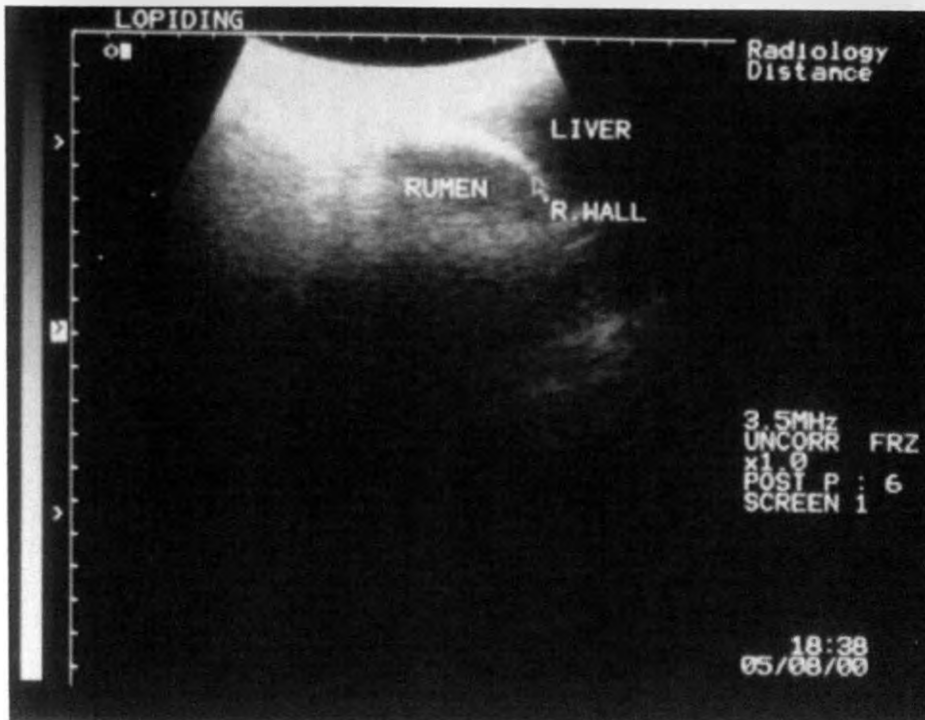


Figure 3.7 A sonogram of a goat abdomen showing rumen, ruminal wall and liver



Figure 3.8 Sonogram of the abdomen shows the appearance of the kidney.
Note the medulla (m) and the cortex (c)

Taenia Hydatigena Cyst

Taenia hydatigena cysts appeared as thin-walled anechoic fluid-filled structures that could not be distinguished from a thin-walled unilocular hydatid cyst in a standing animal. However, they were never located within the liver parenchyma or the lungs, but were in the mesentery. The only distinguishing characteristic feature was that the cyst changed in both shape and size when the animal was scanned on lateral recumbency.

Echinococcus cysts

In all the animals, hydatid cysts were imaged as anechoic fluid-filled space-occupying masses within the liver parenchyma or the lungs. In 12 (80.0 %) animals, the cysts were thin-walled unilocular while in 3 (20.0 %) animals, they appeared as thick-walled unilocular structures (Table 3.3). Calcification appeared as hyperechoic ring in the cyst wall in 1 (6.7 %) animal. Multiple unilocular cysts separated by the liver parenchyma were imaged in 13 (86.7 %) animals. Single multiloculated cysts appeared in 1 (6.7 %) animal (Figure 3.9) while multiple multiloculated cysts were observed in 1 (6.7 %) animal.

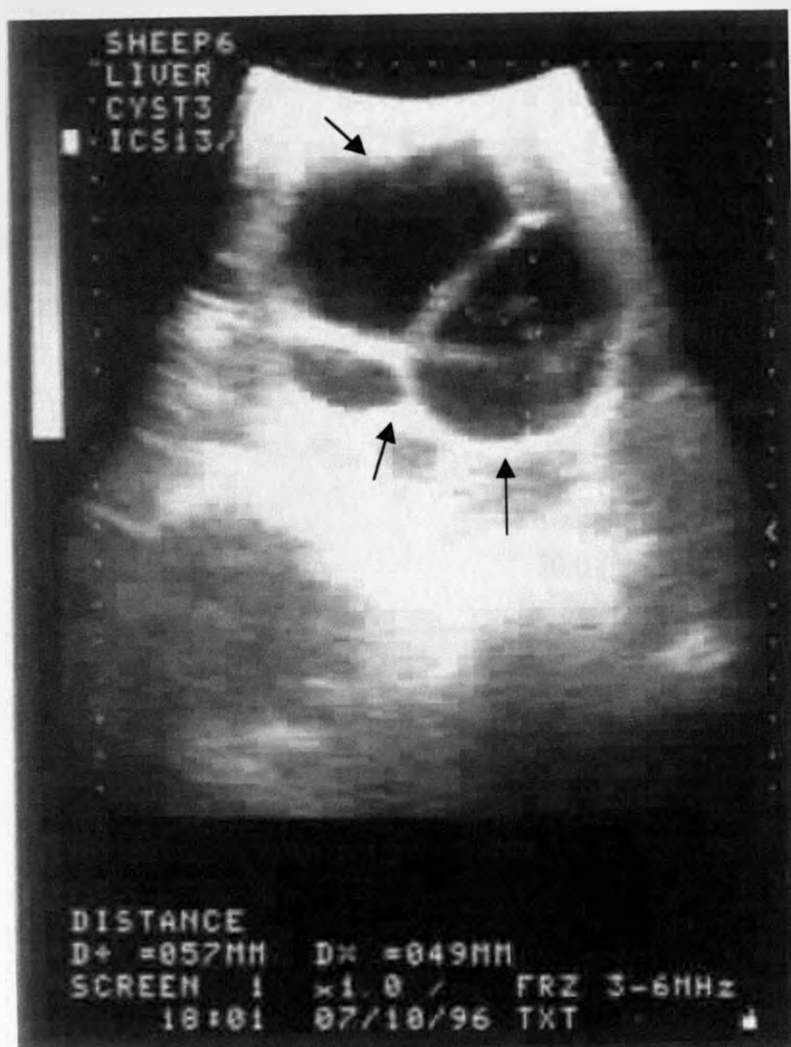


Figure 3.9 *Echinococcus granulosus* cyst from a sheep – WHO classification TCE2 (Arrows)

Table 3.3 Types of cysts observed in the goats

WHO classification	% No. of Animals
TCE 1	80.0 (n = 12/15)
TCE2	6.7 (n = 1/15)
TCE3	86.7 (n = 13/15)
TCE4	20.0 (n = 3/15)

Key: TCE1 - Echinococcus cyst type 1 in the WHO classification

3.2.3 Costs of Ultrasound Examination

The costs of equipment and the recurrent expenditure are as shown in table 3.4.

Table 3.4 **Costs of equipment and recurrent expenditure in ultrasound scanning**

Cost of equipment (\$)		Recurrent expenditure (\$)	
Portable ultrasound machine	25,000.00	Ultrasound gel/ 5 Lts	4.00
Printer	1,700.00	Ultrasound film (1roll, 200 exposures)	50.00
Shaver/clipper	330.00	Labour (2 casuals \$5/day) / day	10.00
Electric cables	84.00	Fuel / Lt	0.50
Electric generator	5,000.00		

A total of 200 animals were scanned per day. To scan these animals, 15ml of ultrasound gel was used per animal, and 5 litres of fuel was required to run the generator for the 200 animals. The shelf life of equipment used was estimated at 5 years.

Cost of examination / goat = direct (Recurrence) costs (a) + indirect (equipment) costs (b)

$$\begin{aligned}a &= (84/5) \times 0.015 + 50/200 + 10/200 + (2 \times 5/200) \times 0.5 \\&= 0.252 + 0.25 + 0.05 + 0.025 \\&= 0.58\end{aligned}$$

$$b = 26.8/200 = 0.134$$

$$a+b = 0.58 + 0.134 = 0.714$$

Cost of ultrasound examination / goat = \$ 0.714*

* Cost of ultrasound examination per animal was inversely proportional to number of animals scanned per day. The number of animals scanned per day was inversely proportional to the shelf life of the ultrasound scanner.

3.2.4 Application of ultrasonography in prevalence studies

A total of 1390 goats (746 males and 644 females) was examined, 43.6 % (606/1390) being from Northwest Turkana, Kenya, and 56.4 % (784/1390) from Toposaland, Southern Sudan. Of the animals from Turkana, 90.4 % (548/606) were females and 9.6 % (58/606) were males. Of the animals from Toposaland, 87.8 % (688/784) were males and 12.2 % (96/784) were females (Table 3.5).

In both Toposaland and Northwestern Turkana, a total of 45 (3.2 %) goats harboured hydatid cysts. Eleven of these goats were from Northwestern Turkana and 34 were from Southern Sudan. The number of male goats with hydatid cysts (29 (2.1 %)) was significantly higher than females (16 (1.2 %)) ($P < 0.05$). Hydatid cysts were present in the liver of 43 (3.1 %) animals and in the lungs of 2 (0.14 %) animals.

Among the animals from Southern Sudan, the prevalence of hydatid cysts was 4.2 % (29/688) in males and 5.2 % (5/96) in females. In the Turkana animals, the prevalence was 2.0 % in females while no cysts were detected in males. The prevalence of hydatid cysts in various villages of Northwest Turkana was: Lopiding 2.6 % (7/272), Aposta 0.5 % (1/184), Lokichoggio 2.3 % (2/86) and Nanam 1.6 % (1/64). The overall prevalence of the disease was 1.8 % in goats from Turkana and 4.3 % in those from Southern Sudan.

Table 3.5 Number of goats scanned from villages of Northwestern Turkana and Toposaland indicating sex of the animal and the organ where hydatid cyst was visualised by use of ultrasound

Village	Number Scanned			Hydatid Positive			
	Male	Female	TOTAL	Male	Female	Lung	Liver
Lopiding	8	264	272	-	7	-	7
Aposta	3	181	184	-	1	-	1
Nanam	-	64	64	-	1	-	1
Lokichoggio	47	39	86	-	2	-	2
Toposaland	688	96	784	29	5	2	32
TOTAL	746	644	1390	29	16	2	43

3.3 DISCUSSION

For any imaging technique to be useful in diagnosis, pertinent features of both normal and abnormal structures need to be visualised and distinguished in an animal. In the present study, ultrasound provided distinctive features of various

organs and structures examined. The liver, one of the most common sites for hydatid cysts, could be visualised with all its structures. Additionally, various parts of the kidney could be visualised. Unlike X-ray, where characteristic cyst structures do not present as a clear image or are absent (Rogan *et al*, 1991), various structures of *echinococcus* cysts could be seen as clear images in the present study.

The agreement between ultrasound and postmortem findings was good ($\kappa = 0.59$). However, the specificity of ultrasound as determined in this study was adversely affected by several factors. All the false negative animals in the first experiment (Section 3.2.1) had hydatid cysts within the lung parenchyma. Air in the lung act as a perfect reflector of the ultrasound beam that prevents imaging of structures deep to aerated lung surface. *Echinococcus* cysts within the lung parenchyma can be visualized only if they are adjacent to the chest wall. Some false negatives are expected with this technique in animals that have cysts deep in the pulmonary parenchyma. This would contribute to a low specificity of the technique.

An explanation for the low sensitivity may be the presence of *Taenia hydatigena* cysts. One third of the false positive animals were a result of *T. hydatigena* infection. *Taenia hydatigena* cysticerci occur in the mesentery and liver of domestic livestock as a single unilocular thin walled cysts which are indistinguishable from unilocular hydatid cysts caused by *E. granulosus* on ultrasound examination (Maxson *et al*,

1996). Although the presence of daughter cysts, multiloculated cysts or separation of the laminated membrane from the cyst wall is used as a diagnostic feature of *Echinococcus* cysts (Pant and Gupta, 1987), single unilocular *Echinococcus* cysts are common.

One reason for the false positives could be the difficulty in establishing, in all cases, an equivocal aetiologic diagnosis of various cyst types, including cystic echinococcosis, a drawback common to most radiological techniques (Hira *et al.*, 1993). The factors that contribute to this problem are the very nature of radiological imaging and the high degree of dependence on the skill and experience of the radiologist performing the scan. From previous authors' opinion (Hira *et al.*, 1993), the latter factor may occasionally dominate.

The sensitivity value of ultrasound technique in this study was in the same range as that found in most serological tests when the tests are performed in humans. These include complement fixation (CT) (Matossian, 1977; Chemtai *et al.*, 1981), immunoelectrophoresis (IEP) (Chemtai *et al.*, 1981), arc-5 double diffusion test (DD5) (Chemtai *et al.*, 1981; Kagan, 1976), and ELISA (Gathura, 1991). It was, however, much higher than serologic tests carried out in livestock species (Lightowlers and Gottstein, 1995). Also the technique proved superior to serology when performed under field conditions. In addition to being non-invasive and

painless, ultrasound could also reveal the number, size, site and condition of the cysts.

Various advantages of ultrasonography as a diagnostic technique for *Echinococcus* cysts have been reported in previous studies involving humans (Macpherson *et al.*, 1987). In the present study, the author experienced similar attributes. The technique could determine the presence, size, nature and exact location of the *Echinococcus* cysts. Although the initial cost of ultrasound equipment was high, the running costs were inexpensive compared to other diagnostic tests. To examine one animal, \$0.714 was required for both equipment depreciation and running costs as compared to \$10 (expendable materials only) for one sample in polymerase chain reaction (PCR) examination (Dinkel *et al.*, 1998). Based on the findings in this study, ultrasound would be a useful technique tool in routine clinical diagnosis of hydatid cysts in small ruminants (sheep and goats).

In previous studies in Turkana District, the prevalence of hydatid cysts in goats was estimated to be < 2 % when using slaughter data (Macpherson, *et al.*, 1985), and 2.5 % by ultrasound examination (Maxson *et al.* 1996). Whereas these values are comparable with the prevalence in the present study (1.8 %), they may be biased because debilitated and sick animals are more likely to be sent for slaughter, and ultrasound examination was performed in only one village. In the

present study, ultrasound examination was performed in four different villages that were randomly selected and on whole flocks of goats, and the prevalence figures obtained for the condition are therefore more likely to be unbiased.

