SOME EPIDEMIOLOGICAL ASPECTS OF *ECHINOCOCCUS GRANULOSUS* AND ISOLATE CHARACTERIZATION IN ANIMALS IN DARFUR STATES

By

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DEDICATION

To my parents, brothers and sisters with love and gratitude

To my wife Ayeda and daughters Balsam and Banan,

I dedicate this work with appreciation and affection

A
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Above all, my thanks giving prayers go to the ALMIGHTY ALLAH for the physical and mental health he has endued and endowed me with.
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ABSTRACT

In this study, various epidemiological aspects of hydatidosis/echinococcosis caused by *Echinococcus granulosus* in camels, cattle, sheep and goats as intermediate hosts, and dogs and foxes as final hosts in Darfur Region, Western Sudan were investigated.

An abattoir survey was carried out for hydatidosis in 565 camels, 4318 cattle, 13727 sheep and 7523 goats slaughtered in the study area during October 2001 to September 2003. New data were presented on prevalence of hydatidosis in animals and humans. The highest rate of infection was found in camels (61.42%), followed by sheep (10.88%), cattle (5.23%) and goats (1.58%). The size of the cysts, the volume of the fluid they contained, the biological status of cysts, their predilection sites in different organs and the intensity of infection in these organs were investigated. Cysts collected from camels and cattle had high fertility rate (73.84 and 27.49% respectively) compared to the low fertility rate of sheep and goats cysts (9.24 and 2.63% respectively). Daughter cysts were observed in fertile cysts removed from camel and cattle lungs and livers. Cysts predilection sites in camels was the lung (65.56%) followed by the liver (34.09%), spleen (0.2%) and kidneys (0.15%), while in cattle, the predilection site was the liver (55.26%) and lungs (44.74%). In sheep and goats, the predilection site was the mesentery (83.13 and 48.69% respectively), followed by the lungs (11.71 and 28.95% respectively) and the liver (5.15 and 22.37% respectively).

The prevalence of the adult parasite (*Echinococcus granulosus*) in 548 stray dogs and 116 wild foxes in Darfur region was investigated. The prevalence rate in dogs was found to be 19.16% but none of the foxes examined was found to be infected with *Echinococcus granulosus*. This high infection rate in dogs coincided with high rate of cyst recovery from camels (61.42%) in the study area.

Experimental transmission of infection through feeding viable cysts from camels to dogs and also from camels, cattle and sheep to foxes was conducted to compare the suitability of dogs and foxes as definitive hosts of *E. granulosus*. The infection was established in all animals fed viable cysts with variable numbers of
adult worms recovered at the end of the experiment. Worms developmental characteristics in dogs and foxes that experimentally infected with hydatid material of the same source (camel) was studied. The average worm burdens in foxes (19552) was less than in dogs (28807) and the proportion of worms with gravid segments in foxes (24.5 – 31%) was also lower than in dogs (37 – 56%). The prepatent period was longer in foxes (69 – 79 days) than in dogs (46 – 55 days). No significant differences in total worm length and dimensions of scolex, hooks and suckers (P>0.05) were found between worms harvested from dogs and foxes.

This is the first record on experimental transmission of *Echinococcus granulosus* to foxes in the Sudan. The results provided evidence that foxes are potential definitive hosts for the camel strain of *E. granulosus* in the Sudan although their role in the epidemiology of hydatidosis is uncertain as none was found naturally infected.

The infectivity of *E. granulosus* of camel/dog strain to local sheep and goats, and to wild gazelles (*Gazella dorcas*) was investigated to monitor the possibility of their respective role in maintenance of the parasite cycle. The results showed that 75% sheep and 25% goats were infected with hydatid cysts. No fertile cysts were recovered from these experimental animals. All cysts encountered were either sterile, calcified or caseated. Most of the cysts in sheep and goats were found in the mesentery.

It is the first time to conduct experimental transmission of *Echinococcus granulosus* to gazelles in the Sudan. None of the two gazelles (*Gazella dorcas*) that inoculated orally with infective eggs of *E. granulosus* of camel origin raised in dogs was found to harbour hydatid cysts. This may be due to host immunity, parasite characteristics or to the small number of animals used in the experiment.

A study on molecular characterization of *Echinococcus granulosus* isolates encountered from different intermediate hosts (camel, cattle and sheep) and from different localities of Darfur region were genotyped by molecular methods. Samples of adult worms of camel, cattle and sheep origin experimentally raised in foxes were also genotyped by the same molecular
methods. Polymerase chain Reaction-Restriction Fragments Length Polymorphism (PCR – RFLP) and mitochondrial cytochrome c oxidase subunit 1 (CO1) sequencing techniques were used to determine the extent and distribution of *Echinococcus granulosus* genetic variation in Darfur region.

The findings of the study indicated that camel strain (G6) is prevalent in Darfur region and was identified in camels, cattle and sheep. Other strains of the parasite were not recorded in this study.
CHAPTER ONE

1.1 INTRODUCTION

Cystic echinococcosis or hydatidosis is an important cyclo-zoonotic parasitic disease caused by a specific tapeworm, *Echinococcus granulosus*. The parasite cycles in a predator/prey relationship between carnivore (definitive) hosts and herbivore (intermediate) hosts. Humans may become infected with hydatid cysts if they inadvertently ingest tapeworm eggs passed in faeces of infected carnivores. Cystic echinococcosis or hydatidosis refers to the existence of the larval cystic stages (hydatid cysts) in normal intermediate hosts, however, the tapeworm stage in the definitive host is referred to as an *Echinococcus granulosus* infection or some variant of that wording (Andersen, 1997).

The disease occurs in all major continents of the world and in some areas it ranks as the leading disease of public health significance (Schantz *et al*., 1995). The disease is particularly important in developing countries where many rural inhabitants live under poor sanitary conditions and in close proximity to their domestic animals, and where large stray dog population, uncontrolled slaughter of meat animals and poor meat hygiene practices exist (Andersen, 1997). In the Sudan studies on cystic echinococcosis in domesticated animals were carried out by various workers (Eisa *et al*., 1962; El Khawad *et al*., 1976; Saad and Magzoub, 1989 a and 1989b; Mohammed and Elmalik, 2000; Elmahdi, 2003). The occurrence of human hydatidosis in the Sudan was reported by Eisa *et al*. (1962), Tola (1987) and Elmahdi (2003).
The epidemiology of the disease is based on two cycles, pastoral (synanthropic) in which the dog is always involved, and sylvatic cycle, which occurs in wild canids and is based on predation or carrion feeding. This cycle has particularly high importance in hunting communities where the infection may be introduced to domestic dogs (Schantz and Schwabe, 1969). The nature of the cycles between wild carnivores and domesticated ruminants, and between dogs and wild ruminants are of vital importance for effective control efforts and can be obtained only by experimental cross-infections carried out with material from different local host species and subsequently characterization studies of any parasite variants may be disclosed (McManus and Smyth, 1979). This will make an attempt to answer the question: Does infection in sylvatic animals indicate a parasite cycle, in these species or is infection in domestic animals acquired from wild animals? Recent applications of molecular techniques have provided a much more valid and discriminatory approach to the identification and characterization of parasite isolates from different host species and from various geographical locations (McManus and Rishi, 1989).

From the work done in the Sudan, the role of wild animals in spreading echinococcosis/hydatidosis to domesticated animals and human has not been investigated, therefore the objective of this study is to provide epidemiological data for subsequent formulation of control strategies by:

1. Investigation of prevalence of echinococcosis in dogs and foxes.
2. Investigation of the prevalence of hydatidosis in slaughter animals (camels, cattle, sheep and goats).

3. Clarifying the role of foxes and gazelles in the transmission of the disease by experimental cross-infections.

4. Characterization of *E. granulosus* strains from different host species in the area by molecular techniques.
1.2 LITERATURE REVIEW

1.2.1 Classification:

According to Rausch (1994a), the systematic arrangement of *Echinococcus granulosus* was accepted as follows:

Kingdom : Animalia  
Phylum     : Platyhelminths  
Class      : Eucestoda  
Order      : Taeniidea  
Suborder   : Taeniata  
Family     : Taeniidae  
Subfamily: Echinococcinae  
Genus      : *Echinococcus*  
Species    : *Echinococcus granulosus*  
Biotypes  : Northern biotype, European biotype.

1.2.2 Genus: *Echinococcus* (Rudolphi, 1801)

Speciation in the genus *Echinococcus* has been discussed by Smyth (1977). At present, four species of the genus *Echinococcus* are recognized on the basis of the standard taxonomic criterion by which cestodes are specifically distinguished. These are *Echinococcus granulosus* (Batsch, 1786), *Echinococcus, multilocularis* (Leuckart, 1863), *Echinococcus oligarthrus* (Diesing, 1863) and *Echinococcus vogeli* (Rausch and Bernstein, 1972). These four species of *Echinococcus* are differentiable in the strobilar as well as the larval stages (Rausch, 1997).

1.2.3 *Echinococcus granulosus* (Batsch, 1786):

1.2.3.1 History and world distribution:

*Echinococcus granulosus* was first described in 1786 by Batsch. The validity of this parasite as belonging to the genus *Echinococcus*
was later confirmed (Rausch, 1968). *Echinococcus granulosus* exists under natural conditions where the final host is the wolf and the metacestode occurs in various species of wild herbivores upon which wolves regularly prey. Through the domestication of various wild animals, a synanthropic cycle became established, domestic ungulates replacing their wild progenitors and the domestic dog replacing the wolf (Rausch, 1968).

*Echinococcus granulosus* has a worldwide geographic range and occurs in all continents including circumpolar, temperate, subtropical and tropical zones (Craig *et al.*, 1996; Schantz *et al.*, 1995) (Figure 1)

1.2.3.1.1 *Echinococcus granulosus* in Asia:

*Echinococcosis* or hydatidosis had been reported in most Asian countries. In Saudi Arabia, it had been reported in camels, cattle and sheep (Farah, 1987) and in baboons (Ghandour *et al.*, 1995). The disease was also reported in Kuwait (Hassounah and Behbehani, 1976) and in Iraq (Molan and Baban, 1992). In the Levant countries, canine echinococcosis and livestock hydatidosis had been reported by various authors (Kamhawi and Abdel-Hafez, 1995; Kamhawi *et al.*, 1995). Human hydatidosis was also reported in the Levant countries (Yarrow *et al.*, 1991; Amr *et al.*, 1994). In Iran: Dalimi *et al.* (2002) reported the presence of the parasite in dogs, jackals and foxes as final hosts and in sheep, goats, cattle and buffaloes as intermediate hosts. The parasite was also reported in wild sheep (*Ovis ammon orientalis*) and gazelles (*Gazella subgutturosa*) (Dar and Alkarmi, 1997). The parasite was also reported in India (Bhattacharya *et al.*, 2000) and in China (Chi *et al.*, 1990).
Fig. 1 Geographical distribution of *Echinococcus granulosus*  
(From Eckert et al., 2001)
1.2.3.1.2 *Echinococcus granulosus* in Europe:

*Echinococcus granulosus* has an uneven geographic distribution with very low prevalence rates in some of the northern and central countries, but with medium or high prevalence in regions of southern, southeastern and eastern regions of Europe. Iceland and Greenland are free of the parasite (Schantz, *et al.*, 1995). Transmission of *E. granulosus* occurs predominantly in synanthropic cycles, involving domestic dogs as definitive hosts and sheep goats, cattle, pigs and equines as intermediate hosts (Eckert *et al.*, 2001). Wolves, red foxes, wild ruminants and wild pigs had been found occasionally to be infected with *E. granulosus* in some countries, but normally do not play a significant role in disease transmission (Schantz *et al.*, 1995). Human hydatidosis had been reported in various countries (Craig *et al.*, 1996; Cebollero *et al.*, 2001). Several strains of *E. granulosus* had been identified in Europe (Thompson and McManus, 2001).