The prevalence rate of hydatid cysts in goats from Northwestern Turkana was lower (1.8 %) than that in goats from Toposaland (4.3 %) ($P < 0.05$). The lower prevalence in Turkana may be attributed to the hydatid control programme that has been in place from 1983 in that area. For a period of 15 years, control efforts have been directed to community education and mass treatment of dogs (Macpherson and Wachira 1997). These may have contributed to lower infection rates in goats from Turkana villages. Such a control programme is absent in Southern Sudan.

In this study, the occurrence of hydatid cysts was much higher in the liver than in the lungs. Previous reports indicate that hydatid cysts have a higher predilection for the lungs than in the liver (Al-Abbassy *et al*, 1980). In the present study, the prevalence rate of hydatid cysts in the lungs may be an underestimation because ultrasound is limited in its detection of lesions in the lungs. Air in the lungs reflects sound waves, which interferes with detection of hydatid cysts within the parenchyma. Only hydatid cysts adjacent to the thoracic wall can be visualised.

The prevalence rate of hydatid cysts in animals as determined by ultrasound may be lower than that determined by slaughter because only superficial lung cysts in one lung were visualised. Also, only part of the liver was seen due to superimposition on the part of the lung. However, ultrasound was the only diagnostic tool available in the present study. Additionally, the human population in the study area is entirely dependent on the animals for milk and blood and therefore mass slaughtering to determine the prevalence of hydatid cysts was not feasible. Ultrasonography proved to be a non-invasive and well-accepted technique for prevalence studies of hydatid cysts in goats.

CHAPTER 4

Determination of cystic echinococcosis infection level in slaughter animals in three selected areas of Northern Turkana, Kenya

4.0 INTRODUCTION

Cystic echinococcosis is a zoonotic disease caused by the larval stages of the parasitic tapeworm *Echinococcus granulosus*. It is distributed in all the continents of the world (Matossian *et al.*, 1977) but the highest incidence in humans has been reported in Northern Turkana, Kenya. The disease manifests as a chronic debilitating condition where fluid-filled cysts form in any part of the body. The cysts vary in size and quantity of fluid, and Turkana patients with cysts containing as much as 24 litres of fluid have been reported (Zeyhle *et al.*, 1999).

Domestic intermediate hosts (cattle, sheep, goats, and camels) are a major reservoir for human cystic echinococcosis caused by *E. granulosus* in Kenya (Macpherson *et al.*, 1989). However, information available on the epidemiology of the disease in the intermediate hosts in Northern Turkana describes how the disease situation was before 1980 (Macpherson, 1981). Thereafter, efforts were made to control the disease through community education, and dog management (dog treatment, dog sterilisation and elimination of stray dogs) (Macpherson and Wachira, 1997). This led to a decrease in human cystic echinococcosis from 8% to 3% but no study was carried out to determine the prevalence of the disease in domestic intermediate hosts. Between 1995 and 1998, control activities were temporarily suspended due to financial constraints. It has therefore become increasingly important to evaluate the situation and determine the trend of the disease. To provide more reliable information that can be used in redesigning control strategies, a prevalence survey of cystic echinococcosis was necessary. The aim of this study was, therefore, to determine the level of cystic echinococcosis infection in slaughter animals in three different agro-ecological zones of Northern Turkana.

4.1 MATERIALS AND METHODS

4.1.1 LOCATION AND DESCRIPTION OF THE STUDY AREA

This study was conducted between 1998 and 2000. The study was carried out in 3 areas namely Lokichogio, Kakuma and Central Divisions of Turkana District.

4.1.1.1 Lokichogio Division

Lokichogio Division covers an area of 9,126km². It lies between latitudes 5° and 6°, and longitudes 34° and 36°. It is in agro-ecological zone V (altitude of 600 M and 900M) and has a mean annual rainfall of 430mm. The annual mean temperature ranges between 22°C and 30°C. Lokichogio Division had 44 butcheries of which only 34 were functioning at the time of the study. All the butcheries were located within Lokichogio town. In addition, there were 14 slaughter slabs and 8 condemnation pits within Lokichogio town.

4.1.1.2 Kakuma Division

Kakuma Division lies between latitudes 4° and 5°, and longitudes 34° and 36°. It covers an area of 10,946km² and is in agro-ecological zone VI (altitude of 400 M and 700M). The rainfall pattern and distribution has been unreliable and erratic over the years but in most times occur once per year between March and May. The annual mean temperature ranges between 24°C and 36°C, while mean annual rainfall is 350mm. At the time of the study, Kakuma had 2 main slaughter slabs and 34 butcheries.

4.1.1.3 Central Division

Central Division lies between latitudes 3° and 4°, and longitudes 34° and 36°. It covers an area of 4,802km² and is in agro-ecological zone VI (altitude 369M and 500M) with an erratic rainfall distribution. The mean annual rainfall varies from as low as 19 mm to 380mm. The annual mean temperature ranges between 28°C and 40°C. At the time of the study, individual butchers carried out livestock slaughter near their respective butcheries. The study was carried out in 42 butcheries.

4.1.2 EXAMINATION OF SLAUGHTER ANIMALS

In the three study areas, a thorough post-mortem examination was conducted on slaughter animals. The lungs, liver, heart, spleen and kidneys were examined in each carcass. *Echinococcus* cysts identified were removed whole and collected in polyethene bags. Each polyethene bag was used for hydatid cysts obtained from one animal and was labelled appropriately to show the species. The cysts were transported to the nearest laboratory and examined within 1 hour.

4.1.2.1 Lokichogio

In Lokichogio, total of 2851 goats, 438 sheep, 264 cattle and 17 camels were examined. Information on the species of livestock slaughtered was obtained from slaughter slabs and butcheries within Lokichogio town. A postmortem of the carcasses was done and hydatid cyst infected animals recorded. Cysts collected were examined at African Medical and Research Foundation (AMREF) laboratory.

4.1.2.2 Kakuma

In Kakuma division, the slaughter animals examined included 223 goats, 6 sheep, 65 cattle and 40 camels. Any cysts that were collected were examined at Kakuma Catholic Mission hospital laboratory.

4.1.2.3 Central

In Central Division, a total of 2678 goats, 144 sheep, 52 cattle and 13 camels were examined. Any cysts that were collected were examined at Lodwar District Hospital laboratory.

4.1.3 CYST VIABILITY STUDIES

Individual cysts were grossly examined for degeneration and calcification. The cysts were then incised and the contents poured into a clean petri dish. The contents were examined under a microscope (X40) for the presence of protoscoleces. Similarly, the germinal layer was put in glycerine between two microscopic glass slides and examined for the presence of protoscoleces. The viability of protoscoleces was determined by exclusion of eosin dye and flame cell motility.

4.1.4 STATISTICAL ANALYSIS

The Data were analyzed with SAS software. Infection differences were compared using student's t-test and generalized linear models (GLIM) procedure. Differences were considered significant at $P < 0.05$.

4.2 RESULTS

A total of 6791 animals were examined at slaughter in the three study areas. These included 5752 goats, 588 sheep, 381 cattle and 70 camels. Lokichogio Division had a significantly higher number of animals slaughtered than either Kakuma or Central Divisions ($P<0.05$). Goats were the most commonly slaughtered animals in all three areas.

The infection of cystic echinococcosis was significantly different ($P<0.05$) within the species in all the areas. Sheep had the lowest prevalence (3.6%) in all the study areas, while camels had the highest prevalence (61.4%) of the disease. Cattle had a higher prevalence of the disease (19.4%) than goats (4.5%).

An assessment of infection per each study area indicated that the prevalence of cystic echinococcosis in cattle was higher in Lokichogio Division (25.3% (67/264)) than in either Kakuma (10.7% (7/65)) or Central Divisions (0% $n=52$). Also, Lokichogio had a much higher prevalence of the disease in sheep (4.6% (20/438)) than Kakuma (0% $n=6$) and Central Divisions (0.69% (1/144)). Similarly, goats slaughtered in Lokichogio Division had higher cystic echinococcosis infection (8.4% (240/2851)) than those slaughtered in either Kakuma (0.45% (1/223)) or Central (0.67% (18/2678)) Divisions. On the other hand, the prevalence of the disease in camels was higher in Central Division (84.6% (11/13)) than either Lokichogio (70.6% (12/17)) or Kakuma (20/40 (50%)) Divisions (Table 4.1). Statistical analysis of the obtained results showed a significant difference ($P<0.05$) in the prevalence rates of cystic echinococcosis between the study areas.

Table 4.1 Percent infection and prevalence of cystic echinococcosis in livestock slaughtered in the three study areas (Lokichogio, Kakuma and Central) of Northern Turkana

	Lokichogio				Kakuma				Central			
	Goats	Sheep	Cattle	Camels	Goats	Sheep	Cattle	Camels	Goats	Sheep	Cattle	Camels
% Infection per organ												
Liver	1.6 (45 ^a /2851 ^b)	1.6 (7/438)	3.03 (8/264)	29.4 (5/17)	0.0 (n=223)	0.0 (n=6)	3.1 (2/65)	17.5 (7/40)	0.04 (1/2678)	0.0 (n=144)	0.0 (n=52)	46.2 (6/13)
Lung	7.4 (212/2851)	3.4 (15/438)	22.7 (60/264)	41.2 (7/17)	0.45 (1/223)	0.0 (n=6)	7.7 (5/65)	52.5 (21/40)	0.63 (17/2678)	0.69 (1/144)	0.0 (n=52)	53.8 (7/13)
Liver & Lung	0.63 (18/2851)	0.46 (2/438)	0.76 (2/264)	11.8 (2/17)	0.0 (n=223)	0.0 (n=6)	0.0 (n=65)	20.0 (8/40)	0.0 (n=2678)	0.0 (n=144)	0.0 (n=52)	23.1 (3/13)
Others*	0.04 (1/2851)	0.0 (n=438)	0.38 (1/264)	11.8 (2/17)	0.0 (n=223)	0.0 (n=6)	0.0 (n=65)	0.0 (n=40)	0.0 (n=2678)	0.0 (n=144)	0.0 (n=52)	7.7 (1/13)
% Prevalence	8.4	4.6	25.3	70.6	0.45	0.0	10.7	50.0	0.67	0.69	0.0	84.6
% Infected animals with viable cysts	35.4	50.0	50.7	83.3	0.0	0.0	42.9	85.0	44.4	0.0	0.0	81.8

Key:

*Others = other body organs (heart, spleen, kidney); ^a = Number of infected animals; ^b = Total number of animals examined

Assessment of organ distribution of cystic echinococcosis indicated that in all the three study areas, lungs and livers were the most frequently infected visceral organs in all the species. In three of the species (cattle, goats and camels) lungs were the most frequently infected while in one (sheep), the liver had high infection rate than lungs. Concurrent infection of both liver and lungs was less common than infections of either liver alone or lung alone. Very few animals had cysts in other organs (heart, spleen and kidney).

Of the total number of infected animals in each species, camels did not show any significant differences in cyst viability between the study areas. However, they had a higher number ($P<0.05$) with viable cysts than all the other animal species examined in this study. Infected goats from Lokichogio and Central Divisions had a higher proportion with viable cysts than those from Kakuma Division. Sheep from Lokichogio Division had the highest proportion with viable cysts while cattle from Lokichogio and Kakuma Divisions had a higher proportion with viable cysts than those from Central division.

4.3 DISCUSSION

Cystic echinococcosis, although one of the most important helminth infections in man, has proved difficult to establish an accurate prevalence status in intermediate hosts in any continent. This is partly due to poor reliability of the available diagnostic tests and high costs of performing these tests under field conditions. Most of the prevalence studies have relied on slaughter data (Baldock *et al.*, 1985; Eugster, 1978; Macpherson, 1981; Jobre *et al.*, 1996). This study employed slaughter survey to try and establish the prevalence status of the disease in intermediate hosts in northern Turkana.

Slaughter survey has been recommended in studies of cystic echinococcosis for various reasons (Baldock, *et al.*, 1985). It is an economical way of gathering information on livestock diseases, particularly subclinical conditions. Also, no satisfactory test exists for cystic echinococcosis in living ruminants (Craig, 1997). In addition, lesions of cystic echinococcosis usually remain for the life of the animal, and therefore, at postmortem, it is possible to tell whether or not an animal was infected. In the present study, slaughter survey provided information on the status of cystic echinococcosis infection in areas where animals could be examined at postmortem after slaughter. However, it had two limitations. First, slaughter animals were not a true random sample of the population at risk because debilitated and sick animals were more likely to be sent for slaughter. Second, not all slaughter was monitored. This was the case in areas far from town centers where there were no butcheries or slaughter slabs, and where home slaughter was carried out. Information on the status of the disease in such circumstances could therefore not be obtained.

In all the animal species, except the camel, Lokichogio Division had the highest level of cystic echinococcosis infection. This may be explained by better environmental conditions that are conducive to the perpetuation of the parasite in Lokichogio Division which are absent in the other two study areas (Kakuma and Central). Whereas Kakuma and Central Divisions are in agro-ecological zones VI, Lokichogio Division is in agroecological zone V. It has a higher mean annual rainfall (430mm) than both Kakuma (380mm) and Central (as low as 19mm) Divisions. Also, it has lower environmental temperatures than the two areas. In a study to determine the transmission dynamics of cystic echinococcosis, Wachira *et al* (1991) found that eggs of *E. granulosus* could survive only a few hours under the high ambient temperatures of Turkana. When exposed to sunlight and high temperatures, they become desiccated and did not hatch even when consumed by intermediate hosts.

Another explanation for the higher prevalence of the disease in Lokichogio may be constant migration of livestock across the border from Southern Sudan. In another study by the same authors (Njoroge *et al*, 2000) ultrasound examination of goats from Southern Sudan showed a higher prevalence of the disease than in those from Turkana villages. Most of these goats were sold and slaughtered in Lokichogio and therefore the high prevalence at slaughter. The number of animals slaughtered that originated from Sudan could, however, not be accurately established in this study.

Other factors that may contribute to the differences in prevalence of cystic echinococcosis include an abundance of infected definitive host and stocking rate of livestock. In previous studies, Macpherson *et al* (1985) found much lower prevalence of *E. granulosus* in dogs from around Lodwar (Central Division). In the same study, Northwestern Turkana had the highest prevalence of *E. granulosus* in dogs. This may explain the high prevalence of the disease in Lokichogio Division. Additionally, due to better climatic conditions, Lokichogio has a higher stocking rate of livestock than either Kakuma or Central Divisions (Ministry of Agriculture and Rural Development (MARD), 1999). This may also contribute to the transmission cycle and hence high prevalence of the disease.

Macpherson (1981) and Macpherson *et al*, (1985) found a prevalence of <2% in cattle, sheep and goats but up to 80% in camels in Turkana. In the present study, the prevalence in sheep and camels were found to be similar to the findings of previous authors. However, in the present study, the prevalence values were higher in cattle (19.4%) and goats (4.5%) than in the previous studies (Macpherson, 1981; Macpherson *et al* 1985). The reasons for the differences between the findings in this study and those of the previous authors remain unclear. It is possible that the disease prevalence in the two species may have increased over the years. However, the more likely explanation for the apparent increase may be that slaughter of animals is currently monitored more closely than before and therefore

cases that were not detected could now be detected. In the previous studies, the number of animals examined were much less than in the present study. Only 10 camels, 10 cattle, 61 sheep and 844 goats were examined (Macpherson, 1981). The previous results may therefore have a higher degree of bias. Based on the findings in the present study, efforts should be made to control transmission of cystic echinococcosis from slaughter slabs and butcheries by safe disposal of *Echinococcus* cysts such that dogs cannot have access to the cysts.

CHAPTER 5

Treatment of cystic echinococcosis with oxfendazole

5.0 INTRODUCTION

Oxfendazole has been widely used in veterinary medicine to control nematode infections. It has also been effective against the intestinal stages of *Echinococcus granulosus* as well as other cestodes in the gastrointestinal tract. When used in the pig, a single dose of 30mg/kg-body weight of oxfendazole has been found to completely eliminate all tissue cysts of *Taenia solium*, an important human tapeworm. Oxfendazole has, however, not been used in treatment of cystic echinococcosis. Trials of oxfendazole in an animal model would provide useful information that would lead to an effective chemotherapy in human cystic echinococcosis.

5.1 MATERIALS AND METHODS

5.1.1 Experimental animals

Naturally infected animals were used in this experiment. Scanning sheep and goats at Kiserian slaughterhouse in Ngong Division of Kajiado District, Kenya identified infected animals. All the animals identified as positive for hydatid cysts were purchased from owners and transported to the Faculty of Veterinary Medicine, Kabete Campus, University of Nairobi. The animals were quarantined for one week before the start of the experiment. During the quarantine period, they were treated against pneumonia with tetracyclines at 20mg/kg-body weight for 4 days. They were also dewormed with a single dose of levamisole at 130mg total dose. The animals were then assigned serial numbers and randomized to the treatment or control group.

5.1.2 Treatment of experimental animals

The treatment group was treated orally with oxfendazole at 30mg/kg-body weight twice a week for 4 weeks while the control group did not receive any treatment. All the animals were provided with feed and water *ad libitum*. Eight weeks after the start of treatment, the animals were euthanised and a complete postmortem examination carried out. The lungs, liver, kidneys and other abdominal organs were examined for hydatid cysts. All visible cysts of the sheep parasite *Taenia hydatigena* were examined, and cysts of *E. granulosus* were dissected and aspirated, and a portion fixed in formalin.

5.1.3 Ultrasound evaluation

Goats were preferred for examination in the study since sheep were difficult to shave down to the skin for an adequate ultrasound examination. Prior to treatment, views of the right lung, right lobe of the liver, and whole abdomen were obtained. The ultrasound examination was repeated 2, 4, and 8 weeks following the initial dose of oxfendazole. Cysts were measured at their greatest diameter and observed for typical signs of degeneration, i.e. decreased size, increased echogenicity, detachment of endocyst, and collapse (Davies et al., 1986). The animals' treatment status was unknown to the ultrasonographer. In order to estimate how closely the ultrasound appearance reflected the actual size, an index of cyst size (maximum height x maximum width) was used for comparison.

5.1.4 Pathology, histology and viability studies

A standard postmortem examination was performed. The lungs, liver, abdominal cavity, kidneys, and spleen were visually inspected and dissected. All surface cysts were dissected intact, and the cyst fluid removed by needle

aspiration. Portions of liver cysts that appeared alive and intact were fixed in formalin and stained with hematoxylin and eosin. Eosin exclusion and observation for flame cell movement determined the viability of *E. granulosus* and *T. hydatigena* (Macpherson, 1981).

5.1.5 Data analysis

Data were normalized by log transformation, and mean values and standard deviations were calculated and compared by student's *t*-test for paired data. Qualitative variables were compared by Fisher's exact test for chi-square variables. Correlation coefficients comparing the size indices of ultrasound measurements with those of postmortem measurements were calculated by the computer program cricket graph. $P < 0.05$ was used to determine statistically significant differences.

5.2 RESULTS

5.2.1 Clinical response

A total of 200 goats and 20 sheep were scanned by ultrasound. Out of these, 12 goats and 5 sheep were entered into the study. The majority of the animals offered were in poor condition, since the owners in this region were more likely to bring sick animals for slaughter. All of the sheep and 30% of the goats had pneumonia and were treated with tetracycline at 20mg/kg for 4 days. One goat died of pneumonia 2 weeks into the study, and autopsy revealed overwhelming pyogenic infection; this animal was not included in the analysis. One animal in the treatment group died 3 weeks after the final dose of oxfendazole, and two control animals died of pneumonia 2 weeks after the final dosing of the treatment group. Postmortem on these animals was performed within 4 hours of

their deaths and the results were included in the study. No complications could be directly attributed to oxfendazole therapy. All animals gained weight, and no differences in weight gain were observed between the treatment and control groups.

5.2.2 Ultrasound evaluation

Ultrasound examination was confined to the right sides of the animals and the abdomens. The lungs were not specifically examined, since ultrasound has poor sensitivity for lung cysts due to the presence of air in this organ. Hair is not completely removed from animals by shaving; thus the limit of resolution of ultrasound in animals is approximately 50mm in any dimension compared to 20mm in humans. Ultrasound identified 92% (36/39) of cysts >50x50mm in size that were located in the right lobe of the liver. There was a 10% false positive rate due to two calcified nonviable cysts in a control animal and two cysts of *T. hydatigena* in the treatment group. The differences in the size indices (maximum height x maximum width) of cysts found by ultrasound compared to postmortem indices were not statistically significant (1797 ± 1269 and $1,842 \pm 1,387$ respectively).

In animals receiving oxfendazole, 47% showed decreased cyst viability within two months. Three cysts decreased in size (Fig. 5.1), while others showed increased echogenicity, complete or partial detachment of the endocyst, or new calcification (Fig. 5.2). There were no changes in identifiable cysts in control animals.



Figure 5.1 Ultrasound appearance of *Echinococcus* cysts showing changes in size after treatment with oxfendazole. Note the decrease in size with time.

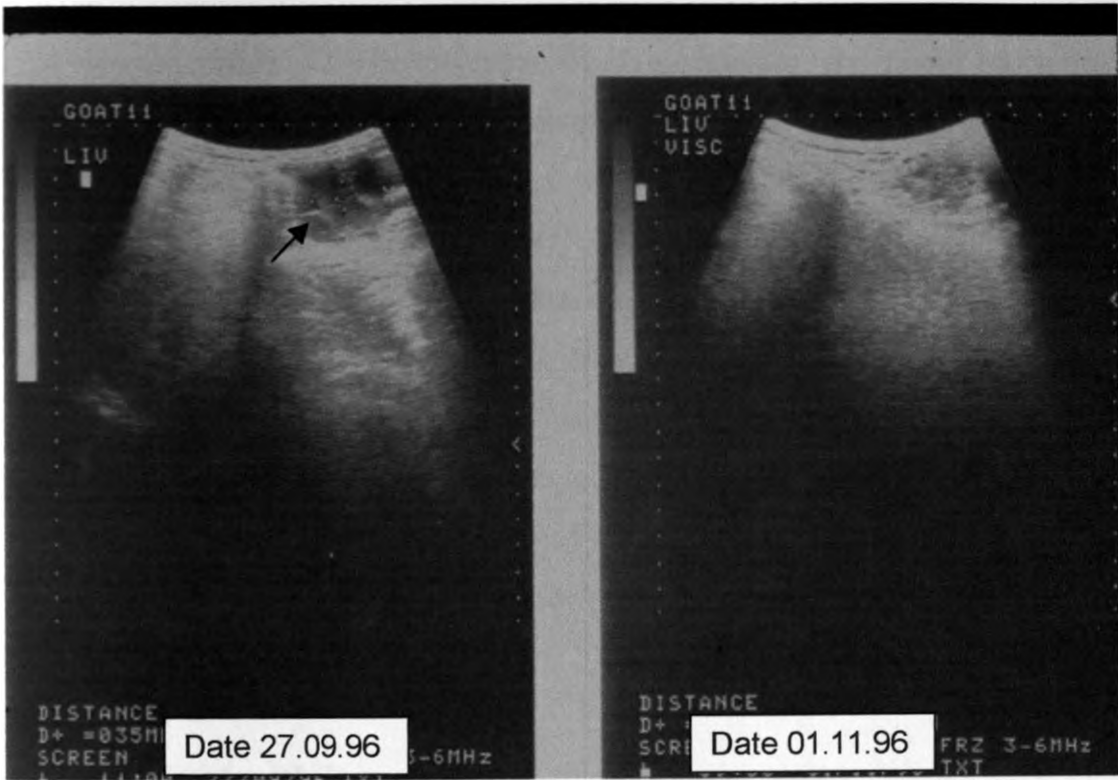


Figure 5.2 Ultrasound appearance of *Echinococcus* cysts showing changes in echogenicity after treatment with oxfendazole. The animal on the left (Date 27.09.96) had undergone treatment for one month. Note the collapse of the endocyst (Arrow) on Date 27.09.96 and hyperechogenicity on Date 01.11.96

5.2.3 Protoscolex and cyst viability

On postmortem, a total of 93 *E. granulosus* cysts from 16 animals were identified. The sheep harbored 52% of the cysts. By dye exclusion and flame cell motility criteria, viable protoscoleces were present in 72% (13/18) of cysts from control animals and 3% (2/75) of cysts from treated animals ($P < 0.000001$; two-tailed fisher's exact test). In treated animals, 35 cysts were found to be degenerate or calcified by visual inspection. The remaining 40 possessed normal appearing membranes attached to the cyst wall. However, only 5% (2/40) of the viable-appearing cysts from treated animals, contained living protoscolices, compared to 93% (13/14) apparently viable cysts in the control animals. None of the six *T. hydatigena* cysts found in the treated animals contained living organisms. Calcifications were found at postmortem in 16% of the animals receiving treatment and 22% of control animals.

5.2.4 Cyst histopathology

In the treatment group, 47% of the cysts (35/75) were identified on postmortem appearing grossly intact and potentially viable. To determine whether the viable-appearing cysts were in some way affected by oxfendazole treatment, sections from nine of them were examined histologically. All showed evidence of marked host cell reaction consisting of infiltration of the adventitial layer with neutrophils, eosinophils, and plasma cells. In addition, inflammatory infiltrate, new space between the liver tissue and cyst wall contained disorganized fibroblasts and mesenchymal cells. In most necrotic areas, the laminate layer could not be collected together with adherent liver tissue and the adventitial layer appeared completely degenerate and was replaced by acute inflammatory cells (Fig.5.3).

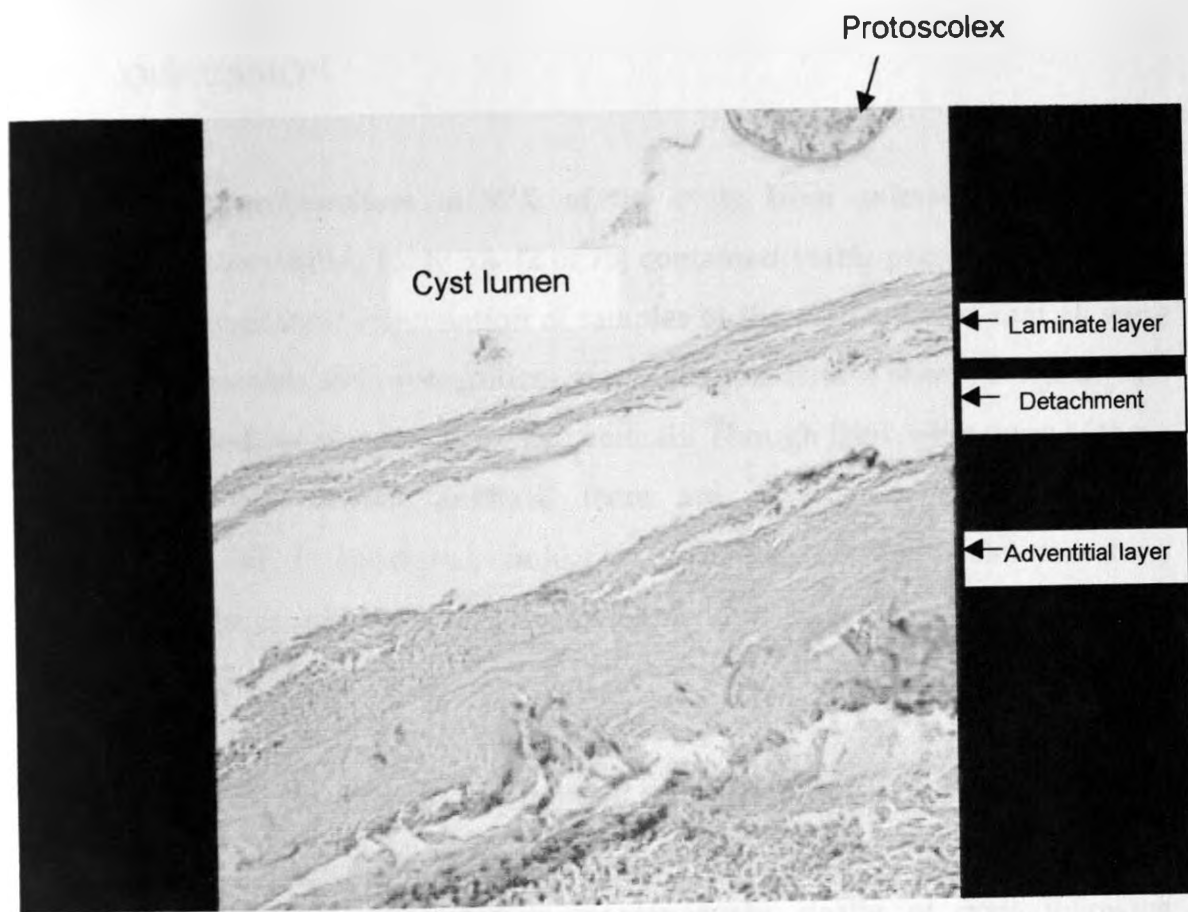


Figure 5.3 Histology of cysts from animals treated with oxfendazole. Note the detachment of the laminate layer and the infiltration of liver tissue with inflammatory cells.