1.2.3.1.3 *Echinococcus granulosus* in the Americas:

In the United States of America, epidemiological surveys showed that hydatid cysts were detected in sheep and dogs (Andersen *et al.*, 1973). Coyotes and deer had been identified as wild reservoir hosts of the parasite in the Central Valley of California (Romano *et al.*, 1974).

In South America, the disease is endemic in Argentina, Uruguay, Brazil, Chile and mountainous areas of Peru and Bolivia (Kamenetzky *et al.*, 2002). The authors reported hydatidosis in cattle, sheep, pigs and goats and adult parasite in dogs. They also identified the sheep, Tasmanian sheep, cattle and camel strains in humans.
Sylvatic cycle in red foxes and hares was also reported (Schantz et al., 1972).

1.2.3.1.4 *E. granulosus* in Australia and New Zealand:

*E. granulosus* is widely distributed on the mainland of Australia in domestic or wild animal hosts, with great differences in prevalence rates among various regions (Jenkins and Craig, 1992; Grainger and Jenkins, 1996; Lymbery et al., 1995). The synanthropic cycle involves domestic dogs and domestic herbivores (predominantly sheep) as hosts, while the sylvatic cycle involves wild dogs, dingoes and red foxes as definitive hosts and macropod marsupials (Kangaroos) as intermediate hosts (Eckert et al., 2001). The only strain of *E. granulosus* involved in cycles of the parasite is the common sheep strain (G1) (Thompson and McManus, 2001). Human cases of cystic echinococcosis had been reported by Jenkins and Power (1996).

In 1996, the Island State of Tasmania was provisionally declared free of *Echinococcus* infection with respect to dogs and sheep, and in 1999 it was anticipated that New Zealand could be hydatid-free (Heath et al., 1999).

1.2.3.1.5 *Echinococcus granulosus* in Africa:

Echinococcosis/hydatidosis caused by *E. granulosus* had been reported in most African countries (Andersen, *et al*., 1997). Camels, sheep, goats and cattle were found infected in all countries in North Africa and are considered to be the main source of infection of *E. granulosus* for dogs, which are the main definitive hosts (Abou-Eisha *et al*., 1999; Ibrahim and Gusbi, 1997). In Sub-Saharan Africa wide variation in the prevalence of *E. granulosus* in dogs and livestock
were reported. Areas of high endemicity are known to occur in Eastern Africa including at least part of Sudan, Ethiopia, Kenya and Uganda (Macpherson and Wachira, 1997). Large parts of western central and southern Africa apparently have lower prevalence but an accurate assessment is difficult due to lack of recent and comprehensive data. The parasite is transmitted predominantly in a synanthropic cycle involving dogs and various livestock animals (camels, cattle, sheep and goats), but wildlife-cycles exist involving a number of wild carnivores and wild ruminants and pigs (Macpherson and Wachira, 1997). *E. granulosus* infections were reported in jackals (Macpherson, 1986), spotted hyaena, wild cat, cape hunting dogs, cape silver fox and lions (Verster and Collins, 1996; Young, 1975). Various prevalence rates in wild intermediate hosts species were reported from many African countries south of the Sahara (Macpherson *et al*., 1983).

Cystic echinococcosis in humans continues to be a significant public health problem in most African countries (Macpherson, *et al*., 1989b; Shambesh, 1997).

Several strains of *E. granulosus* (sheep, cattle, camel, horse and lion strains) had been identified in various parts of Africa (Macpherson and Wachira, 1997).

1.2.3.1.6 *Echinococcosis/hydatidosis in the Sudan:*

In the Sudan, echinococcosis is caused by *E. granulosus*. The adult parasite is found in the dog and the larval stage is found in camels, cattle, sheep, goats and man.

Studies on animal echinococcosis in the Sudan were carried out by various workers. Abdel Malek (1959) made a list of the parasites of
domesticated animals in the Sudan. The list included a single case of bovine liver hydatidosis in Kosti, 3 cases of lung, liver and spleen cysts in camels in Khartoum and Omdurman and 4 cases of *E. granulosus* in the small intestines of dogs in Khartoum and Kosti. Eisa *et al.* (1962) reported prevalence rates in dogs of 86.5% in Kopoeta District and 26.6% in Torit District, while none of the dogs examined in Pibor District of Upper Nile Province were found to harbour *E. granulosus*. The prevalence rates of 25, 19.4 and 33.3% in cattle, sheep and goats respectively were reported in Equatoria and Upper Nile Provinces (Eisa *et al.*, 1962). El Khawad *et al.* (1976) recorded the prevalence rates of 6.2 and 9.3% in cattle and goats respectively in Equatoria Province and 7.6% in cattle in Bahr-el-Ghazal. In Khartoum, Eisa *et al.* (1977) found 6.06% of 33 dogs examined harbouring *E. granulosus*. The numbers of parasites they collected ranged between 34 and 3200 per animal. El Khawad *et al.* (1978) found that 24.8, 12.2 and 10.0% of cattle, sheep and goats respectively, slaughtered in Western Sudan were infected with hydatid cysts. El Khawad *et al.* (1979) carried out a survey in the central region of the Sudan and reported the prevalence rates in camels, sheep, cattle and goats as 35.3, 8.16, 4.28 and 3.17% respectively and 3.03% in dogs in Omdurman town. Among 141 camels slaughtered at Tampool area, 64 (45.4%) were found to harbour hydatid cysts (Saad *et al.*, 1983). Idris (1985) reported that the incidence rate in dogs in Khartoum Province was 17.51%. Saad and Magzoub (1986) carried out a survey of adult parasites in 50 stray dogs in Tampool (Butana area) and they found the parasite in 51% of the examined dogs where they reported 24800 worms in one dog. Tola (1987) investigated hydatidosis of camels, cattle and sheep in Butana, Khartoum and
Sudanese camels exported to Egypt. He reported that the average prevalence rate in camels, cattle and sheep was 56.4, 2.1 and 2.0% respectively. Saad and Magzoub (1989a and 1989b) conducted a survey of hydatidosis in areas representing all regions of the Sudan except the south. The overall prevalence rates they reported were 48.69, 3.84, 12.9 and 4.4% in camels, cattle, sheep and goats respectively. They observed that with the exception of two, all cysts encountered in sheep and goats were calcified or semi-calcified, while 42.4% of the examined cysts from camels were fertile and the fertility of those from cattle were exceptionally high (29%). They also observed that the liver was the preferred site for the cysts in cattle. High prevalence rate of 67.74% was reported in camels in El Obied (Saad et al., 1989). In Eldamer Province, Elhussien and Ali (1990) reported that the prevalence rate in camels was 37%. El Sawi (1994) found that 8.9% and 4.21% of 1362 sheep and 164 goats respectively slaughtered at Omdurman Central Abattoir were infected with hydatid cysts. El Sawi and Saad (1995) found that 43.9% of camels slaughtered at Omduram Slaughterhouse were infected with hydatid cysts. Elansary and Hamad (1997) examined 400 head of sheep in Kassala slaughterhouse and reported that 122 (30.5%) were infected with hydatid cysts. They reported a high fertility rate (26.2%) of the cysts encountered. Mohammed and Elmalik (2000) carried out a survey in Nyala town. They reported prevalence rates of hydatidosis in camels and cattle slaughtered at Nyala Slaughterhouse as 79.51 and 6.42% respectively. They also examined 26 stray dogs and reported that 26.92% showed *E. granulosus* infection. Elmahdi (2003) carried out an abattoir survey for hydatidosis in camels, cattle and sheep in
Omdurman, Tampool and Medani. He reported the highest rate of infection in camels (45%), followed by sheep (7%) and cattle (3%).

The occurrence of human hydatidosis in the Sudan was first reported by Christopherson in 1909 (Saad, 1985). He gave his experience of 7 years service in the Sudan where he came across 6 cases. Two of the patients were Egyptians who got the disease from Egypt. The other 4 cases originated in the Sudan. Eisa et al. (1962) reported the occurrence of hydatidosis as an endemic disease amongst Taposa tribe of Kapoeta District of the Equatoria Province of Southern Sudan. They showed that several operations for removal of hydatid cysts were performed at Kapoeta and Torit hospitals. Cahill et al. (1965) conducted a serological study in Southern Sudan and reported 13.1% positive cases, hence they reported that the disease was not uncommon in the south. Ower and Bitakarmine (1975) reported that about half of the human cases of the disease, which occur in Uganda, were immigrants from Southern Sudan. Tola (1987) observed a prevalence rate of about 1.2% in human population in Khartoum Province. Community-based mass ultrasound surveys in Southern Sudan showed high hydatidosis prevalence in nomadic people living in those areas (Macpherson et al., 1989b). Studies conducted in Southern Sudan reported a prevalence ranging from 0.5% to 3.5% in humans (Magambo et al., 1996). Elmahdi (2003) conducted an ultrasound survey of 300 subject by a portable scanner. He diagnosed a single case of human hydatidosis in central Sudan.

1.2.3.2 The biology of Echinococcus granulosus:

The adult worm (3 – 6 mm long) inhabits the small intestines of carnivore definitive hosts such as dogs, wolves, jackals or foxes; and
the metacestode (hydatid cyst) occurs in various domestic ungulates (camels, cattle, sheep, goats, pigs and horses) and wild ungulates (wildebeest, buffalo, grant gazelles, zebra, giraffe and warthog) in some parts of the world (Thompson, 1979). The adult worm possesses 3 – 4 proglottids. The terminal one is gravid and usually about half the length of the worm. The scolex bears four muscular suckers and two rows of hooks on the rostellum. The hydatid cyst is a fluid-filled unilocular bladder with an outer acellular laminated layer and an inner nucleated germinal layer, from which fluid is secreted and brood capsules containing protoscolices develop. The brood capsules may become detached and float freely in the fluid of the cysts, being called hydatid sands (Soulsby 1982). Occasionally daughter cysts develop within the hydatid cyst. The size of the cysts and number of protoscolices they contain vary with host and organ involved (Gemmell et al., 1987a). The eggs are typical taeniid eggs and are readily infective when passed in the faeces of infected definitive hosts. The eggs are highly resistant to environmental factors but are sensitive to desiccation and high temperatures (Laws, 1968).