5.3 DISCUSSION

In this trial, protoscolices in 97% of the cysts from animals treated with oxfendazole were killed (only 3% (2 of 75) contained viable protoscolices). Also, gross and microscopic examination of samples of the cysts showed that all were damaged. The cysts and protoscolices of a related parasite of sheep, *T. hydatigena*, were also killed in oxfendazole treated animals. Though there were none of these parasites in the control animals, there are no reports of spontaneous degeneration of *T. hydatigena*, unlike *E. granulosus*. Oxfendazole, therefore, possesses significant activity against intermediate stages of many species of tapeworms.

Interpretation of these results is somewhat complicated, however, by the variable presentation and course of natural hydatid disease. Although calcification is one way in which some cysts resolve spontaneously, deaths of cysts following chemotherapy may also produce calcification. Some spontaneously calcified cysts will, however, still have viable protoscolices. It is likely that some calcified cysts in both the treatment and control groups were already dead. Another consideration is that maturation and development of protoscolices is highly variable, and they may take years to emerge (Gemmell *et al.*, 1986). In cysts with no evidence of protoscolices, these forms of the parasite may have been resorbed or were not present at the start of the therapy. The histological examination of a sample of the cyst walls revealed that all had some damage, which is another indication of drug efficacy.

CHAPTER 6

Comparative study of albendazole and oxfendazole in treatment of cystic echinococcosis

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6.0 INTRODUCTION

To date, there is no standard and effective treatment of cystic echinococcosis. In cases where albendazole has been reported to be successful in treatment of cystic echinococcosis, very high dosage rates had to be administered for long periods of time (20mg/Kg bwt/day for 30 - 60 days)(Chen *et al.*, 1994). Even in such cases, the efficacy rate of albendazole in humans has been reported to be 30-60% (WHO, 1996). Elsewhere in this study, oxfendazole has been found to have an efficacy rate of 97.3% in sheep and goats (Chapter 6). It is important to compare the efficacy of albendazole and oxfendazole in treatment of cystic echinococcosis. Only well planned clinical trials in the same animal species can compare the efficacy of the two drugs. Randomized studies in humans have always been a problem in developing countries where cystic echinococcosis is endemic (Aktan *et al.*, 1998). The aim of this study was to compare the efficacy of albendazole and oxfendazole in treatment of cystic echinococcosis using naturally infected sheep and goats.

6.1 MATERIALS AND METHODS

6.1.1 Experimental animals

Naturally infected animals were used in this experiment. Scanning sheep and goats at various villages of Lokichogio Division, Northwestern Turkana, Kenya, identified infected animals. All the animals identified as positive for hydatid cysts were purchased from owners and transported to the African Medical and Research Foundation (AMREF) - camp, Lopiding. The animals were quarantined for two weeks before the start of the experiment. During the quarantine period, they were treated against pneumonia with tetracyclines at 20mg/kg-body weight for 4 days. They were also dewormed with a single dose of levamisole at 130mg

total dose. The animals were then assigned serial numbers and randomized to either of three groups, two treatment groups or control group.

6.1.2 Treatment of experimental animals

The treatment groups were treated orally with either albendazole or oxfendazole at 30mg/kg-body weight twice a week for 4 weeks while the control group did not receive any treatment. All the animals were provided with feed and water *ad libitum*. Eight weeks after the start of treatment, the animals were euthanised and a complete postmortem examination carried out blindly. The lungs, liver, kidneys and other abdominal organs were examined for *Echinococcus* cysts. The cysts were dissected and aspirated, and a portion fixed in formalin.

6.1.3 Evaluation of hematological changes

Hematological examination and liver and kidney function tests were carried out to determine the overall health of the animals. The tests were carried out as described by Jain (1986). Blood was obtained from each animal once per week by jugular venipuncture using an 18-G needle. The samples were collected in two types of vials.

1. 5ml of blood into a vial without any anticoagulant for determination of aspartate aminotransferase (AST) and blood urea nitrogen (BUN).
2. 5 ml of blood into a vial containing EDTA for determination of haemoglobin (Hb) concentrations and packed cell volume (PCV).

6.1.3.1 Aspartate Aminotransferase (AST) Test

Aspartate aminotransferase (AST) activity was used to determine the functional status of the liver. To 1.0ml of AST reagent¹ in a test tube, 0.1ml of serum was added. The resulting solution was mixed by gently inverting the tube and then sucked into the spectrophotometer cuvette. The absorbance was read from the spectrophotometer at wavelength 340nm.

6.1.3.2 Blood Urea Nitrogen (BUN) Test

Blood urea nitrogen (BUN) was used to determine the functional status of the kidney. To 1.0ml of the BUN reagent¹ in a test tube, 0.01ml of the serum sample was added. The solution was immediately mixed by gentle inversion and then sucked into the spectrophotometer cuvette. Absorbance was read from the spectrophotometer at wavelength 340nm.

6.1.3.3 Haemoglobin (Hb) Concentration

Hemoglobin (Hb) concentration was determined by the cyanmethaemoglobin method as reported by Jain (1986). EDTA blood was diluted 1:50,000 in isoton. Six drops of Zap-O- globin were added to lyse the cells and convert haemoglobin to cyanmethaemoglobin. The contents were then poured through the coulter haemoglobinometer to read Hb concentration in grammes per 100ml (g/dl).

¹ The AST reagent contains the following active ingredients: 200mmol/L L-Aspartate, 12mmol/L 2-oxoglutarate, 600U/L malate dehydrogenase, 0.25mmol/L NADH, phosphate buffer(pH 7.8±0.1) and 0.05% sodium azide (preservative)

²The active ingredients of BUN reagent include: 8mmol/L 2-oxoglutarate, 0.25mmol/L NADH, 50,000U/L urease, 1,500U/L GLDH, phosphate buffer (pH 8.0±0.1), and 0.05% sodium azide added as preservative.

6.1.3.4 Packed Cell Volume (PCV)

This was determined in a high-speed microhaematocrit centrifuge. A capillary tube was filled with EDTA blood to 3/4 full and sealed on one end with plasticin. It was then centrifuged at 10,000 rpm for 5 minutes. The PCV was read using a microhaematocrit reader.

6.1.4 Ultrasound Evaluation

Prior to treatment, views of the right lung, right lobe of the liver, and whole abdomen were obtained. The ultrasound examination was repeated 2, 4, and 8 weeks following the initial dose of either albendazole or oxfendazole. Cysts were observed for typical signs of degeneration, i.e. decreased size, increased echogenicity, detachment of endocyst, and collapse (Davies *et al.*, 1986). The animals' treatment status was unknown to the ultrasonographer.

6.1.5 Pathology, histology and viability studies

A standard postmortem examination was performed. The lungs, liver, abdominal cavity, kidneys, and spleen were visually inspected and dissected. All surface cysts were dissected intact, and the cyst fluid removed by needle aspiration. Portions of liver cysts that appeared alive and intact were fixed in formalin and stained with hematoxylin and eosin. Eosin exclusion and observation for flame cell movement determined the viability of *E. granulosus* (Macpherson, 1981).

6.1.6 Data analysis

Data were normalized by log transformation, and mean values and standard deviations were calculated and compared by student's *t*-test for paired data. Qualitative variables were compared by Fisher's exact test for chi-square variables. Correlation coefficients comparing the size indices of ultrasound measurements with those of postmortem measurements were calculated by the computer program cricket graph. $P < 0.05$ was used to determine statistical significance.

6.2 RESULTS

6.2.1 Experimental animals

A total of 472 animals were examined on ultrasound. Out of these, 15 animals with cystic echinococcosis were entered into the experiment. The animals were randomly allocated into 3 groups of 5 animals each. Two groups were subjected to treatment (with either albendazole or oxfendazole) while the third group was the control.

6.2.2 Hematological findings

6.2.2.1 Aspartate Aminotransferase (AST) Activity

The aspartate aminotransferase (AST) activity was used to determine the status of the liver function during treatment period. In all the three groups of animals (albendazole, oxfendazole and control group), there were no significant

variations ($P>0.05$) in aspartate aminotransferase (AST) activity. The AST levels were 24.28 ± 4.95 iu, 24.64 ± 4.40 iu, and 24.76 ± 5.17 iu in albendazole, oxfendazole and control groups respectively (Table 6.1).

6.2.2.2 Blood Urea Nitrogen (BUN)

The mean blood urea nitrogen levels in both the treatment groups and the control group are shown in Table 6.1. During the treatment period, there were no significant changes ($P>0.05$) within and between different experimental groups. The mean BUN levels were 26.58 ± 6.57 mg/dl, 24.62 ± 6.52 mg/dl, and 24.91 ± 4.99 mg/dl in albendazole, oxfendazole and control groups respectively.

6.2.2.3 Haemoglobin (HB) Concentration

There were fluctuations of haemoglobin concentration within different experimental groups during treatment period. The fluctuations were present in all the three groups (albendazole, oxfendazole and control). However, there were no significant differences between the groups ($P>0.05$). The mean haemoglobin concentrations were 9.86 ± 1.24 mg%, 9.22 ± 1.25 mg% and 9.31 ± 1.45 mg% in albendazole, oxfendazole and control groups respectively (Table 6.1).

6.2.2.4 Packed Cell Volume (PCV)

The mean packed cell volume (PCV) did not vary significantly within each group ($P>0.05$). Additionally, there was no significant variation between the different experimental groups ($P>0.05$). In albendazole, oxfendazole and control groups, the mean PCV were $29.64 \pm 3.74\%$, $27.12 \pm 3.77\%$, and $27.48 \pm 4.62\%$ respectively (Table 6.1).

Table 6.1 Hematological findings of animals with cystic echinococcosis after treatment with either albendazole or oxfendazole and the control group

	Hematological Findings							
	Mean AST (±Std) iu	Standard Error	Mean BUN (±Std) mg/dl	Standard Error	Mean HB (±Std) mg%	Standard Error	Mean PCV (±Std) %	Standard Error
Albendazole	24.28 ± 4.95	0.99	26.58 ± 6.57	1.31	9.86 ± 1.24	0.25	29.64 ± 3.74	0.75
Oxfendazole	24.64 ± 4.40	0.88	24.62 ± 6.52	1.30	9.22 ± 1.25	0.25	27.12 ± 3.77	0.75
Control	24.76 ± 5.17	1.03	24.91 ± 4.99	1.00	9.31 ± 1.45	0.29	27.48 ± 4.62	0.92

6.23 Ultrasound Findings

In animals receiving albendazole, 4 showed decreased cyst viability compared to 3 in oxfendazole group ($P<0.05$). The cysts showed increased echogenicity, complete or partial detachment of the endocyst, new calcification, or decrease in size. There were no changes in identifiable cysts of control animals.

6.24 Findings of Post mortem examination, viability studies and histology

A total of 49 cysts were harvested at postmortem (Appendix VI). Twenty-three (23) of the cysts were from the albendazole group while 15 and 11 were from oxfendazole and control groups respectively. Grossly, 4 (17.4%), 7 (46.7%) and 1 (9.1%) cysts were degenerate from albendazole, oxfendazole and control group respectively. The degenerate cysts had yellow to blackened adventitial layer and were filled with turbid yellowish fluid. Additionally, there were significant differences ($P<0.05$) in the viability of the cysts between the different experimental groups. Microscopic examination of protoscolices for eosin dye exclusion and flame cell motility showed that 60.9% (14/23) of the cysts from albendazole group had dead protoscolices compared to 93.3% (14/15) and 27.3% (3/11) for oxfendazole and control groups respectively (Table 7.2). The viable-appearing cysts showed evidence of marked host cell reaction consisting of infiltration of the adventitial layer with neutrophils, eosinophils, and plasma cells.

TABLE 6.2 Microscopic findings of *Echinococcus* cysts from animals treated with either albendazole or oxfendazole and the control animals

Type of treatment	Cysts with viable/Non-viable protoscolices	
	<u>Non-viable</u>	<u>viable</u>
Albendazole	60.9% (14/23)	39.1% (9/23)
Oxfendazole	93.3% (14/15)	6.7% (1/15)
Control	27.3% (3/11)	72.7% (8/11)

6.3 DISCUSSION

In the present study, the efficacy of albendazole was 60.9%. This is comparable with the efficacy of the drug in humans (WHO, 1996). In humans, albendazole has been reported to have an efficacy of 30-60% when administered daily at the dosage rate of 20mg /kg body weight. In the present study, the dosage rate was 30mg/kg-body weight administered twice a week. Different administration rates (bi-weekly versus daily) and different dosage rates may have resulted in similar efficacy due to species differences. Sheep and goats are ruminants while man is monogastric and therefore drug metabolism is expected to be different.