1.2.3.3 The life-cycle:

The life-cycle of *Echinococcus granulatus* takes place in a similar fashion to that of many other tapeworms that have a predator/prey relationship. Eggs are passed in the faeces of an infected definitive host and may subsequently be ingested by a grazing intermediate host, hatch into embryos in the intestine, penetrate the intestinal lining, and are then picked up and carried by blood throughout the body to major filtering organs; mainly liver and/or lungs although other organs may be involved (Heath, 1971). After the
developing embryos localize in a specific organ or site, they transform and develop into larval hydatid cysts in which protoscolices are produced via asexual reproduction. The hydatid cyst stage requires one year’s time to grow sufficiently and produce protoscolices capable of infecting the carnivore host (Smyth, 1964). The definitive hosts acquire the infection by ingesting hydatid cysts when an infected intermediate host is slaughtered, died or preyed. Protoscolices attach to intestinal lining and develop into mature adult tapeworm in about 40 – 45 days post infection depending on parasite strain and various host factors (Thompson, 1995). Man becomes infected by ingesting tapeworm eggs passed from an infected carnivore. This occurs most frequently when individuals handle infected carnivores or inadvertently ingest food such as uncooked vegetables or drink water contaminated with faecal material containing tapeworm eggs (Figure 2).

1.2.3.4 The epidemiology:

The adaptability of *E. granulosus* to a wide variety of host species and the repeated introduction of domestic animals from some parts of the world to others has resulted in the present broad cosmopolitan distribution of the parasite in all major climates. Its life cycle is complex involving two hosts and a free-living egg stage. The dynamics of transmission of the parasite are determined by the interaction of factors associated with these two hosts, the external environment and socio-ecological factors.

Intraspecific variations, with differences in infectivity to both definitive and intermediate hosts and differences in other biological properties of the parasite are of fundamental importance in
Fig. 2  Schematic life-cycle of *Echinococcus granulosus* showing synanthropic and sylvatic (dotted lines) cycles (Adapted from Andersen et al., 1997).
determining the epidemiology of the parasite (Gemmell, et al., 2001). It is customary to consider the epidemiology as being based on two cycles, pastoral and sylvatic. In the pastoral cycle, the dog is always involved, being infected by feeding on ruminants’ offals containing hydatid cysts. The domestic intermediate host will vary according to the local husbandry. This cycle is the primary source of human hydatidosis, the infection being by accidental ingestion of oncospheres from coats of dogs or from food or water contaminated by dog faeces. The sylvatic cycle occurs in wild canids and wild ungulates and is based on predation or carrion feeding. This cycle is less important as a source of human infection, except in hunting communities where the infection may be introduced to domestic dogs by the feeding of infected viscera of wild ruminants (Schantz and Schwabe, 1969).

At any one time the parasite population consists of 3 sub-populations: adult in the definitive host, larvae in the intermediate host, and eggs in the environment.

1.2.3.4.1 The adult worms in the definitive host:

1.2.3.4.1.1 Domestic definitive hosts:

Although adult E. granulosus worms occur in a number of different species of carnivores in Africa south of Sahara, the domestic dog is undoubtedly the most important definitive host in the domestic cycle (Eisa, et al., 1962). The prevalence of E. granulosus infection in dogs is an index of the extent of infection in a local area and of the relative degree of risk of human infection. Primarily the number of protoscolices ingested determines the number of worms harboured by the dog.
Considerable knowledge has now been gained concerning the protective immune response to adult and larval tapeworm infections (Rickard and Williams, 1982). Dogs by their lingual-anal grooming habits have abundant access to tapeworm eggs but appear only to acquire immunity to *E. granulosus* from the ingestion of protoscolices (Gemmell, 1990). This immunity is weak and develops slowly (Jenkins and Rickard, 1985). The heterogeneity of susceptibility of dogs to infection with *E. granulosus* affects the number of worms that establish in different hosts and their sizes (Jenkins and Rickard, 1986). The number of eggs produced by each segment ranges between 100 and 1500 (Wachira *et al.*, 1991).

Other definitive hosts shown to interact with the domestic cycle of the parasite include silver-backed jackals, golden jackals and cape hunting dogs (Verster and Collins, 1966; Macpherson *et al.*, 1983; Macpherson, 1986).

### 1.2.3.4.1.2 Wild definitive hosts:

The first record of wild carnivores as definitive hosts of *E. granulosus* infections in Africa was made by Ortlepp (1937). He found the parasite in the cape hunting dog (*Lycaon pictus*) and lion (*Panthera leo*). The wildlife cycles of *E. granulosus* exist between these animals and their prey species in certain areas.

The cape hunting dog has been found to be a definitive host in the wild (Nelson and Rausch, 1963; Verster and Collins, 1966) and under experimental infection with material of sheep and cattle origin (Verster, 1965). The discovery of adult *Echinococcus* in a lion prompted Ortlepp (1937) to describe a new species of the parasite for this host, *E. felidis*, subsequently redesignated by Verster (1965) as
the subspecies, *E. granulosus felidis*. Rausch (1967) regarded *E. granulosus felidis* as synonymous with *E. granulosus*. Other records of infected lions were made by many workers in Africa (Young, 1975; Dinnik and Sachs, 1972). Macpherson et al. (1984) had suggested that the form of *E. granulosus* in the lion and its prey species be regarded as a distinct strain in which the lion is the definitive host, and zebra, wildebeest, warthog, buffalo and occasionally other antelopes act as intermediate hosts. The parasite has subsequently been found to occur in the golden jackal (*Canis aureus*), the silver-backed jackal (*canis mesomelas*), the cape silver fox (*Vulpes chama*), spotted hyaena (*Crocuta crocuta*) and African wild cat (*Felis lybica*) in different African countries (Verster and Collins, 1966; Macpherson *et al.*, 1983; Macpherson, 1986). Infections by *E. granulosus* in foxes of the genus *Dusicyon* had been reported from different localities in Argentina (Schantz *et al.*, 1972). Jones and Walters (1992) carried out a survey of wild foxes in mid-Wales and they found only two of 197 animals examined to be infected. Dingoes were found to be the major definitive hosts in Southern Queensland, Australia (Bladock *et al.*, 1985).

In addition to these natural infections, several experimental infections of wild carnivores had been undertaken. Experimental infection of lions with material from zebra has resulted in successful infections (Young, 1975). The silver-backed jackal has been found to be readily infected experimentally with hydatid material of human, cattle and sheep origin (Macpherson, *et al.*, 1983). Experimentally *E. granulosus* of sheep origin from Australia and several European countries had been shown to attain sexual maturity and produced
shelled eggs in the European red fox (Vulpes vulpes; Thompson, 1983). South American red fox of the genus Dusicyon was also found to be infected experimentally with E. granulosus of Argentine sheep origin and development of the worms and egg infectivity were compared favourably to that of dogs (Schantz, et al., 1976).

In some studies the foxes used experimentally had represented different geographic populations and different subspecies therefore lacking genetic uniformity (Thompson, 1977). Red foxes in Great Britain have been successfully infected experimentally (Cook, 1989), as have those of British origin in Australia (Thompson, 1983), but also in Great Britain, Clarkson and Walters (1991) found that foxes were poor final hosts for E. granulosus, and they observed as well that breeds of dogs differed in degree of susceptibility to infection. The spotted hyaena was found to be non-susceptible to experimental infection, whereas material from the same source was highly infective to dogs and sliver-backed jackals (Macpherson et al., 1983).

1.2.3.4.2 The larva in the intermediate host:

The intensity, infectivity and availability of the eggs in the environment, local circumstances of livestock husbandry, feeding behaviour of the intermediate host, and the slaughter policy together determine the number of infective organism entering the host (Gemmell, 1976). However, the number of these that become established is strictly controlled by the host natural and acquired resistance to infection. According to Varela-Diaz et al. (1974), natural resistance causes parasite mortality at all stages during the parasite development and its extent may be related to the parasite strain and physiological condition of the host. They reported that hydatid cyst
may only produce a low level of antigenic stimulation, perhaps insufficient to induce a host response adverse to cyst survival, but strong immunity was induced following parenteral (intramuscular) injection of lambs with artificially activated embryos of the parasite and a significant reduction in the total cyst counts and absence of viable cysts from the challenge infection was observed. This immunity can be maintained throughout the life of the host by continuous ingestion of eggs but may wane within 6 – 12 months in the absence of reinfection (Gemmell and Johnstone, 1981). Little immunity can be transferred from mother to offspring through colostrum (Sutton, 1979), but without significance in the field unless the sheep were in a hyperendemic area (Dempster and Harrison, 1995). *E. granulosus*, has become adapted to a large variety of both wild and domestic intermediate host species distributed all over the world (Macpherson and Wachira, 1997).

**1.2.3.4.2.1 Domestic intermediate hosts:**

In many parts of the world, *E. granulosus*, is perpetuated predominantly by a domestic cycle involving an array of livestock species which include cattle, camels, sheep, goats, pigs, donkeys and horses. Regional foci of infection seem to be defined by lifestyle rather than livestock distribution (Macpherson *et al.*, 1989b). In Africa, hydatid infection rates in livestock have been observed to vary depending on farming practices (Verster and Collins, 1966). Countries with known hyperendemic infections in Sub-Saharan Africa include Kenya, Nigeria, Somalia, Sudan, Swaziland and Uganda (FAO, 1993). Both susceptibility to infection and cyst fertility rates are essential factors in determining the importance of different intermediate hosts.
to local maintenance of *E. granulosus*. The susceptibility of cattle to infection is variable (Eisa, *et al.*, 1962; Macpherson, 1985). Where camels are kept, more than half their population is infected and levels of infection in camels are much higher in relation to other domestic intermediate hosts (Macpherson and Wachira, 1997). Hydatid cysts in camels, goats and sheep are usually fertile and the three hosts appear to be the most important intermediate hosts of *E. granulosus*, in Sub-Saharan Africa (Macpherson, 1985). However, Saad and Magzoub (1989b) reported that most cysts encountered in sheep and goats were calcified or semi-calcified. The role of pigs as intermediate hosts of *E. granulosus*, is limited by the common farming practice of raising them indoors, which reduces contact with the definitive hosts (Verster and Collins, 1966). Donkeys and probable all equines are poorly susceptible to infection with *E. granulosus* in Africa (Dada, *et al.*, 1981).

1.2.3.4.2.2 Wild intermediate hosts:

It was not until 1962 that the first wild intermediate hosts were reported in Africa. Verster (1962) recorded hydatid cysts in the cape molerat (*Georynchus capensis*), warthog (*Phacochoerus aethiopicus*) and wildebeest (*Connochaetes taurinus*). Since then, large numbers of wild intermediate hosts species have been identified. Most of these infections were reported in animals, which lived in national parks or game conservation areas (Dinnik and Sachs, 1969). The most commonly reported wild intermediate hosts in many African countries include buffalo (*Syncerus caffer*), warthog, wildebeest, zebra (*Equus burchelli*), Impala (*Aepyceros melampus*), Giraffe (*Girrafa spp.*), grant’s gazelle (*Gazella granti*), blue duiker (*Cephalophus monticolae*),
water buck (*Kobus* spp.), topi (*Damaliscus korrigum*), Puku (*Adenota wardonji*), Oryx (*Oryx algazal*), hartebeest (*Alcelaphus buselaphus cokii*), hippopotamus (*Hippopotamus amphibius*), Kudu (*Tragelaphus strepsiceros*), Colobus monkeys (*Colobus* spp.) and baboon (*Papio* sp.) (Verster, 1962; Woodford and Sachs, 1973; Macpherson *et al*., 1983; Young, 1975). Cysts are generally fertile in these hosts, and wildlife cycles are thought to occur in a number of national parks in African countries (Krecek *et al*., 1990). Wild intermediate hosts were also reported in other parts of the world (Ghandour *et al*., 1995; Dar and Alkarmi, 1997).

**1.2.3.4.3 Domestic: Wildlife interactions:**

The introduction of commercial game ranching of wild herbivores in many African countries, mainly to satisfy the appetite of tourists for exotic meats and for the sport of hunting have provided opportunities for dogs to be exposed to hydatid cysts from intermediate hosts (Schantz and Schwabe, 1969). Dogs infected with the domestic strains of *E. granulosus* may contaminate the grasslands and range lands that livestock and wildlife share, particularly in East Africa. In this region transhumance pastoralists live in close proximity to wild animals that share the same habitat with domestic ones, thereby facilitating the transmission of a large number of diseases including echinococciosis (Macpherson, 1994). More than six species of carnivores have been found infected with *E. granulosus* (Macpherson, 1986). It is believed that the source of infection to wild carnivores is from predation or the scavenged carcasses of infected domestic livestock (Macpherson *et al*., 1984). These wild canids may contaminate the grasslands and range lands that livestock and
wildlife share, but their significance in the domestic life cycle of the parasite is unknown.

1.2.3.4.4 Human: wildlife interaction:

The interaction of human and wildlife is largely restricted to poachers, hunters, those people who have settled along the borders of the conservation areas, and, to a lesser extent, tourists who visit the national parks in ever-increasing numbers (Macpherson et al., 1983). The Turkana (Kenya) will, on occasion, eat jackals and hyaenas; the handling of these animals coupled with poor hygiene, the high E. granulosus infection rates in jackals, and lack of knowledge about the disease may place such people at risk of exposure to E. granulosus infection from these wild carnivores (Macpherson et al., 1983). Many nomadic and pastoral groups in Sub-Saharan Africa do not bury their dead, and human may provide a source of infection for scavenging wild carnivores and dogs. This demonstrates an epidemiological pattern of transmission such as dog-human-dog cycle in Turkana District of Kenya (Macpherson 1983; Macpherson et al., 1983). Additionally, protoscolices from cysts removed from human have been found to be infective to silver-backed jackals (Macpherson et al., 1983).

1.2.3.4.5 The eggs in the environment:

The crowding of animals during grazing on contaminated soil and the extent to which soil is contaminated by dogs faeces are important environmental factors (FAO/UNEP/WHO, 1981). Local meteorological factors affect the survival of eggs as well as the activity of the agents concerned in egg dispersal and availability (Wachira, et al., 1991). Desiccation is lethal (Laws, 1968) and the end
points of temperature are of the order of +4°C to –70°C (Gemmell, 1990). The eggs of *E. granulosus* survived for more than 200 days at 7°C, 50 days at 21°C, but less than a week at 40°C (Gemmell, 1977). Agents responsible for egg dispersion into the environment have not yet been fully identified but suggested agents include wind, birds, rainfall, arthropods, and earthworms, as well as animal feet (Gemmell, 1997).

Experimental evidence is now available that blow flies (Calliphoridae) are important transport hosts for the eggs of *Taenia hydatigena*, *T. ovis*, and presumably *E. granulosus* and *E. multilocularis* (Lawson and Gemmell, 1990).

### 1.2.3.4.6 Dynamics of transmission

The perpetuation of echinococcosis depends upon the common presence of the parasite and the definitive and intermediate hosts. The continued existence of host and parasite populations depends upon the fine balance of various interacting regulatory forces (Anderson and May, 1978). Studies on dynamics of host/parasite systems had indicated that such characteristics as over dispersion of parasite numbers within the host population and the development of host immunity act as important stabilizing factors (Anderson and May, 1978). Factors contributing to the dynamics of transmission include intrinsic, extrinsic and socio-ecological factors (Roberts *et al.*, 1986).

#### 1.2.3.4.6.1 Parasite contribution to the dynamics of transmission:

**1.2.3.4.6.1.1 The biotic potential:**

The parasite’s major contribution to the dynamics of transmission is its biotic potential (Gemmell *et al.*, 1986a). The biotic
potential and hence the transmission dynamics may vary widely in different ecological and climatic zones (Gemmell, *et al.*, 1987a). They also recorded that *E. granulosus* has about 1/100 and 1/30 the biotic potential of *Taenia hydatigena* and *T. ovis* respectively.

1.2.3.4.6.1.2 Host specificity:

The ability of taeniids to establish themselves and develop normally in the final host is influenced by the genetic characteristics of the carnivore as well as of the cestode (Rausch, 1967). Some strains exhibit a marked degree of host specificity; that the number of worms is related to the rate of development in a particular host species (Verter, 1965). *E. granulosus* has remarkably low intermediate host specificity; that hydatid cysts have been described in man, camels, cattle, sheep, goats, equines, pigs, and a large number of wild intermediate hosts (Macpherson, 1985).

1.2.3.4.6.1.3 Strains of *E. granulosus*

The essence of the term “strain” as applied to *Echinococcus* refers to intraspecific grouping of a genetically distinguishable isolates which share significant biological features pertaining to the epidemiology or control of hydatidosis (Thompson and Lymbery, 1988). The criteria used in identification of strains from various animal hosts take into consideration morphological, biological, biochemical, epidemiological and developmental features *in vivo* and *in vitro*, pathological and serological differences, and recently the characteristics of the genome using molecular techniques for DNA analysis (Bowles and McManus, 1993a, b, c; Thompson *et al.*, 1995; Eckert and Thompson, 1997). The larval stage amplification, associated with high rates of self-fertilization of adults and with the
natural selection excreted by the hosts, may have strong impact on *E. granulosus* genetic differentiation (Kamenetzky *et al.*, 2002). Morphological and biological studies have provided extremely useful information for strain identification, but they may be influenced by host and environmental factors and may not necessarily reflect distinctness at the genetic level (Bowles and McManus, 1993c). Currently, nine genetically distinct population (genotypes) assigned G₁ to G₉ which differ in host range, developing rate, infectivity to humans, pathogenicity and antigenicity had been identified in *E. granulosus* (Thompson *et al.*, 1995; Thompson and McManus, 2001). All these genotypes appear to be adapted to a particular life-cycle pattern and host assemblage (Scott *et al.*, 1997). The known strains of *E. granulosus* in the world based on review articles by Schantz *et al.* (1995), Eckert and Thompson (1997) and Eckert *et al.* (2001) include sheep strain (G₁), Tasmanian sheep strain (G₂), Buffalo strain (G₃), Horse strain (G₄), cattle strain (G₅), camel strain (G₆), pig strains (G₇, G₉), cervid strain (G₈) and lion strain (Table 1). At least five of these strains are thought to exist in Sub-Saharan Africa (Wachira *et al.*, 1993b). These include the sheep, cattle, camel, horse and lion strains.

1.2.3.4.6.1.3.1 Sheep strain (G₁):

The sheep strain is globally the most widespread of *E. granulosus* strains (Schantz *et al.*, 1995). It has special significance in parts of South America, Southern and Eastern Europe, Northern and Eastern Africa, and parts of Asia and Australian region. Several studies have shown that the sheep strain of *E. granulosus* differs morphologically and biologically from other strains (Thompson *et al.*, 1995).
<table>
<thead>
<tr>
<th>Strain</th>
<th>Africa</th>
<th><em>Zebra, wildebeest, wart hog, lion, Jackal, Cape</em></th>
<th><em>Buffalo, antelopes, camel, goat, cattle, sheep</em></th>
<th><em>Sheep, cattle, pigs, camels, macropods, man</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lion</strong></td>
<td>?</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Cervid</strong></td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Pig</strong></td>
<td>Yes</td>
<td>Low or no</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Cattle</strong></td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Low or no</td>
</tr>
<tr>
<td><strong>Horse</strong></td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Buffalo</strong></td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Intermediate hosts</strong></td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

**Geographic Distribution**

*Adapted from Thompson, 1995; Thompson and McManus, 2001: 2002.*
Molecular studies have confirmed the genetic uniformity of this strain from a range of endemic areas (McManus and Rishi, 1989; Bowles and McManus, 1993a, b, c; Kamenetzky et al., 2002). Dog, fox, dingo, wolf, jackal and hyaena were the known final hosts (Rausch, 1995; Thompson, et al., 1995). The intermediate host range of this strain is not restricted to sheep, but may also include goat, cattle, buffalo, yak, camel, pig, and macropodes (Thompson et al., 1995; Eckert and Thompson, 1997). Epidemiological evidence confirmed by isoenzyme and molecular studies have indicated that the sheep strain is infective to humans (Bowles and McManus, 1993a, c; Kamenetzky et al., 2002). Humans may play an active intermediate host role in maintenance of the sheep strain in some endemic regions of Africa where human corpses are rarely buried, such as what occurs in Turkana, Kenya (Macpherson, 1983).

1.2.3.4.6.1.3.2 Tasmanian sheep strain (G2):

A sheep strain occurring in Tasmania involves in its cycle dogs and possibly foxes as final hosts and sheep as intermediate hosts. Morphological, biological and recent molecular studies support its recognition as a distinct strain (Thompson et al., 1995). This strain was identified in cattle and human in Argentina by molecular studies (Kamentzky et al., 2002).

1.2.3.4.6.1.3.3 Buffalo strain (G3):

Buffalo plays an important role as intermediate hosts of *E. granulosus* in many Asian countries. Molecular studies had shown that buffaloes are susceptible both to the sheep and the cattle strains of *E. granulosus* (Bowles and McManus, 1993a). Whether or not some special morphological, biological and genetic features observed in
previous studies of *E. granulosus* isolates of buffalo origin are indicators for strain distinctness remains to be determined (Thompson *et al.*, 1995).

### 1.2.3.4.6.1.3.4 Horse strain *(G₄)*:

The horse strain of *E. granulosus* previously described as a subspecies *E. granulosus equinus*. This strain occurs in several countries of Europe, in the Middle East, South Africa and New Zealand (Eckert and Thompson, 1997). Thompson and McManus (2002) suggested the elevation of this strain to species level and be designated as *E. equinus*. Comparative studies of isolates from various European countries, South Africa and New Zealand demonstrated the morphological uniformity of this strain (Kumaratilake *et al.*, 1986). The genetic distinctness was confirmed in independent studies using different techniques (Scott and McManus, 1994; Siles-Lucas *et al.*, 1994). The final host range of this strain includes dog, lion, jackal and cape hunting dog (Macpherson, 1986). The intermediate host range is narrow and includes only horse and other equines (Thompson *et al.*, 1995). There is evidence that this strain has low or no infectivity in its larval stage to sheep, cattle and man (Eckert and Thompson, 1988; Thompson and Lymbery, 1988).