In the group treated with oxfendazole, protoscoleces were either dead or absent in 93.3% of the cysts. Additionally, protoscoleces were either absent or dead in 27.3% of the cysts in the control group. These findings are similar to those of other authors. In a recent study, Dueger *et al.* (1999) reported the mean aggregate of dead protoscoleces to be 99%, 93% and 68% for sheep treated daily, weekly and monthly with oxfendazole at 30mg/kg body weight. Similarly, they found dead protoscoleces in 32% of the cysts. The findings in the present study are, however, different from those found earlier (Chapter 5) by the same author. In a previous study using two groups of animals (oxfendazole and control), protoscoleces were dead in 97.3% of the cysts from the treatment group while none were dead in the control group. The differences in the findings of the two studies may be difficult to explain because the later study tried to mimick the former one.

The findings of this study indicate that oxfendazole has a higher efficacy (93.3%) than albendazole (60.7%) when administered at the same dosage rate (30mg/kg-

body weight) and for the same period (twice weekly for 4 weeks). These findings may be explained by the differences in pharmacokinetics of the two drugs. In a comparative analysis of albendazole and oxfendazole using high performance liquid chromatography (HPLC), Lanusse *et al* (1995) found the plasma disposition of albendazole metabolites to be markedly different from that of oxfendazole derivatives. Albendazole sulphoxide (the active metabolite in albendazole) exhibited faster absorption and a higher C_{max} than oxfendazole. Furthermore, while albendazole sulphoxide declined relatively rapidly in plasma reaching non-detectable concentrations at 60-hrs post-albendazole administration, oxfendazole was found in plasma for upto 144 hrs post-treatment. However, more studies are necessary to determine the rate of penetration into *Echinococcus* cyst and levels of both albendazole sulphoxide and oxfendazole within the cyst.

CHAPTER 7

Discrimination of *Echinococcus granulosus* strains found in various mammalian species and man in Turkana, Kenya by polymerase chain reaction (PCR)

7.0 INTRODUCTION

In previous studies with polymerase chain reaction (PCR) to characterize various different strains of *Echinococcus granulosus* present in Turkana, Kenya, primers that are specific to *Echinococcus* were not used. It is possible that other strains of the parasite exist but could not be identified by the methods used. Primers specific to cestodes as well as the sheep strain of *E. granulosus* have been developed (Dinkel, 1998; Dinkel *et al*, 1998). The aim of this study was to further screen and differentiate *E. granulosus* strains present in man and livestock in Turkana using cestode specific and sheep strain specific primers.

7.1 MATERIALS AND METHODS

7.1.1 Collection of Samples

Collection of the cestode materials was carried out in Turkana District and slaughterhouses near Nairobi between May 1998 and August 2000. *Echinococcus* cysts were collected in polythene papers and transported to laboratory for processing. Each cyst was incised and the endocyst, hydatid fluid, and protoscoleces harvested in a petri dish. Protoscoleces were harvested in a 10ml sample bottle using a pipette and rinsed with phosphate-buffered saline (PBS). Each sample bottle was labelled appropriately to show the type of animal species

and the organ from which it was obtained. Each isolate was stored separately in 70% ethanol until required for DNA extraction.

7.1.2 Determination of *Echinococcus* strains by PCR

The work on PCR was carried out at the University of Hohenheim, Germany in the month of October 2000 (Appendix I). The work involved two main steps namely extraction of DNA and and polymerase chain reaction (PCR).

7.1.2.1 Extraction of *Echinococcus* DNA

Extraction of the DNA involved digestion of the samples, isolation of the parasite DNA and determination of the amount of DNA in each sample.

7.1.2.1.1 Digestion of the *Echinococcus* samples

Each sample was transferred from the 10ml collection tube into a microtiter eppendorf, centrifuged at 5000 rpm for 1 minute and 70% ethyl alcohol preservative removed. Any remaining ethyl alcohol in the sample was removed by centrifuging the sample with 500ul-digestion buffer at 5000 rpm for 1 minute (then removing the buffer). 500ul Digestion buffer², 60ul Proteinase K and 10ul

² 100ml of digestion buffer was prepared by mixing 1ml 1M Tris HCl (pH 7.5), 2ml 0.5M EDTA (pH 8.0), 5ml 5M NaCl, 10ml 2% SDS and 86ml Distilled water.

DTT³ were then added to the sample. The sample was mixed with electric shaker (vortex Genie 2®). The suspension was then incubated at 56°C under movement overnight.

7.1.2.1.2 Isolation

After digestion of the sample, the resulting solution was centrifuged at 13,500rpm for 1 minute. The supernatant was transferred into a new tube and the pellet discarded. Phenol chloroform was then added and the suspension centrifuged at 13,500 rpm for 15 minutes. The aqueous phase (500ul) was transferred into a new tube, and 50ul 3M Na Acetate (pH 5) and 1000ul absolute (100%) ethyl alcohol added. The solution was mixed by inverting the tube and incubated at -20°C overnight before being centrifuged at 13,500 rpm at 4°C for 30 minutes. The supernatant was then discarded, 1000ul of 70% ethyl alcohol added and further centrifugation done at 13,500 rpm at 4°C for 10 minutes. The supernatant was discarded, the sample short centrifuged for 30 seconds and any remaining ethanol removed with a pipette to leave the DNA pellet as dry as possible. The sample was then incubated at 55-60°C for 30 minutes to vaporize any ethyl alcohol remaining. The DNA was resuspended in 200ul Tris-EDTA buffer (pH 7.6) and incubated at 4°C overnight.

³ Proteinase K reduces proteins to amino acids while DTT (Dithiothreitol) reduces the S-S bridges

7.1.2.1.3 Determination of amount of DNA

The amount of DNA isolated in each sample was determined by measuring the optical density of the DNA isolates. The isolates were warmed in a rotating water bath at 50°C for 10 minutes. 4ul of each sample was then diluted with 200ul Tris-EDTA buffer (pH 7.6). Optical density was determined with a photometer (UV Lambda BIO®, Perkin Elmer) and the amount of DNA in each sample recorded in ug/ml.

7.1.2.2 Polymerase Chain Reaction (PCR)

Primers for known DNA sequences for *E. granulosus* were used in the present study. The target sequence for amplification was part of mitochondrial 12S rRNA gene. A PCR procedure described by Dinkel *et al.* (1998) was used with some modifications. The PCR was conducted in two steps, one involving a cestode specific primer and the other involving a primer specific to *E. granulosus* sheep strain. In the first step, the primer pair P60.for. and P375.rev. amplified a 373bp fragment of the DNA. A total of 250ng of DNA was added to a reaction mixture containing 10mM Tris-HCl buffer, 2.5mM MgCl₂, 200uM dNTPs, 40pmols/ul P60.for., 40pmols/ul P375.rev. and 2.5U Taq polymerase (AGS GmbH, Heidelberg, Germany). Thermal cycling of the amplification mixture was performed in an automated Gene Amp PCR System 9700® (Perkin Elmer)

(Appendix II). A cycle represents denaturation for 30 seconds at 94°C, anealing for 60 seconds at 52°C, and elongation for 40 seconds at 72°C (Figure 7.1).

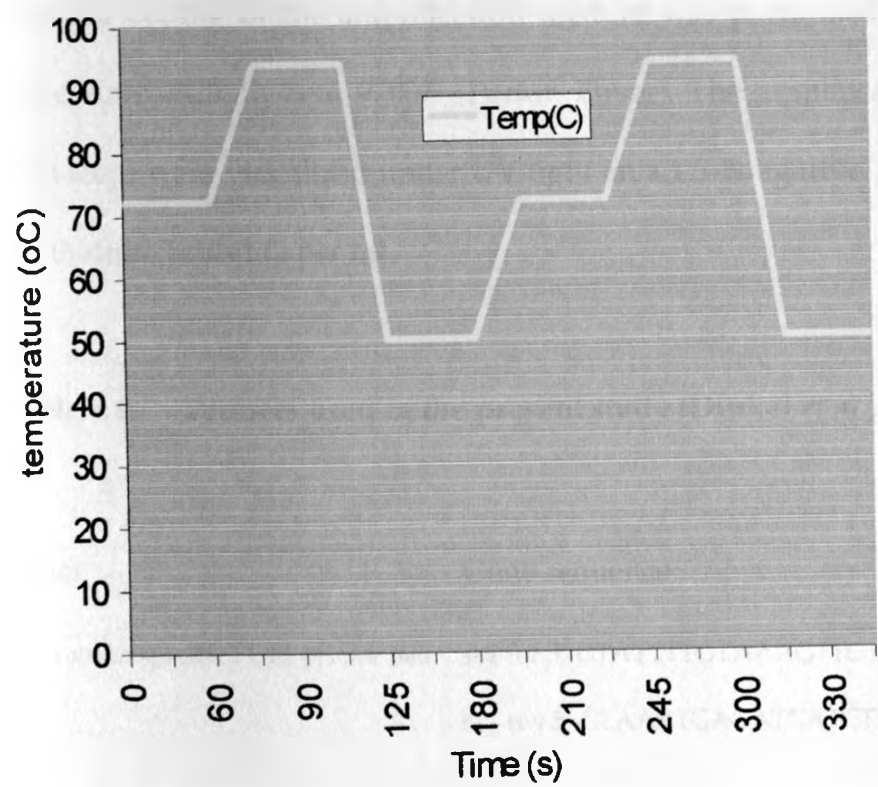


Figure 7.1 PCR temperature protocol

In the second step, the primer pair, Pss1.for. and Pss1.rev. (Table 7.1) differentiated various isolates in the *E. granulosus* sheep strain. A total of 250ng of the DNA isolate was added to a reaction mixture containing 10mM Tris-HCl buffer, 2.0mM MgCl₂, 200uM dNTPs, 50pmols/ul Pss1.for., 50pmols/ul Pss1.rev. and 2.5U Taq polymerase (AGS GmbH, Heidelberg, Germany). Thermal cycling of the amplification mixture was performed in an automated Gene Amp PCR System 9700® (Perkin Elmer). The amplification products for both steps were visualized under UV light on a 1.5-% agarose gel containing 1ug of ethidium bromide per ml.

Table 7.1 Primers used in the present study (Dinkel *et al.*, in preparation)

Primer	Gene sequence
Sheep strain specific PCR/primer pair	ss1 for 5'-GTATTTTGTAAAGTTGTTCTA-3'
	ss1 rev 5'-CTAAATCACATCATCTTACAAT-3'
Sheep strain DNA probe	E. gran.ss 5'-ACTACACAAACITAAATTAAATTATTC-3'
Camel strain DNA probe	E. gran.cs 5'-ACTACACAAACTCAAACCATTA-3'

7.2 RESULTS

7.2.1 Distribution of sources of Samples

A total of 349 samples were collected. Among these, 17.5% (61) were of human origin while the rest were from domestic intermediate host species. Samples from the domestic intermediate hosts included 19% (67) cattle, 30% (104) camel, 5% sheep, 27% (94) goats and 1% (4) pigs. The samples were obtained from liver, lung, spleen or mesentery (Table 7.2). About 86.2% of all the samples were from Turkana while 13.8% were from either Dagoretti or Kiserian. All the pig samples were obtained from Dagoretti area while all the camel and human samples were from Turkana.

Table 7.2 Hydatid cyst samples (protoscolices) used in the PCR experiment and their origin

Origin	liver	lung	spleen	mesentery	Source			Total
					<u>Turkana</u>	<u>Dagoretti</u>	<u>Kiserian</u>	
Human	58	-	-	3	61	-	-	61
Cattle	8	59	-	-	32	35	-	67
Camel	17	80	7	-	104	-	-	104
Sheep	16	3	-	-	17	-	2	19
Goats	20	73	1	-	87	-	7	94
Pigs	-	4	-	-	-	4	-	4
Total								349

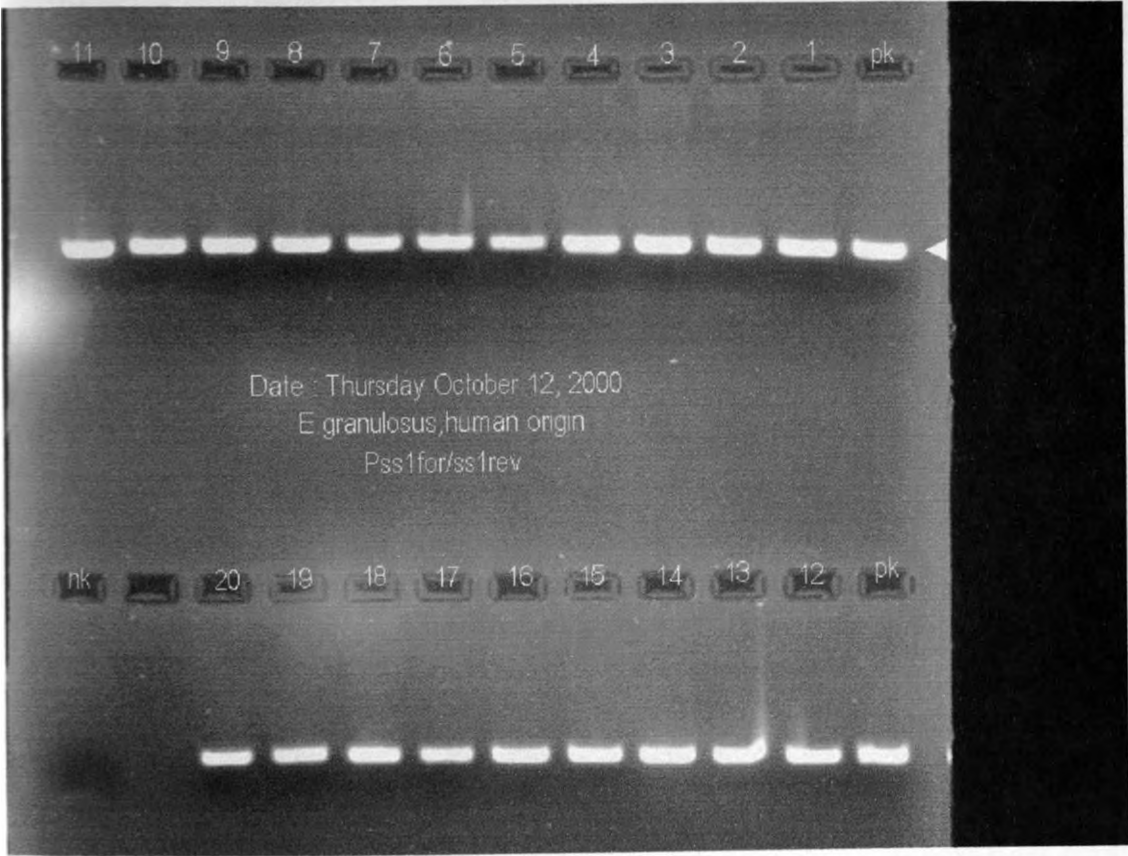
7.2.2 PCR amplification products

All the 61 human samples produced a characteristic banding pattern of the sheep strain *E. granulosus* while using cestode specific and sheep strain specific primer. All the 19 samples of sheep origin that were examined had sheep strain *E. granulosus* (Fig. 7.2). Of the camel samples examined, 21 had sheep strain *E. granulosus* while 83 had camel strain *E. granulosus* (Fig. 7.3). Samples of cattle origin had a mixed infection; 8 samples had camel strain while 59 had sheep strain *E. granulosus* (Fig. 7.4). Three samples obtained from pigs had sheep strain *E. granulosus* while one sample had camel strain.