### 1.2.3.4.6.1.3.5 Cattle strain *(G₅)*:

A cattle strain of *E. granulosus* is known to occur in various Central European countries, in Russia, South Africa, India, Sri Lanka and Argentina (Eckert and Thompson, 1997; Kamenetzky *et al.*, 2002). The only known definitive and intermediate hosts of epidemiological significance are dogs and cattle respectively. This strain differs from other *E. granulosus* strains in morphology and also
in biological features (Thompson et al., 1984). The distinctness of this strain has been confirmed by identification of peculiar and uniform genetic characteristics (Bowles and McManus, 1993a, c; Kamenetzky et al., 2002). This strain was confirmed to be infective to humans (Kamenetzky et al., 2002).

Recently, cattle strain was proposed to be elevated to species level designated as *E. ortleppi* (Thompson and McManus, 2002).

**1.2.3.4.6.1.3.6 Camel strain (G₆):**

*Echinococcus granulosus* obtained from African camels exhibited rapid development in dogs and showed various distinct morphological features. Morphologically the isolate from the camel could be readily distinguished from isolates of horse and sheep but certain similarities existed with the cattle strain (Eckert et al., 1989). Molecular characterization of *E. granulosus* isolates from camels originating from the Middle East, Kenya, Sudan and Somalia have confirmed their genetic distinctness on the one hand and their genetic heterogeneity from the cattle and sheep strains on the other (Bowles and McManus, 1993c; Wachira et al., 1993b). Dogs are proven final hosts for the camel strain of *E. granulosus* (Eckert et al., 1989) and camels, goats and cattle were reported as intermediate hosts (Wachira et al., 1993b; Kamenetzy et al., 2002). Molecular investigations of human cases of cystic hydatid disease from various countries showed that the camel strain is infective for humans (Zhang et al., 2000; Kamenetzky et al., 2002).

**1.2.3.4.6.1.3.7 Pig strains (G₇, G₉):**

The pig strain (G₇) of *E. granulosus* was known to occur in various countries (Schantz et al., 1995; Kamenetzky et al., 2002). The
isolate from Poland has unique DNA characteristics and could be distinguished from isolates of sheep, cattle, horse and camel origin (Siles-Lucas et al., 1994). The dog is the natural final host (Eckert and Thompson, 1988). *E. granulosus* eggs of the pig strain (G7) were highly infective for pigs but had a much lower infectivity for lambs and calves (Eckert and Thompson, 1988). There was little information on the potential infectivity of the pig strain for humans (Pawlowski et al., 1993). The finding of Scott et al. (1997) had shown that *E. granulosus* infecting Polish patients shared close molecular affinity with a genotype (G7) of pig origin but exhibited some clear differences. Therefore, it was designated as a new genotype (G9). They suggested that two pig genotypes may exist.

**1.2.3.4.6.1.3.8 Cervid strain (G8):**

A cervid strain of *E. granulosus* has a wide distribution in holarctic zones and also occurs under favourable conditions at lower latitudes in North America and Eurasia (Rausch, 1995). The natural cycle is predominantly perpetuated by the predator prey relationship between the wolf and ungulates of the family Cervidae (elk, reindeer, and red deer; Rausch, 1995). Epidemiological evidence suggested that this form was infective to humans but with low pathogenicity (Eckert and Thompson, 1988; Rausch, 1995).

**1.2.3.4.6.3.1.3.9 Lion strain:**

In East Africa strobilar stages of *E. granulosus* have been found in wild canids (hunting dogs, hyaena, jackals) and in a felid, the lion, and the metacestodes occur in several species of wild ungulates including warthog, zebra, buffalo, and wildebeest (Macpherson, 1986; Rausch, 1995). Special morphological features of *Echinococcus*
granulosus from lions and the existence of a wildlife cycle suggest that a distinct strain may be involved (Eckert and Thompson, 1997), but no detailed genetic characterization; at present separated on the basis of morphological, biological and epidemiological features (Thompson and McManus, 2001).

1.2.3.4.6.2 Socio-economic and cultural factors:

The distribution of E. granulosus is influenced by economic, agricultural, educational levels, social or cultural factors, but it usually relates most directly with the degree of association individuals have with their domestic livestock (Schantz et al., 1995).

Many factors including human behaviour, host, environmental contamination, livestock management, and slaughtering practices influence the prevalence of cystic echinococcosis (Macpherson, 1994). Ignorance about transmission factors by population at risk, prevailing lifestyle with poor sanitation, and lack of clean culinary water increase the risk of human infection (Gemmell, 1997). Schwabe and Abou-Daoud (1961) reported high incidence in Lebanese shoemakers who tan leather with dog faeces. They also noticed the much higher prevalence in Christians than Muslims in the Middle East because of the different attitudes to dogs. Home slaughtering of animals at times of religious or social festivals help to maintain a highly infected dog population and children mostly play freely with dogs, which facilitates early transmission of the disease (Andersen et al., 1997).

In the Turkana district of Kenya, several interacting factors contribute to the high rate of infection in the people. Of particular importance are the specially trained dogs that are kept by women
during child-rearing period to clean the face or anal area of small children when they soil themselves, and to dispose of vomitus and faeces expelled by the children within the dwellings (Macpherson, 1983). The Turkana might become infected by eating the intestines of dogs or other carnivores (Macpherson, 1983). Women may also come into contact with dog faeces when they decorate their bodies with a mixture of mud and ochre which may be contaminated with dog faeces, or by their use of dog faeces in the lubricant which is used to prevent damage to the skin of the neck and shoulders by the massive weight of necklaces that the women accumulate year by year (French and Nelson, 1982).

They also reported that one of the factors which accentuates the risk for human infection is the use of dog faeces as medicaments in the preparation of dressings on cuts and septic sore, and for the treatment of rheumatism.

1.2.3.5 Diagnosis:
1.2.3.5.1 Diagnosis in definitive hosts:

Identification of *E. granulosus* in dogs or other definitive hosts is extremely important for both epidemiologic studies and surveillance of hydatid control programmes (FAO/UNEP/WHO, 1981).

1.2.3.5.1.1 Autopsy:

Careful examination of the small intestines at autopsy, performed by an expert person, is the most accurate method of *Echinococcus* diagnosis in dogs and other definitive hosts (Schantz, 1997). Intestinal examination is, however, often quite difficult when worm burdens are low and specially when tapeworms are also
immature. Direct contact with dogs in echinococcal endemic regions by workers or investigators will be a potential biohazard, therefore care must be taken when opening the intestines and when examining, identifying, counting or preserving specimens. Autopsy procedures have been described in the FAO/UNEP/WHO Guidelines (1981). A sample of *Echinococcus* tapeworms should be fixed in 70% ethanol when fresh or retained from formualin-fixed intestines, for morphological examination and/or DNA Profiling to identify strain characteristics.

1.2.3.5.1.2 Coproparasitological detection:

Direct identification of taeniid eggs in faecal smears, from perianal tape swabs or in concentrated canine stool samples is not species-specific and lacks sensitivity (Craig *et al.*, 1988). All taeniid eggs, including *Echinococcus*, are morphologically identical so the presence in faeces of whole proglottids of *Echinococcus* is diagnostic. Eggs of *Echinococcus* were directly species-specifically identified by an indirect fluorescent antibody test (IFAT) using an *Echinococcus* oncosphere specific monoclonal antibodies (Craig *et al.*, 1986c). Although highly specific, this test was cumbersome and impractical for testing large numbers of dogs, as well as being dependent on perianal contamination and hatchability of eggs.

1.2.3.5.1.3 Arecoline purgation:

Arecoline purgation with arecoline hydrobromide (2 mg/kg) or arecoline acetarsol (3 mg/kg) is still the main method of antemortem diagnosis of canine echinococcosis. This method, however, has significant drawbacks including its biohazardous nature, logistic practical problems, potential side effects, and low sensitivity (Wachira
et al., 1990) and it is also labour intensive. The importance of arecoline purgation is that it is 100% specific and is, therefore, likely to be always at least a confirmatory tool for use in epidemiological studies or control surveillance (Craig, 1997). A detailed description of the dosing technique, methods for examining faecal samples, and precautions for handling infected dogs and faeces has been published (FAO/UNEP/WHO, 1981; Eckert et al., 2001).

1.2.3.5.1.4 Coproantigen detection:

The newest technical advance in diagnosis of *Echinococcus* infections in dogs is the detection of specific antigens in the faeces. Several tests based on antibody sandwich ELISA techniques, have been described to detect naturally or experimentally acquired *Echinococcus sp.* infection in dogs (Jenkins et al., 2000). They do not give false-positive reactions with faeces of dogs infected with *Taenia spp.* or other helminth infections (Allan et al., 1992). Testing of faecal samples from wild canids and even wild lions (Deplazes et al., 1992), will also be very useful in epidemiologic studies, especially as fixation of stool samples in 5% formalin does not appear to decrease coproantigen test sensitivity (Allan et al., 1992). The sensitivity is closely related to the worm burden in infected dogs (Allan et al., 1992).

The source of tapeworm antigen in faeces could be scolex, tegumental turnover, and/or degradation products from detached proglottids, proglottids excretory-secretory production, and possibly from egg-derived antigens, though the latter appears not to be the case (Allan et al., 1992).
1.2.3.5.1.5 Serology:

In dogs, serum antibodies to metacestode antigens were detectable using ELISA after experimental infection with *E. granulosus* (Gasser *et al.*, 1988). Minimal cross reactivity was seen in serum samples of dogs experimentally infected with *Taenia* spp.; however, in field studies in a hyperendemic areas, serologic tests were not useful for differentiating currently infected dogs from those presumed to have been infected previously (Jenkins *et al.*, 1990). The protoscolex antigen-ELISA for serum antibodies (IgG) was tested against arecoline-purged dogs and gave a serologic sensitivity of only 35% for *E. granulosus* (Craig *et al.*, 1995a).

1.2.3.5.1.6 DNA probes:

The first definitive report of development of a DNA detection test for echinococcosis was directed towards *E. multilocularis*. Mathis *et al.* (1996) followed up and improved the DNA test for *E. multilocularis* by increasing the efficiency and purity of egg preparation from faecal samples. With this approach the specificity of PCR was 100% and a sensitivity of 94% was obtained. Similar studies have not been carried out for *E. granulosus* but should be comparable if species specific primers were used (Craig, 1997).

1.2.3.5.2 Diagnosis in intermediate hosts:

1.2.3.5.2.1 Postmortem examination:

Domestic animals are usually examined for hydatidosis at licensed slaughterhouses during meat inspection. The cysts can often be visually detected in organs. However, they should be identified microscopically in the laboratory (Soulsby, 1982).
1.2.3.5.2.2 Immunodiagnosis of hydatidosis in livestock:

The immunodiagnosis of animal echinococcosis was reviewed by Craig, (1997). They reported wide variation in their results, hence he concluded that serologic tests on animals sera are not very sensitive and tend to give a high level of false positive results due to cross reactions with other parasitic infections.

1.2.3.5.2.3 Diagnosis in humans:

1.2.3.5.2.3.1 Imaging methods:

Imaging methods for detection of space-occupying masses include: ultra-sonography (US), computed tomography (CT), radiology (x-ray) and magnetic resonance imaging (MRI) are the primary approaches for clinical diagnosis of cystic echinococcosis (Sinner, 1991). Ultrasound has emerged as the screening technique for cystic echinococcosis with greatest sensitivity, specificity and clinical correlation (Houston, 1991). The technique was found to detect higher prevalence of echinococcal cysts infection and higher positive predictive values when compared with serologic tests for screening (Macpherson et al., 1987).

1.2.3.5.2.3.2 Immunodiagnosis:

The immunological diagnosis of human cystic echinococcosis for detection of antibodies or circulating antigens in serum or urine was proved to be useful for diagnosis of active infection in cystic echinococcosis patients (Porija et al., 1997; Liance et al., 2000). The method is used for confirmation of presumptive clinical diagnosis and also for post-operative and post-chemotherapeutic follow-up (Craig et al., 1996; Sbihi et al., 2001). The major criteria for determining the
test of choice for a given situation are the sensitivity, specificity, reproducibility, predictive values, persistence, ease of performance, and the amount of antigen required (Sbihi et al., 2001). The immunologic tests applied for diagnosis of cystic echinococcosis in man include complement fixation test (CFT), Latex agglutination test (LA), indirect haemagglutination test (IHA), indirect fluorescent antibody test (IFAT), immunoelectrophoresis (IEP), double diffusion test (DD), Radio immunoassay (RIA), and various techniques of Enzyme-linked Immunosorbent Assay (ELISA). Skin (Casoni) test, although it is not a serologic test, yet it is considered in this group because of its closeness to this subject.

**1.2.3.5.3 Strain characterization:**

Strain identification is complex and is based on a combination of criteria, notably morphological, biological, biochemical, epidemiological features and recently on characteristics of the genome using molecular techniques for DNA analysis (Bowles and McManus, 1993a, b, c; Thompson et al., 1995).

Although morphological and biological studies have provided extremely useful information for strain identification, it has to be considered that these features are variable. They may be influenced by host and environmental factors and may not necessarily reflect distinctness at the genetic level (Bowles and McManus, 1993c). Several molecular techniques are now available which allow a direct characterization of the genome of the parasite using genetic markers and have the advantage that they are not confounded by variability induced by the host or the environment (Bowles and McManus, 1993c). Fresh, frozen or ethanol preserved material of adult or
metacestode stages is needed for such examination. Genetic variation in *E. granulosus* has been investigated in either the mitochondrial (mt DNA) genome or the nuclear ribosomal (r DNA) genome (Bowles and McManus, 1993c).

Recent molecular studies have involved the following techniques:

1. **Restriction Fragment Length Polymorphism (RFLP)** of ribosomal DNA or other genomic regions (McManus and Rishi, 1989; Bowles and McManus, 1993a; Siles-Lucas *et al.*, 1994). Genomic DNA of the unknown parasite is extracted from the parasite, purified to varying degrees, digested by restriction endonuclease enzymes, the resulting fragments are electrophoretically separated on an agarose gel, transferred single stranded onto nitrocellulose or nylon filter and hybridized with a specific DNA probe in a southern Blot approach (RFLP-SB). Recently, the RFLP technique has been modified and simplified by linking it with the polymerase chain reaction (RFLP-PCR or PCR-linked RFLP).

2. **Gene sequencing (GS):** The nucleotide sequence of the mitochondrial cytochrome c oxidase subunit 1 (CO₁) and of the NADH dehydrogenase 1 (ND₁) genes are determined using PCR (Bowles and McManus, 1993b, c).

3. **The random amplified polymorphic DNA-PCR (RAPD-PCR)** is a technique by which genomic DNA is amplified by PCR using a single oligonucleotide primer of arbitrary nucleotide sequence (Scott and McManus, 1994; Siles-Lucas *et al.*, 1994). This technique is relatively simple, it requires only small amounts of
DNA and is rapid. However, reliable results are only obtainable under carefully controlled conditions, especially with regard to the quality of template DNA. Therefore, it is recommended that the RAPD-PCR should be used simultaneously with one of the other DNA techniques (Scott and McManus, 1994).

1.2.3.6 Treatment:

1.2.3.6.1 Treatment of final hosts:

Arecoline hydrobromide given orally was found to be effective in the treatment of *E. granulosus* infection dogs (Lloyd *et al.*, 1991). Praziquantel was found to be extremely effective against both immature and adult stages in dogs (Thakur, *et al.*, 1978) but it has no ovicidal activity (Thakur *et al.*, 1979). Total clearance of worms only occurred in dogs given the highest dose (Arru *et al.*, 1990).

1.2.3.6.2 Treatment of intermediate hosts:

Ivermectin injected directly into the cysts resulted in cysts collapse and death of protoscolices with ultrastructural changes on the germinal layers. However, this effect was restricted to the ivermectin-treated cysts (Ochieng-Mitula and Burt, 1996). Casado *et al.* (2002) found that the efficacy of ivermectin and albendazole in combination is much better than either drug when used alone.

Surgery is the treatment that has the potential to remove cystic echinococcosis in human and leads to complete cure (WHO, 1996). However, surgery may be impractical in patients with multiple cysts localized in several organs. It is also a hazardous procedure because spillage of the cyst fluid may cause anaphylactic shock or dissemination of protoscolices resulting in secondary hydatidosis.
(WHO, 1996). This risk can be minimized by injecting a protoscolicide into the cysts to kill the germinal membrane and protoscolices. Chemotherapy and PAIR (Puncture – Aspiration – Injection - Reaspiration) offer an alternative treatment, especially in inoperable patients and for cases with high surgical risk (WHO, 1996). In PAIR, puncture should be done with precaution under ultrasonic guidance then aspiration of substantial amount of cyst fluid, followed by injection of a protoscolicidal substance and reaspiration of the cyst fluid after 15 – 20 minutes. Chemotherapy with benzimidazoles (albendazole and mebendazole) for human cystic and alveolar echinococcosis has become more frequently used with significant success (Davis et al., 1989). Albendazole was used for multiple cysts, for those not amenable to surgery and also pre-surgically to reduce the risk of recurrence and after surgery (Morris and Taylor, 1988). The response to combined long-term treatment with albendazole and praziquantel was better and much quicker compared to either agent (Morris et al., 1990).

1.2.3.7 Control of *Echinococcus granulosus*:

A good knowledge of the local epidemiology of echinococcosis is essential to the success of a control programme. It assists in determining the best control polices to pursue (Gemmell, 1997), therefore, any approach to the control of echinococcosis should recognize the multiplicity of interacting extrinsic and intrinsic factors as well as the impact of socioecological factors on the dynamics of transmission. Options for control include horizontal and vertical approaches. The former emphasizes long-term primary healthcare (education, sanitation and upgrading of meat inspection) with the aim
to reduce disease transmission. The vertical approach is targeted to the reduction of the parasite biomass by reducing the tapeworm population (dog-dosing) or reducing the dog population. The vertical approach can be divided into four phases: planning, attack, consolidation and maintenance of eradication (Gemmell et al., 2001).

1.2.3.7.1 The control in dogs:

The reduction of the total parasite biomass through 6 weekly mass dog dosing with Praziquantel, eradication of unwanted and stray dogs, immunization of dogs against *E. granulosus*, and the regular programme to spay bitches will reduce the infection pressure to humans and livestock (Macpherson and Wachira, 1997). Herd et al. (1975) had shown a significant suppression of egg production in dogs immunized with secretory antigens derived from adult *E. granulosus* grown *in vitro*.

1.2.3.7.2 Safe meat hygiene practices:

Slaughtering of meat animals at abattoirs and destruction of infected organs play a major role in interrupting the transmission cycle. The effective supervision of disposal of condemned offals by burning, boiling or deep burial, forms an important part of echinococcosis control. Dogs should be prevented from entering abattoirs. Illegal slaughtering must be prosecuted and special precautions must be taken when home slaughtering is carried out for social ceremonies (Gemmell et al., 2001).

1.2.3.7.3 Health education:

Health education is a basic component of any programme for control of *Echinococcus granulosus* and cystic echinococcosis. It
requires the motivation and participation of various population groups and has to take into consideration the beliefs, perceptions, behaviours, expectations, traditional habits, cultural and religious traditions, customs and needs of the people. The educational material should address local problems in order to be effective and have the needed impact on governmental officials, political decision-makers, managers, farmers health professionals, butchers, abattoir workers, dog owners, school-age and other educationally deprived children, field and laboratory workers and every one involved directly or indirectly in a control programme of echinococcosis. The full socio-economic impact which may be considerable in endemic areas has to be brought out clearly in order to alert the community on the need for control. The educational materials include audio-visual aids (Video films, television programmes), Mass Media, posters, pamphlets, pictures, brochures, colouring books and preserved adult E. granulosus and hydatid cysts (Macpherson and Wachira, 1997; Gemmell et al., 2001).
CHAPTER TWO
GENERAL MATERIALS AND METHODS

2.1 The study area:

Darfur region is the territorial area that comprises the three states of Northern Darfur, Southern Darfur and Western Darfur, with their capitals being El-Fasher, Nyala and El Geneina, respectively. The region has an international border with Central African Republic, Chad and Libyan Arab Jamahiriya. It is also sharing national borders with Northern Kordofan and Bahr-el-Ghazal States. It extends between latitudes 10°N and 19°N and longitudes 22°E and 27°E, occupying a total area of 138,150 square miles (Fig. 3). The region is inhabited by 4746456 inhabitants (Department of Statistics, 1994).

2.1.1 The climate:

Northern Darfur State is characterized by desert and semi-desert climate, while Southern and Western Darfur Sates are characterized by the Sahelo-Sudanian zone of Savannah grasslands and woodlands in which rainfall isohyetes run on an east-west axis, with rainfall decreasing to the north (Kerven, 1987). The rainy season (Kharief) usually starts in June and ends in October. July and August are the wettest months of the year with a total rainfall of above 29% of the total annual rainfall (398 – 402 mm). The temperature reaches its maximum (37 to 41°C) in the summer (Saief) as from March to June and drops to lowest readings (15 to 17°C) in the winter (Shitta) during December to February. Annual mean relative humidity was estimated as 35.6% and reaches its peak (81.3%) in July and August.
2.1.2 The soil:

Three types of soil are recognized in Darfur region. The continental sand (Goz), the soil of low fertility which spreads over much of Northern Darfur State and Parts of Southern and Western Darfur States. The silt, which is the most fertile of all Darfur soils, is found along the banks of rivers and valleys. The clay soil is found in all the riverine lands. The clay marshes of Bahr-el-Arab in the extreme south of Darfur region provide summer grazing for cattle of the Baggara tribes. In the west is Jebel Marra mountains.