Table 7.3 Strains of *E. granulosus* from isolates of different intermediate hosts in Kenya

Origin	Sheep strain	Camel strain	Total
Human	61	-	61
Cattle	59	8	67
Camel	21	83	104
sheep	19	-	19
Goat*	59	18	77
pigs	3	1	4

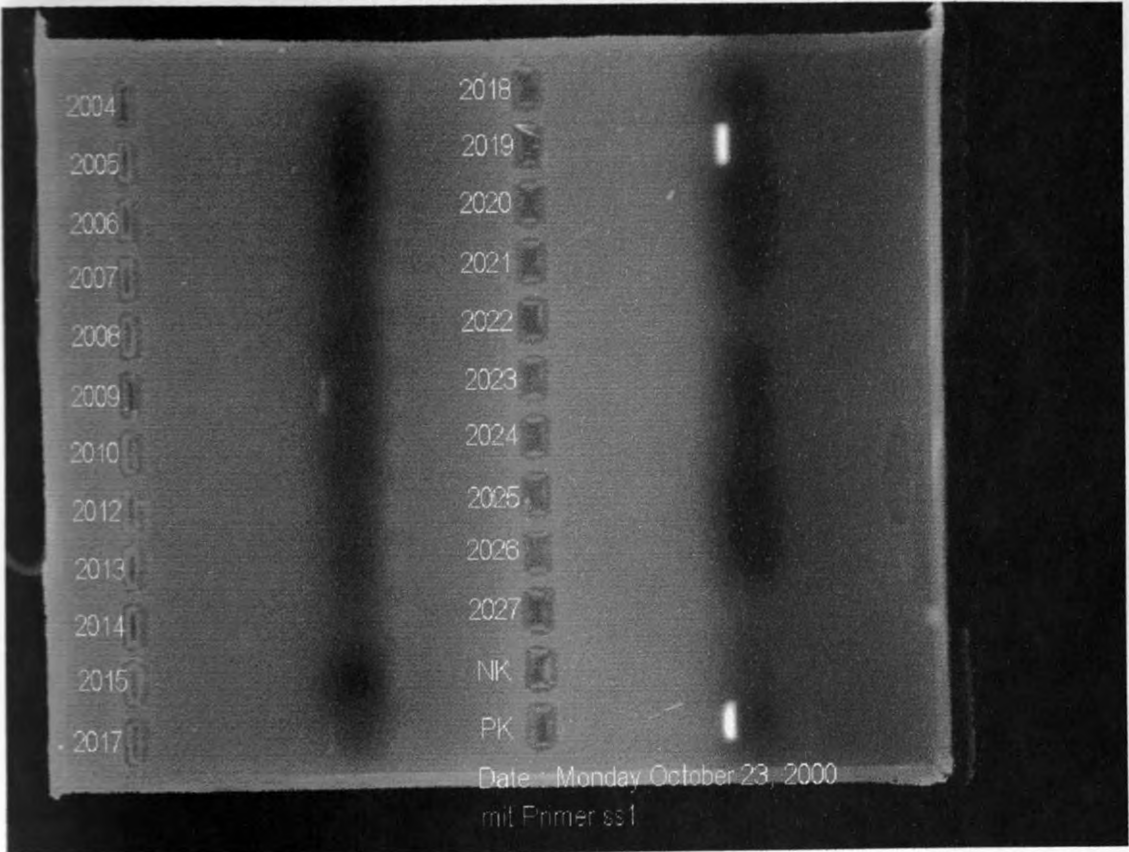
* Some of the goat samples were not analysed for technical reasons



Key:

- Pk positive control
- Nk negative control
- Pss for/ss1rev forward and reverse sheep-strain specific primer
- 1 - 20 sample numbers 1 to 20

Figure 7.2 PCR results of *E. granulosus* from samples of human origin, using a sheep-strain specific primer. All the samples had sheep strain *E. granulosus* (Note the characteristic banding pattern)



Key:

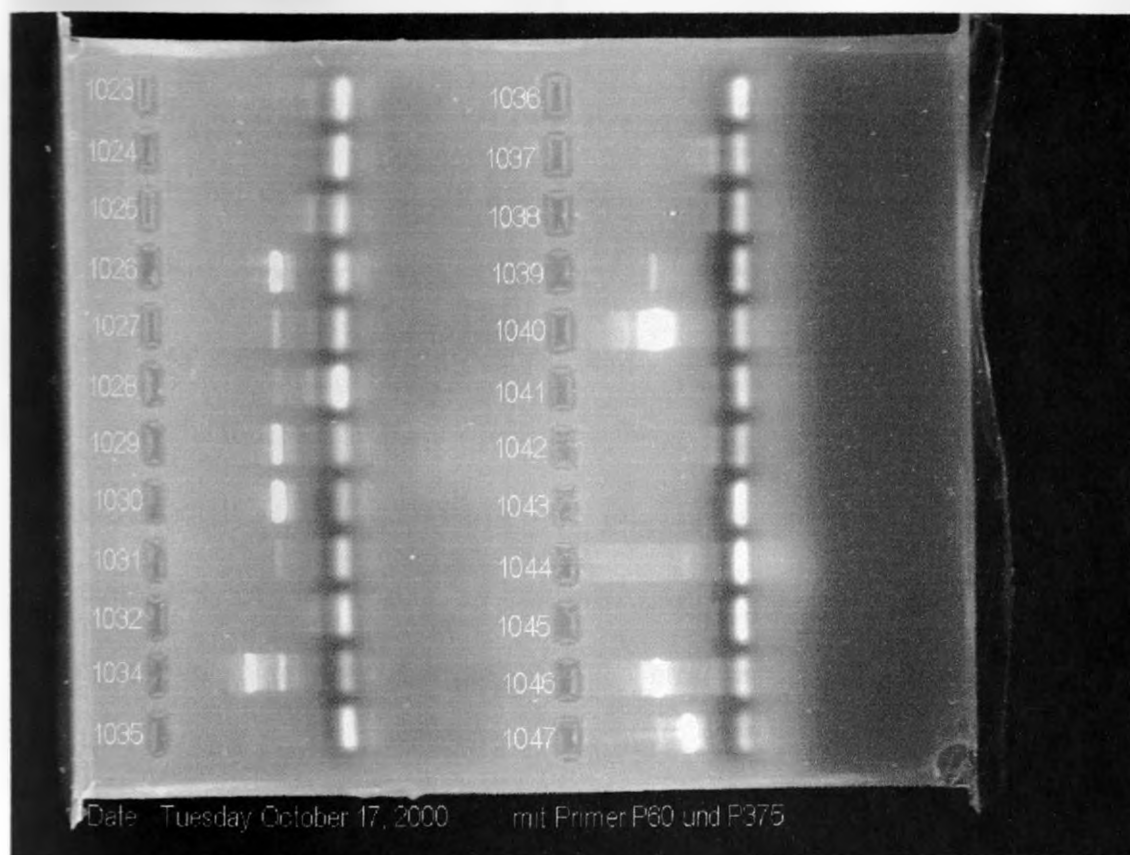
Pk positive control

Nk negative control

Pss for/ss1rev forward and reverse sheep-strain specific primer

2004 - 2027 samples numbers 2004 to 2027

Figure 7.3 PCR results of *E. granulosus* from samples of camel origin, using a sheep-strain specific primer. All the samples except one (no.2019) had camel strain confirmed by DNA sequencing. Sample 2019 had sheep strain *E. granulosus* (Note the characteristic banding pattern of one sample)



Key:

MitPrimer P60 und P375 cestode specific primers
2023 - 2047 sample numbers 2023 to 2047

Figure 7.4 PCR results of *Echinococcus granulosus* from samples of cattle origin, using a cestode specific primer. All the samples had *E. granulosus* cestodes. (Note the characteristic banding pattern). In some cases, the primer was not fully utilised during the PCR reaction, thus forming a second band.

7.3 DISCUSSION

In the past, identification of various strains of *E. granulosus* was based on a limited number of morphological features. These features are however variable and may not necessarily reflect distinctness at the genetic level (Hobbs *et al.*, 1990). Other methods of identification, biochemical and immunological analysis, are similarly limited in their usefulness because of the potential for host and environmentally induced variation (McManus *et al.*, 1994). However, development of molecular techniques (e.g. PCR) has allowed a direct characterisation of the genome of *E. granulosus* and the findings are not influenced by variability induced by the host or the environment (Eckert and Thompson, 1997). This study utilised PCR technique.

In a previous study on strain differentiation, two strains (camel and sheep strains) were identified in isolates from Turkana (Wachira *et al.*, 1993). In the present study, PCR analysis did not detect any additional genetic variations from the findings of Wachira *et al.* (1993). Two characteristic patterns similar to those found by the previous authors were produced in all cases. The patterns corresponded to either the sheep-dog strain or the camel-dog strain. It appears therefore that there are only two strains of *E. granulosus* in Kenya, which are infective to cattle, sheep, camel and man. In goat isolates, sheep strain was identified in more samples than camel strain. This was in contrast to previous

report that showed goats to be infected more by the camel than sheep strains (Wachira *et al*, 1993). The difference in the present study and that of Wachira *et al* (1993) could not be explained but may be as a result of goats grazing with sheep in the present study, and therefore exposure of goats to sheep strain.

Most of the previous authors have reported the camel strain *E. granulosus* only in camels and goats (Macpherson and McManus, 1982; McManus and Macpherson, 1984; McManus *et al.*, 1987; McManus and Rishi, 1989). Additionally, Wachira *et al* (1993) identified camel strain in only one cattle isolate. In the present study, 8 cattle isolates were found to have camel strain, suggesting that the camel strain may be more prevalent in cattle than previously thought. The difference may also be attributed to the source of the isolates. Wachira *et al* (1993) examined only 5 cattle isolates from Turkana while in the present study, 32 isolates were examined.

Previous reports indicated that man is refractory or poorly susceptible to infection with the camel strain *E. granulosus*. All the human isolates examined in the present study had sheep strain *E. granulosus*. However, a single human isolate examined earlier from Turkana was found to have camel strain *E. granulosus*, suggesting that humans may be as susceptible to the camel strain as other animal species (Dinkel, Personal communication).

CHAPTER 8

Evaluation of ethyl alcohol in treatment of cystic echinococcosis using puncture, aspiration, introduction, reaspiration (PAIR) technique

8.0 INTRODUCTION

In the past, the puncture of echinococcal cysts in humans was strongly discouraged because of risks of anaphylactic shock and spillage of the fluid with subsequent peritoneal seeding of protoscolices to form new cysts. However, with numerous cases of either accidental or deliberate *Echinococcus* cyst puncture (Brunetti *et al.*, 1995; Salama *et al.*, 1995; Akhan *et al.*, 1996), these risks have not been reported. Minor complications such as pruritus, rash and abdominal pain have been reported in 0.5% - 22% of the cases (Bret *et al.*, 1988; Xiaozhi *et al.*, 1994). Puncture of the *Echinococcus* cyst and aspiration of the cyst fluid, coupled with addition of a scolicalidal solution to kill the protoscolices, has therefore become one of the surgical techniques used in treatment of cystic echinococcosis in humans (Filice and Brunetti, 1997; Zeyhle, Personal communication, 2000).

Some of the scolicalidal agents that have been used to kill the protoscoleces include cetrimide bromide, hypertonic saline, hydrogen peroxide, and ethyl alcohol. Whereas cetrimide bromide has been effective against protoscoleces, it has been reported to have an adverse effect of causing methaemoglobinuria (Barake *et al.*, 1980). Hydrogen peroxide has equally been effective but is known to cause collapse of the patient after injection (Belghiti *et al.*, 1986). Use of hypertonic saline is not known to have any side effects but a 4% rate of recurrence was reported (Gargouri *et al.*, 1990). In experimental animals using rats, hypertonic saline caused mortalities of 70-90%. Some rats had convulsions due to intracranial bleeding, necrosis and myelinolysis (Kayaalp *et al.*, 2001a). However, detailed information on the efficacy and possible adverse effects of ethyl alcohol when used in Puncture, aspiration, introduction, and re-aspiration (PAIR) technique is lacking. The aim of this study was to evaluate 95% ethyl alcohol,

(the concentration used in humans) in the PAIR technique using sheep and goat models.

8.1 MATERIALS AND METHODS

8.1.1 PAIR Procedure

Hair was shaved from the abdomen of sheep and goats, and a coupling gel applied. Ultrasound examination was performed with a real time B-mode scanner with a 3.5 mHZ linear assay transducer with electronically variable focus. Scan images were recorded on a graphic printer.