2.1.3 Vegetation:

The predominant vegetation formations within the region are entirely confined to the low-rainfall woodland Savannah ecological zone. It consists of deep rooted trees (shade trees, thorny trees and bushes). Temporary vegetation is governed primarily by rainfall.

2.1.4 Human activities:

Agriculture and rearing of livestock are the main activities of the people in the rural areas. Darfur region supports the greatest proportion of livestock in the Sudan. Southern and Western Darfur States possess the largest numbers of livestock, whereas Northern Darfur State being semi-desert or desert support the lowest numbers. The animal population of Darfur region is estimated to be 4,682,530 cattle, 9,165,300 sheep, 7,207,500 goats, 687,788 camels, 75,000 – 1,000,000 equines beside a considerable number of other domestic and wild animals (Musa, 1995).

2.1.5 Animal husbandry systems:

Two types of animal husbandry systems are practiced the nomadic and the sedentary type of animal husbandry. About 80% of
the animals are kept under nomadic system. Livestock owners move with their animals over large distances on seasonal basis in pursuit of water and pasture for their animals. They move by the end of the rainy season southwards along fixed routes known as *(Maraheel)*. They spend most of the summer at the banks of Bahr-el-Arab. Some nomads move with their animals deep into Bahr-el-Ghazal State where the green pasture is available together with sufficient water. Their animals intermix freely and share common waters and pastures with the Dinka animals from the south and also with Misseriyya animals, which come from Kordofan States. Some nomads cross the Sudan border into the Central African Republic and Chad *(Mohammed, 1999)*. During their movements, livestock share common pasture and waters with wild animals like wolves and foxes that may contaminate waters and pasture with their faeces. Nomads also keep dogs that play an important role in guarding the livestock during their movements. With the onset of the rainy season, the nomads start moving back to the north. They stay around Nyala town during most of the rainy season, during which their animals graze together with those of sedentary farmers on the same pasture.

The sedentary animal owners live mostly on the banks of valleys where they have small farms and a sedentary animal rearing system. They only move within short distances to graze their animals on their farms residues during summer. Surface or underground water is available in the valley.

Camel nomads also move with their camels on seasonal basis. They spend the dry season around Nyala and El Daein towns. With the onset of the rainy season, they move northward to Malha, Kutum
and Mellit in Northern Darfur State. In November, they move to Wadi Hawar (Northwest Sudan), where they spend the whole winter. During this season, their camels graze on a characteristic semi-desert vegetation of an annual growth called gizu plants, which due to its nature and to the cold desert Winter, remain green from the end of the rainy season until the following April. During this period camels do not drink and thirst is quenched by the moisture provided by these plants.

Lately the region has been riddled with devastating violence of tribal conflicts and armed banditry. This disturbed considerably the map of human and livestock distribution in the region. Eventually, it was estimated that more than a million people were forced to leave their home places and resettle somewhere within the region or elsewhere outside the region. Modes of life have also changed. Some herdsmen become farmers or paid labourers in towns.

2.1.6 Wildlife in Darfur region:

It is generally accepted that wildlife had been more abundant in Darfur especially in its southern part than it is today. The ranges of desert wildlife species have been very much reduced due to increased domestic animal populations, increased poaching and drought conditions (Fadlalla, 2003). He also reported that dorcas gazelle (*Gazella dorcas*), addax (*Addax nasomaculatus*), dama gazelle (*Gazella dama*) and oryx (*Oryx dammah*) were present in few numbers in several localities in Northern Darfur. In Western Darfur State, greater kudu (*Tragelaphus strepsiceros*), duiker (*Cephalophus spp.*), red-fronted gazelle (*Gazella rufifrons*), porcupine (*Hystrix cristata*), lion (*Panthera leo*), leopard (*Panthera pardus*), hyaenas
(Hyaena hyaena and Crocuta crocuta), fox (Vulpes pallida), baboon (Papio anubis), crivit monkey (Cercopithecus aethiops) and patas monkey (Cercopithecus patas) were reported to occur (Hassabala and Nimir, 1985). The wildlife species reported in Southern Darfur State include: Elephant (Loxodonta africana), giraffe (Giraffa camelopardalis), hippopotamus (Hippopotamus amphibius), buffalo (Syncerus caffer), giant eland (Taurotragus derbianus), roan antelope (Hippotragus equinus), tiang (Damaliscus lunatus), waterbuck (Kobus ellipsiprymnus), reedbuck (Redunca redunca), kob (Adenota kob), bushbuck (Tragelaphus scriptus), oribi (Ourebia ouribi), duikers (Cephalophus spp.), red-fronted gazelle (Gazella rufifrons), warthog (Phacochoerus aethiopicus), black-backed jackal (Canis mesomelas), fox (Vulpes pallida), hyaenas (Hyaena hyaena and Crocuta crocuta), lion (Panthera leo), leopard (Panthera pardus), wild dog (Lycaon pictus), patas monkey (Cercopithecus patas), baboon (Papio anubis) and ostrich (Struthio camelus) (Wilson, 1979). Many species have disappeared from Darfur region because of habitat depletion, overgrazing, agricultural extension, poaching and possession of firearms by natives (Hassaballa and Nimir, 1985). The present status of wildlife in Darfur region is unknown, as there is no recent surveys to update information about the animals, but is believed that civil war has a negative impact on wildlife in that some species might have moved to the neighbouring countries to avoid the disturbance (Malek, 2002).

2.1.6.1 Fauna of Radom National Park:

The park was declared as a national park in 1980. It is located in Southern Darfur State bordered on the west by the Sudan-Central
African Republic international border and on the south by the state boundary of Southern Darfur and Northern Bahr-el-Ghazal. It is one of the largest parks in the Sudan. It covers an area of 11344 sq.Km of clay plains with scattered hills and rock out crops. It is located between longitude 23° 40”N and 24° 10”N and latitude 8° 20”E and 9° 35”E.

Diverse wild animals inhabit the park. An important features of this area are the wet meadows (dahal) that provide water and green fodder for the wildlife population throughout the dry season and are sites of late dry season wildlife aggregation (Hashim, 1995). During the dry season, the animals are not protected and as such some of them migrate to neighbouring Central African Republic due to heavy poaching. However, some of the existing larger game species of the park include: giant eland, reedbuck, hartebeest (*Alcelaphus buselaphus*), roan antelope, tiang, buffalo, giraffe, greater kudu, oribi, hippopotamus, lion, leopard, wild dog and python (*Python sebae*). Other animals are migratory so they are distributed in forests, mountains, banks of the valleys and semi-desert areas. These include various types of gazelle (red-fronted, dorcas and dama gazelles), monkeys, hyaenas, foxes and wild birds (Malek, 2002).

In Darfur region, foxes (locally named *Ba-ashoom or Abu Halima*) were considered as crop pests. Farmers practice traditional methods of pests management especially for foxes by using toothed metallic traps called (*Kajjama*) or by digging animals burrows. The exposed animals are killed. Rats, squirrels and foxes are consumed by certain communities. Foxes hides are used for making shoes and their
fats as medicines. These traditions increase human health hazards created by diseases of these animals (Ibrahim, 1999).

2.2 Field investigation:

2.2.1 Hydatidosis in intermediate animal hosts:

The study was conducted through the period October 2001 to September 2003 to investigate the prevalence of hydatidosis in domesticated intermediate hosts. Slaughterhouses of Nyala, El Fasher and Zalingei, and some village slaughter slaps (rural weekly markets) representing different localities of Darfur region were selected for the investigation.

2.2.1.1 Samples collected:

Carcasses with cyst-like lesions on the surfaces of target organs (liver, lungs, spleen, kidneys, heart and mesentery) and palpable nodules deeper in the lungs (suspected hydatid cases) were examined carefully for the presence of hydatid cysts. The origins of the slaughtered animals were found difficult to trace. The hydatid cysts found were removed carefully by dissection from the affected organs and the surrounding tissues using sharp knives and scalpels. The cysts collected were counted and placed in suitably identified plastic bags then taken to the laboratory in clean buckets for further examination. For long distances they were taken in vacuum icebox. The age, sex, species, organ involved and locality of each animal examined were recorded.

2.2.1.2 Laboratory examination of samples:

The laboratory examination of samples was carried out at the Department of Preventive and Public Health, Faculty of Veterinary Science, University of Nyala.
The volume of cyst fluid was measured with a 20-ml syringe and the diameter of the cyst was measured by using an ordinary ruler taking the average of two diameters at right angles across the wheal. Few drops of cyst fluid aspirate were placed on a glass slide and examined microscopically for the presence of protoscolices. Cysts without protoscolices were cut open and the walls scraped, the membranous material found was pressed between glass slides and examined microscopically. The biological status of the cysts was evaluated grossly and microscopically according to FAO/UNEP/WHO (1981) as fertile, sterile, caseated or calcified. The viability of protoscolices from fertile hydatid cysts was determined by:

a) Morphology and movement on glass slide upon microscopic examination.

b) Stain exclusion using 1% aqueous eosin.

c) Evagination process using normal saline or fresh dog bile.

Fertile cysts required for DNA analysis were rinsed three times in saline and stored in 70% ethanol in tightly closed bottles until subjected to DNA extraction.

2.2.2 Echinococcosis in definitive hosts:

2.2.2.1 Echinococcosis in dogs and foxes:

After animals were killed, their abdominal cavities were open under protective measures. The small intestine was isolated, ligated with tight double adjacent ligatures at both ends and then removed, placed in identified plastic bags and collected in plastic-covered buckets to be taken to the laboratory at Faculty of Veterinary Science, University of Nyala. Specimens collected from far distances were stored in 5% formalin solution. All animals had an identification sheet
indicating sex, origin and reference number. The intestines were open lengthwise and emptied, the mucosal surface scraped with spatula to free attached worms and examined with a hand lens to ensure that no parasites remain attached. Intestines were then rinsed in tap water and the contents washed through a sieve, collected in phosphate buffered saline, placed in black basin container and examined under a binocular magnifiers. The worms were collected by Pasteur pipettes, separated from faecal and intestinal debris by washing in tape water and then identified, counted, evaluated for sexual maturity then preserved in 70% ethanol or in 10% formal saline in securely capped and labeled bottles.

2.2.3 DNA analysis for strain characterization:

A total of 56 isolates (hydatid cysts) collected from different localities of Darfur region from different intermediate hosts (Table 2) and samples of adult worms harvested from experimentally infected dogs and foxes were examined by RFLP-PCR and gene sequencing.

2.2.4 Infective material for *E. granulosus* transmission:

Hydatid cysts were collected from the lungs, livers and mesentery of infected camels, cattle and sheep slaughtered at Nyala slaughterhouse. The cysts were aspirated with sterile needles and the protoscolices were then kept in clean sterile jars. Only fertile cysts with clear fluid and viability of at least 75% were used to infect the animals.