Each animal was then put under heavy sedation, using 0.5mg/kg bwt xylazine HCl (Xylalin®, Apharmo B.V. Netherlands) IV and 0.025mg/kg bwt atropine sulphate given SC to minimise hypersalivation. Each animal was also given 0.8mg/kg bwt Dexamethasone (Agrar®)(Agrar, Holland B.V.) to control possible anaphylactic shock due to cyst rupture and 20mg/kg bwt tetracycline to control cyst infection. The animal was then covered with sterile drapes to minimise external contamination. Contamination of the surgical site by ultrasound transducer was minimised by wiping the surfaces with a swab containing 70% surgical spirit. After locating the *Echinococcus* cyst with ultrasound, a stab incision was made on the skin to allow penetration of the canula. An 18G 6-inch metallic canula was used to puncture the cyst per cutaneously under ultrasound guidance (Figure 8.1). The canula was attached to a 60ml syringe and the cyst fluid was aspirated with the syringe. The aspirated cyst fluid was stored in sterile kidney dish and examined in the laboratory within 15 minutes.

The cyst fluid aspirated was replaced with 95% ethyl alcohol. The alcohol introduced into the cyst was left for 20 to 30 minutes before reaspiration of the cyst fluid/alcohol mixture. The animals were then maintained for one month before scanning with ultrasonography to determine the changes in shape and size of the cysts.

8.1.2 Controls

Animals in this group were subjected to similar treatment as those in the test group except that no ethyl alcohol was introduced into the cyst. Puncture of the cysts was done in a similar manner to the test group and the cyst fluid aspirated. The cysts were then left without introducing any scolicial reagent. The animals were maintained for one month under close observation. After one month, ultrasound scanning was carried out and then animals euthanised for post mortem examination.

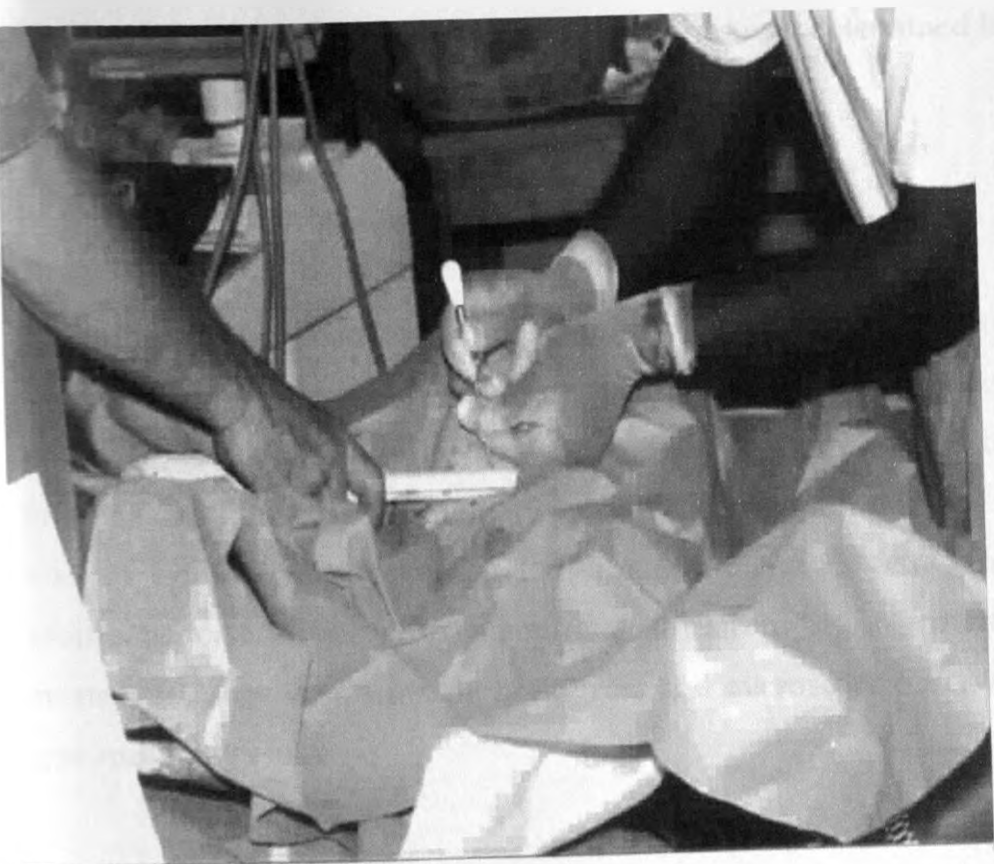


Figure 8.1 Puncture of *Echinococcus* cyst under ultrasound guidance

8.1.3 Laboratory analysis of the cyst fluid

Aspirated fluid from the cyst was examined under a light microscope at low magnification (X40). Viability of the protoscoleces was determined by flame cell motility and eosin dye exclusion (Macpherson, 1981).

8.1.4 Interpretation of the findings

Both ultrasound appearance and post mortem findings were used to evaluate the efficacy and possible adverse effects of 95% ethyl alcohol. Each cyst was examined as a separate entity and the overall interpretation was based on the findings of all the cysts. A comparison of the treatment group and the controls was carried out using cysts subjected to puncture and 95% ethyl alcohol as test group and cysts subjected to puncture alone as controls. Interpretation of post mortem findings was based on both gross and microscope observations of the cyst and liver tissues.

8.2 RESULTS

A total of 6 animals (4 sheep and 2 goats) were used in this study. In these animals 7 cysts were punctured and injected with 95% ethyl alcohol (test group) while 9 cysts were only punctured (controls).

8.2.1 Ultrasound findings

In both test and control groups there was collapse of the endocyst after cyst puncture. One month later, the cysts showed decrease in size, increased echogenicity, and complete or partial detachment of the endocyst (Fig. 8.2).

8.2.2 Post mortem findings

In the test group, the cysts were grossly degenerated with marked fibrosis of the surrounding liver tissue. Incision of the cysts revealed turbid yellow cystic fluid and degenerated endocysts. On microscopic examination of the cyst fluid, the protoscoleces were dead, with detached hooks.

In the control group, the cysts appeared grossly intact but flaccid. Incision of these cysts showed clear fluid with intact endocysts. However, microscopic examination of the cyst fluid showed that the protoscoleces were dead with detachments of hooks.

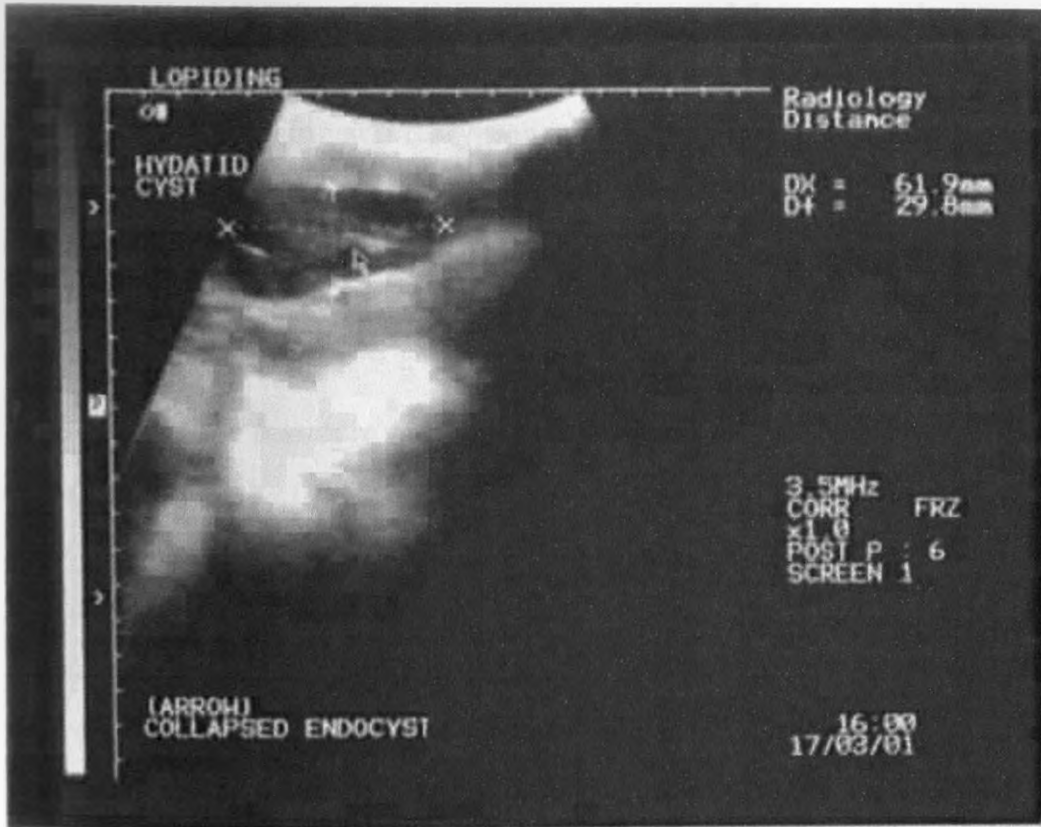


Figure 8.2 A sonogram of an *Echinococcus* cyst after puncture shows collapsed endocyst (arrow).

8.2.3 Histopathological findings

In all the cysts injected with 95% ethyl alcohol there was marked host cell reaction consisting of infiltration of the adventitial layer with neutrophils, eosinophils, and plasma cells. In addition, the liver tissue was severely destroyed and replaced with young and disorganized fibroblasts and mesenchymal cells. In most necrotic areas, the laminate layer could not be collected together with adherent liver tissue and the adventitial layer appeared completely degenerate and was replaced by acute inflammatory cells.

In the cysts where puncture alone was carried out, there was detachment of the laminate layer of the cyst from the adventitia. Additionally, inflammatory cells were observed in the adventitia and the liver tissues. However the degree of inflammation was markedly less than in the test group. Inflammatory cells were identified only in small parts of the liver tissues while most of the tissues were intact with hepatocytes being predominant in an organised appearance.

8.3 DISCUSSION

In the past, 95% ethyl alcohol has been assumed to be a safe scolicalidal agent for use in human patients. It has been widely used in the PAIR technique in different hospitals (Filice *et al.*, 1999). However, its safety and possible adverse side effects have not been adequately studied (Palowski, 1997; Filice *et al.*, 1999). One of the difficulties that studies in humans are faced with is that once treated, a human being cannot be subjected to surgery to study the side effects of a scolicalidal agent. The only indicator of any side effects of a scolicalidal agent would be deterioration of the patient and the clinical picture. This difficulty can only be overcome by using an appropriate animal model where a post mortem

examination can be carried out. The present study is one of the first studies of the effects of 95% ethyl alcohol using sheep and goat models.

In the present study, all the cysts subjected to puncture and 95% ethyl alcohol injection were severely degenerated and the protoscolecess were dead, a desired outcome for any scolicial agent. However, it adversely caused severe damage of liver tissues, a feature reflected by, grossly, severe fibrosis and, by histopathology, marked infiltration of the tissues by inflammatory cells that replaced normal hepatocytes. This was absent in the control group where the protoscolices were also dead as a result of puncture alone. These findings suggest that 95% ethyl alcohol may be causing severe liver damage in human patients who are subjected to the PAIR technique. What may not be clear from the present study is whether the damage to the liver by 95% ethyl alcohol significantly affects its functions, and whether the liver is able to heal and resume its efficiency in function. The findings also suggest that puncture alone may be sufficient to kill the protoscoleces, possibly due to detachment of the endocyst from the host wall.

CHAPTER 9

General discussion and conclusions

In the present study, various parameters that determine the suitability of a diagnostic test were determined on ultrasonography. These included sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and kappa statistic. The PPV is the probability that an animal tested has the disease given that a positive result is received. The NPV is the probability that the animal tested is non-diseased given that a negative result is received. Both PPV and NPV are not good indicators of the suitability of a diagnostic test because they vary according to the prevalence of the disease in the studied population (Macpherson, 2001b). As the prevalence of the disease approaches zero, the PPV will also approach zero and most of the "positive" cases will be "false positives". Conversely, the NPV will be very high at low prevalence and there will be few "false negative" results. On the other hand, the sensitivity and specificity may or may not vary with the prevalence of the disease and are therefore good indicators of the suitability of a diagnostic test. The higher the sensitivity and specificity of a test, the greater the PPV will be at any given prevalence of the disease.

Kappa is defined as the degree of clinical agreement between two tests. In the present study the kappa was calculated between postmortem as the gold standard and ultrasound as the diagnostic technique under evaluation. Kappa has been reported to give a more accurate information of a diagnostic test than both sensitivity and specificity (Sacket *et al.*, 1985). However, unlike other diagnostic tests, ultrasound is very much dependent on the ability of the observer to detect lesions than the ability of the test itself. The sensitivity, specificity and kappa calculated in the present study would, therefore, best indicate the ability of the ultrasonographer to detect *Echinococcus* cysts than the ability of the technique itself. Further studies need to be carried out to determine both intra- and inter-observer variations in ultrasound diagnosis.

The prevalence of cystic echinococcosis was determined by both ultrasonography and slaughter data in the present study. Both methods provided valuable information that can be used to develop control strategies in Northern Turkana. Ultrasound allowed assessment of the disease even in remote areas where slaughter is not monitored. However, it had a limitation of not detecting cysts deep in the lung parenchyma. On the other hand, slaughterhouse data provided information on the disease status in animals presented for slaughter. Cysts in all the body organs could be examined, including those in the lungs. However, the usefulness of the slaughter data was limited in that the animals taken for slaughter are not a true representation of all the animals in the study area. Future studies should gather a comprehensive data by employing both techniques (slaughter data and ultrasound survey).

The differences between the prevalence findings in the present study and those of Macpherson (1981) were interesting. In the present study, much higher prevalence of the disease was found in the intermediate hosts than Macpherson (1981) reported 20 years earlier. It has been established that drought is cyclic in Turkana, and occurs after every 10 years. There were severe droughts in 1970, 1980, 1990 and 2000. Macpherson collected his data soon after the 1980 drought (Macpherson, Personal communication, 2001) while in the present study, the data were collected before the drought in the year 2000. It is possible that drought plays a major role in the transmission of cystic echinococcosis in Turkana. Before the drought, the intermediate hosts (cattle, sheep, goats and camels) usually harbor the parasite *E. granulosus* in their body as protoscoleces within cysts. The onset of drought leads to massive death of livestock, starting with the weak and old animals that have *Echinococcus* cysts. Dogs feed on these carcasses and become heavily infected. Humans become infected when they get into close contact with the infected dogs. Collection of prevalence data in intermediate hosts before and after a period of drought would, therefore, result

in high and low prevalence of the disease, respectively. Further studies of the prevalence of the disease in dogs, before and after a period of drought, would need to be carried out to support the findings of both the present and previous studies in the intermediate hosts.

Various methods of cystic echinococcosis treatment have been developed and used in humans. However, there is no standard method of treatment applicable in all hospitals and medical set-ups. Some authors have argued that cystic echinococcosis is basically medical, not surgical, whose appropriate drugs have yet to be developed (Aktan, 1999), primarily because when the surgical treatment of a specific disease is clear-cut, there is always a surgical technique of choice. The etiology of the disease is well defined and there is no other parasitic disease on earth for which the primary treatment is surgical. In the present study, two drugs (albendazole and oxfendazole) were compared. Oxfendazole was found to be a promising chemotherapeutic agent for cystic echinococcosis. The high efficacy of oxfendazole has been attributed to its longevity in serum as well as its ability to alter the immunobiology of the host-parasite interaction (Dueger *et al.*, 1999). Oxfendazole has been postulated to cause changes in the cyst membrane which permit a loss of host tolerance and/or decreases the ability of the parasite to protect itself from the host immune response (Dueger *et al.*, 1999). The direct effect of oxfendazole may not kill the parasite, but rather it is the collateral immune damage occurring after the drug dissipates that kills the parasite over a period of weeks. This mode of mechanism is absent in albendazole and other chemotherapeutic agents that have been used in treatment trials of cystic echinococcosis and may explain the large number of degenerated cysts found in oxfendazole group compared to either albendazole or control groups in the present study.

Additionally, oxfendazole has been reported to cause both humoral and cellular immunity in sheep (Stankiewicz *et al.*, 1994; Dueger *et al.*, 1999). This might explain the death of two animals in the present study, and up to 24% of animals treated daily in the study by Dueger *et al.* (1999). Although there are differences in drug metabolism between ruminants and humans, the findings in the present and previous studies suggest that treatment trials in humans using oxfendazole would require careful monitoring of the patients to counter any adverse effects of the drug.

Human surgeons generally accept the need for scolicalidal agents. In a questionnaire study conducted on 500 surgeons (Kayaalp *et al.*, 2001b), 97.9% of those who responded believed that scolicalidal agents should be part of the PAIR technique in treatment of cystic echinococcosis. However, there was a wide diversity on the type of agent, and a lack of consensus on the concentration and the waiting periods. In the present study, there was no significant difference in the viability of protoscoleces between the group treated with 95% ethyl alcohol and the control (group where puncture alone was done) when the waiting period was 5 minutes. The findings of the present study suggest that surgeons may not need to use scolicalidal agents at all after puncture of the cyst in the PAIR technique. The mere puncture of the cyst detaches the endocyst from the host ectocyst and therefore cuts the flow of nutrients, thus starting a natural process of death for the endocyst and the protoscoleces after several weeks.

CHAPTER 10

References

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APPENDICES

Appendix I

Requirements for *Echinococcus* PCR laboratory

1. Laboratory space

- Preparation room 1 - where digestion and isolation of DNA is carried out.
- Preparation room 2 - where DNA is mixed with primers and buffers.
- PCR room - where thermal cycler is placed and gel electrophoresis is done.

NB: movement should be unidirectional between preparation rooms and PCR rooms. Contamination originates from the PCR room to preparation rooms.

2. Equipment

Preparation Room 1:

- Microcentrifuge - Revolution capacity upto 15,000 rpm
- Cooling microcentrifuge - Revolution capacity upto 15,000 rpm
- Vortex electric shaker
- Mini hybridization oven - should allow movement of samples under incubation at 56°C
- Safety/ sterile chamber
- Fridge - to attain a temperature of 4°C
- Deep-freeze (-20°C to -80°C)
- Incubator (55°C - 60°C)
- Water bath
- Optical density photometer
- 1.5ml microcentrifuge tubes
- Pipettes - 100ul, 200ul, 1000ul
- Pipette tips - 1500ul (blue), 200ul (yellow), 20ul (white).

Preparation room 2:

- Deep-freeze
- Sterile chamber

PCR Room

- Thermal cycler

3. Materials and reagents

- Sterile phosphate buffered saline (1 x PBS; 8g NaCl, 0.2g KCl, 1.44g Na₂HPO₄, 0.24g KH₂PO₄ per liter with pH adjusted to 7.4 with HCl)
- Digestion buffer (10mM Tris-HCl, pH 8.0, 100mM NaCl, 25mM EDTA, 0.5%SDS)
- Proteinase K
- Phenol
- Chloroform/ isoamyl alcohol (24:1)
- 3 M sodium acetate (pH5.2)
- Absolute ethanol, ice cold
- 70% ethanol, ice cold
- Tissue paper
- Ultrapure water
- DTT (Dithiothreitol)

Appendix II

PCR Instruments

Instrument	Supplier
GeneAmp® PCR Instrument Systems DNA thermal cycler 480®	Perkin-Elmer Biosystems
• 48-sample block • provides well-to-well and cycle-to-cycle reproducibility • non-heated lid	
GenAmp 9700®	
• 96-sample block • heated lid • excellent well-to-well and cycle-to-cycle reproducibility	
PCR Express (Hybaid)®	Perkin-Elmer Biosystems
Eppendorf Gradient Mastercycler®	Perkin-Elmer Biosystems
• 96-0.2ml and 77-0.5ml universal block • 96-well plate • reproducibility	
RoboCycler® Temperature Cylers	Stratagene
• 40-well or 96-well sample blocks • 4 temperature blocks • 1 cooling block • 3 programmable blocks(for denaturation, anealing and extension steps) • robotic arm (that transfers tubes from one block to the next)	
ABI PRISM® 7700 Sequence Detection System	Perkin-Elmer Biosystems
• Suitable for PCR kinetics measurement • 96-well block • performs both thermal cycling and fluorescence detection • based on laser excitation	
LightCycler™ Instrument	
• Allows monitoring of PCR products accumulation • Allows melting curve analysis • Suitable for rapid product analysis and mutation detection studies	

Appendix III

Animals positive for hydatid cysts on both ultrasound (US) and postmortem (PM) examination

Animal			Ultrasound		Postmortem		comment
No	Sex	Age	location	size(MM)	location	size(MM)	
G6	F	>1	R. lung	55x22	R. liver	5x5	th
G6	F	>1	R. liver	26x38	R. lung	8x8	-
G6	F	>1	R. liver	31x40	L. lung	21x21	-
G7	F	>1	R. liver	25x30	L. liver	8x9	-
G7	F	>1	-	-	R. lung	15x15	-
G14	F	>1	R. liver	TNTC	R. liver	TNTC	th
G51	F	>1	L. liver	TNTC	L. liver	TNTC	-
G57	F	>1	ics	13x18	L. liver	20x18	-
G57	F	>1	ics	29x30	R. lung	40x35	-
G57	F	>1	ics 9	36x36	-	-	th
G63	F	>1	ics 10	13x26	L. lung	15x15	-
G63	F	>1	ics 12	-	R. lung	15x12	-
G65	F	>1	ics	TNTC	R. liver	TNTC	-
G71	M	>1	Visceral	19x25	R. liver	15x20	th
G126	F	>1	ics	TNTC	Ap. lobe	TNTC	th
G130	F	>1	ics10	40x43	Ap. lobe	60x60	-
G130	F	>1	ics10	29x32	R. lung	17x17	-
G130	F	>1	ics 9	20x19	R. liver	4x4	-
G130	F	>1	ics 8	19x17	L. liver	6x4	-
G138	F	>1	ics	TNTC	R. liver	TNTC	-
G231	F	>1	ics 7	12x20	R. liver	25x20	-
G236	F	>1	ics 11	45x50	L. liver	15x15	-
G236	F	>1	ics 8	19x21	R. liver	14x10	-
G237	F	>1	ics	TNTC	L. liver	TNTC	-
G238	F	>1	ics	TNTC	L. liver	TNTC	-
G241	F	>1	ics	TNTC	L. liver	TNTC	-
G245	F	>1	ics	TNTC	R. liver	TNTC	-
G248	F	>1	ics 8	12x11	L. liver	10x10	-
G248	F	>1	-	-	L. lung	10x10	-
G257	F	>1	ics 9	65x57	R.liver	65x65	-
S266	F	>1	ics 11	10x16	L. lung	40x45	-
S267	F	>1	ics	TNTC	R. liver	TNTC	-
S268	F	>1	ics	TNTC	L. liver	TNTC	-
G272	F	>1	ics 6	40x52	L. liver	40x45	th

Key:

G - goat; S - sheep; F - female; M - male; >1 - more than one year of age; ics - intercostal spaces; TNTC - too numerous to count; L. - left; R. - right; th - *Taenia hydatigena* cyst was also present

Appendix IV

Animals falsely identified as negative for hydatid cysts on ultrasound (US) examination but positive on postmortem (PM) examination

No.	Animal		Ultrasound		Postmortem	
	Sex	Age	location	size(MM)	location	size(MM)
G31	M	>1	-	-	L.lung	5x5
G35	M	>1	-	-	R.lung	20x5
G62	F	>1	-	-	L.Liver	20x15
G68	F	>1	-	-	L.lung	10x10
G72	F	>1	-	-	R.lung	30x30
			-	-	L.lung	5x5
			-	-	L.lung	20x15
G85	M	>1	-	-	L.liver	5x5
G90	M	>1	-	-	R.liver	5x5
G136	F	>1	-	-	R.liver	40x40
			-	-	L.liver	5x5
			-	-	R.lung	5x5
G139	M	>1	-	-	R.lung	30x30
			-	-	R.lung	23x23
S149	F	>1	-	-	R.liver	TNTC
G157	F	>1	-	-	L.liver	3x3
S160	F	>1	-	-	R.liver	TNTC
G175	M	>1	-	-	R.lung	20x20
G244	F	>1	-	-	R.liver	5x5
			-	-	R.lung	10x10
			-	-	L.lung	40x35
G252	F	>1	-	-	L.liver	10x12
			-	-	L.lung	35x30
G258	F	>1	-	-	R.lung	15x20
			-	-	L.lung	20x20
G270	M	>1	-	-	L.lung	60x40
G273	F	>1	-	-	R.Liver	TNTC
G276	M	>1	-	-	R.liver	20x20
			-	-	L.liver	20x20
G276	M	>1	-	-	R.lung	15x10
G277	F	>1	-	-	R.lung	40x30
G282	F	>1	-	-	R.liver	10x10

Key:
G - goat; S - sheep; F - female; M - male; >1 - more than one year of age; ics - intercostal spaces; TNTC - too numerous to count; L. - left; R. - right; th - taenia hydatigena cyst was also present

Appendix V

Animals falsely identified as positive for hydatid cysts on ultrasound (US) examination but negative on postmortem (PM) examination

Animal			Ultrasound		Postmortem		comment
No.	Sex	Age	location	size (MM)	location	size (MM)	
G24	M	>1	liver	15x22	-	-	th
G25	M	>1	liver	22x24	-	-	th
G33	F	>1	liver	28x46	-	-	-
G104	M	>1	liver	28x31	-	-	-
G192	F	>1	ics 8	23x25	-	-	-
G224	F	>1	ics 10	17x24	-	-	-
G224	F	>1	ics 11	9x12	-	-	-

Key:

G - goat; S - sheep; F - female; M - male; >1 - more than one year of age; ics - intercostal spaces; TNTC - too numerous to count; L. - left; R. - right; th - taenia hydatigena cyst was also present

Appendix VI

Postmortem findings of animals used in albendazole and oxfendazole drug trial

Albendazole				
G5	10x10	Lt lung	dead	-
	15x12	Lt lung	-	calcified
	20x15	Rt liver	dead	degenerate
	20x20	Lt liver	alive	-
G10	15x10	Lt liver	dead	-
G18	60x60	Lt lung	dead	formalin sample
	10x10	Lt lung	dead	-
	15x10	Lt lung	-	calcified
	30x40	Lt lung	dead	degenerate
	15x15	Lt lung	-	calcified
	50x40	Rt lung	alive	formalin sample
	90x70	Rt lung	alive	formalin sample
	15x20	Lt lung	alive	degenerate
	25x20	Lt lung	alive	-
	20x20	Lt lung	dead	degenerate
	15x15	Rt lung	alive	-
G23	20x15	Rt lung	alive	-
	10x15	Rt lung	dead	-
	10x10	Rt lung	alive	-
	20x15	Rt liver	alive	-
	10x10	Rt liver	-	calcified
	5x5	Rt liver	-	calcified
	30x30	Rt lung	dead	degenerate
G29				
Oxfendazole				
G9	20x20	Diaphragm	dead	degenerate
	5x5	Rt. liver	-	calcified
G14	50x60	Rt. liver	alive	formalin sample
	40x40	Spleen	dead	-
G21	15x10	Lt liver	dead	degenerate
	10x10	LN line	dead	-
	5x10	Rt. liver	dead	-
G24	40x40	Lt liver	dead	degenerate
	14x20	Rt lung	dead	degenerate
	10x20	Lt lung	-	calcified
G30	30x25	Rt liver	dead	degenerate
	10x10	Lt liver	-	calcified
	10x10	Lt liver	-	calcified
	5x5	Rt liver	-	calcified
	15x10	Lt liver	-	calcified
Control				
G3	30x40	Rt. liver	alive	formalin sample
G12	10x17	Lt liver	-	calcified
	20x30	Rt. lung	alive	-
	36x40	Rt. Liver	alive	ethyl alcohol sample
G15	30x35	Rt. Liver	alive	sample taken
	20x20	Rt. Lung	alive	-
	5x4	Lt. Lung	-	calcified
G16	40x30	Rt. liver	alive	-
G26	15x15	Lt liver	-	calcified
G28	25x30	Lt lung	alive	-
	15x10	Rt lung	alive	-