2.2.5 Administration of protoscolices:

Experimental animals were infected orally with protoscolices aspirated from cysts, counted using McMaster slide, mixed with sterile minced meat and fed to them.
Table 2: Intermediate hosts, organs involved and geographical origin of *E. granulosus* isolates.

<table>
<thead>
<tr>
<th>Organ</th>
<th>Southern Darfur</th>
<th>Northern Darfur</th>
<th>Western Darfur</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cams</td>
<td>Cattle</td>
<td>Sheep</td>
</tr>
<tr>
<td>Lung</td>
<td>7</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Liver</td>
<td>4</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Spleen</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Kidney</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mesentery</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td>6</td>
<td>8</td>
</tr>
</tbody>
</table>
2.2.6 Experimental transmission of *E. granulosus* from dogs to sheep, goats and gazelles:

Six lambs, six kids one month old and four gazelles (*Gazella dorcas*) approximately two months old were used in the experiment. *Echinococcus granulosus* worms harvested from the puppies experimentally infected were used to infect the animals. The number of *E. granulosus* worms with gravid segments per one ml of the suspension in saline were counted with the aid of a hand lens. Approximate number of eggs per one ml of worm suspension was evaluated microscopically. Sheep and goats were kept in separate pens and were fed grass and concentrates twice daily, but water was available *ad libitum*. The pens were cleaned every week and the drinking troughs cleaned with each change of water. Gazelles were kept in a pen in Nyala Zoo. They were managed in similar manner to sheep and goats.
CHAPTER THREE
Field investigations on echinococcosis/hydatidosis

3.1 Introduction:

Cystic echinococcosis or hydatidosis is an important cyclo-zoonotic parasitic disease caused by adult or larval stage of the tapeworm, *Echinococcus granulosus*. The parasite cycles in a predator/prey relationship between carnivore (definitive) hosts and herbivore (intermediate) hosts. Human may become infected with hydatid cyst if they accidentally ingest tapeworm eggs passed in the faeces of infected carnivores. *Echinococcus granulosus* has a worldwide geographical distribution that occurs in all continents of the world and in some areas it ranks as the leading disease of public health significance (Schantz *et al*., 1995, Eckert *et al*, 2001). The disease is particularly important in developing countries where many rural inhabitants live under poor sanitary conditions and in close proximity to their domestic animals (Andersen *et al*., 1997). In the Sudan, studies on cystic echinococcosis in domesticated animals were carried out by various workers (Eisa *et al*., 1962; El Khawad *et al*., 1976; Idris, 1985; Saad and Magzoub, 1989a and 1989b; Mohammed, 1997, Mohammed and Elmalik, 2000; Elmahdi, 2003).

The occurrence of human hydatidosis in the Sudan was reported by many workers (Eisa *et al*., 1962; Tola, 1987; Magambo *et al*., 1996; Elmahdi, 2003).

The epidemiology of the disease is based on two cycles, pastoral (synanthropic) in which the dog is always involved and sylvatic cycle which occurs in wild canids based on predation or
carrion feeding. Synanthropic and sylvatic cycles may be independent or interlinked (spill-over situation). The spectrum of *E. granulosus* hosts species in these cycles depends on the regional or local differences in the availability of various hosts species and strains of the parasite. Eisa *et al.* (1962) pointed out the possibility of wild carnivores, harbouring the parasite in the southern region of the Sudan but no further studies were conducted to demonstrate the role of various wild animals in maintaining the transmission cycle of the disease.

The work done in the Sudan had not covered Darfur region extensively. The magnitude of echinococcosis/hydatidosis in domesticated animals and man and the role of wild carnivores, especially foxes in the epidemiology of the disease had not been investigated.

The objectives of this study are therefore to elaborate on epidemiological determinants of the disease occurrence by:

1. Determining the prevalence of adult worms in dogs and foxes.
2. Search for cystic stage in camels, cattle and small ruminants to clarify their potential role in transmission cycle.
3. Formulating a possible control strategy based on information deduced from the above data.

**3.2 Materials and methods:**

**3.2.1 Study area:**

This study was conducted in Darfur region which is described in Chapter Two.
3.2.2 *Hydatidosis in the intermediate animal hosts:*

The areas selected for investigations, numbers and species of animals examined in these areas were shown in Chapter Two.

The numbers of animals examined in the slaughterhouses and villages were shown in Table (3).

The examination of livers, lungs, spleens, hearts, kidneys and mesentery was done much beyond the scope of routine meat inspection. Procedures for removal of encountered hydatid cysts, their transportation and laboratory examination were described in Chapter Two. The biological status of the cysts was evaluated grossly and microscopically according to FAO/UNEP/WHO (1981) as:

1. **Fertile**: containing protoscolices.
2. **Caseated**: containing caseous material.
3. **Calcified**: the wall hardened and cyst become stony in texture.

The viability of protoscolices from fertile hydatid cysts was determined according to FAO/UNEP//WHO (1981) by the following criteria

1. **Morphology and movement**: A few drops of hydatid fluid from each fertile hydatid cyst were placed on a glass slide and examined microscopically for morphology. Viable protoscolices appear oval or ovoid in shape, well invaginated, greenish in colouration, have rostellar hooks and have persitaltic-like movement in hydatid fluid. Dead ones appear yellow-brown in colour and immotile.
Table 3: Number and species of animals examined for hydatidosis.

<table>
<thead>
<tr>
<th>State</th>
<th>Camels</th>
<th>Cattle</th>
<th>Sheep</th>
<th>Goats</th>
</tr>
</thead>
<tbody>
<tr>
<td>North Darfur</td>
<td>146</td>
<td>786</td>
<td>4880</td>
<td>1483</td>
</tr>
<tr>
<td>South Darfur</td>
<td>374</td>
<td>2562</td>
<td>6249</td>
<td>3432</td>
</tr>
<tr>
<td>West Darfur</td>
<td>45</td>
<td>970</td>
<td>2598</td>
<td>2608</td>
</tr>
<tr>
<td>Total</td>
<td>565</td>
<td>4318</td>
<td>13727</td>
<td>7523</td>
</tr>
</tbody>
</table>
2. **Settling phenomenon in pooled hydatid fluid:** One to two ml fluid from each fertile hydatid cyst was poured in test tubes and left to stand in a vertical position for 5 minutes then examined for settling phenomenon of viable protoscolices in pooled hydatid fluid. Dead ones float or stay suspended.

3. **Stain exclusion:** A few drops of 1% aqueous eosin was added to 0.3 ml hydatid fluid from each fertile hydatid cyst on a glass slide, left for 5 minutes then examined microscopically for staining. Dead protoscolices stain readily with eosin, while viable ones do not take up the stain.

4. **Evagination process:** Some of invaginated protoscolices were removed from each fertile hydatid cyst and placed in a test tube containing 0.9% NaCl solution or fresh dog bile. The test tube was shaken to ensure thorough mixing of solution with protoscolices, then left vertically for 15 minutes. A few protoscolices from the test tube were mounted on a glass slide and examined microscopically. Viable protoscolices move outwards and inwards until eventually evaginate completely looking like the scolices of the adult worms with suckers and hooks, in dead ones no evagination took place.

### 3.2.3 Echinococcosis in definitive hosts:

#### 3.2.3.1 Echinococcosis in dogs:

A total of 548 adult stray dogs (304 males and 244 females) were shot killed by policemen during a rabies control programme in the study area. The postmortem procedures as described in Chapter Two for removal of small intestines and their processing for laboratory examination and harvesting of worms encountered. The worms were separated from faecal and intestinal debris by washing in
tap water and then identified, counted, evaluated for sexual maturity by the presence of shelled-egg in the gravid segments and then preserved in 70% ethanol or in 1% formal saline in securely capped labeled bottles.

3.2.3.2 Echinococcosis in foxes:
 Permission to capture foxes was obtained from Wildlife Conservation and Management, Southern Darfur State. A total of 116 foxes (72 males and 44 females) were wild caught by baited traps or shot killed by traditional rural hunters and farmers. The autopsy procedures, transportation of small intestines and their examination in the laboratory were the same as described for dogs.

3.3 Results

3.3.1 The prevalence in intermediate hosts:
3.3.1.1 The prevalence in camels:
 Out of 565 camels examined, 347 (151 male and 196 females) were found to be infected with hydatid cysts giving a prevalence rate of 61.42%. The prevalence rates in Northern, Southern and Western Darfur States were shown in Table (4).

<table>
<thead>
<tr>
<th>Animal species</th>
<th>Prevalence Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goat</td>
<td></td>
</tr>
<tr>
<td>Sheep</td>
<td></td>
</tr>
<tr>
<td>Cattle</td>
<td></td>
</tr>
<tr>
<td>Camel</td>
<td></td>
</tr>
</tbody>
</table>

Out of a total of 2018 hydatid cysts encountered in examined animals, 1323 (65.56%) were found in the lungs, 688 (34.09%) in the liver, 4 (0.20%) in the spleen and 3 (0.15%) in the kidneys. Out of the 347 infected camels, 130 (37.46%) were infected in the lungs, 127 (36.60%) in the liver, 84 (24.21%) in both organs, 3 (0.86%) in the
<table>
<thead>
<tr>
<th>No. examined</th>
<th>No. infected</th>
<th>% infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Southern Darfur</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3432</td>
<td>6249</td>
<td>2562</td>
</tr>
<tr>
<td>41</td>
<td>797</td>
<td>158</td>
</tr>
<tr>
<td>1.19</td>
<td>12.75</td>
<td>6.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>62.57</td>
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<tr>
<td></td>
<td></td>
<td>374</td>
</tr>
<tr>
<td>Western Darfur</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2608</td>
<td>2598</td>
<td>970</td>
</tr>
<tr>
<td>51</td>
<td>261</td>
<td>42</td>
</tr>
<tr>
<td>1.96</td>
<td>10.05</td>
<td>4.33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>52.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7523</td>
</tr>
<tr>
<td>Northern Darfur</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7523</td>
<td>13727</td>
<td>4318</td>
</tr>
<tr>
<td>119</td>
<td>1494</td>
<td>226</td>
</tr>
<tr>
<td>1.58</td>
<td>10.88</td>
<td>5.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>61.42</td>
</tr>
</tbody>
</table>

liver and spleen, 2 (0.58%) in the lungs and spleen, and 1 (0.29%) in the lungs and kidney. No cysts were observed in the
heart or in the mesentery. The range of hydatid cysts observed in the inspected organs was shown in Table (5).

The cysts diameter ranged from 1.5 cm to 8.3 cm, while the cysts volume ranged from 2.3 ml to 154 ml. Daughter cysts were observed in six fertile hydatid cysts in two livers and three lungs. The largest cysts were observed in the lungs (Fig. 4). The biological status of hydatid cysts found in examined animals was shown in Table (6).

3.3.1.2 The prevalence in cattle:

Out of a total of 4318 cattle (1101 males and 3217 females) examined, 226 (59 males and 167 females) were infected with hydatid cysts, giving a prevalence rate of 5.23%. The prevalence rates in Northern, Southern and Western Darfur States were shown in Table (4). Out of a total of 371 hydatid cysts encountered in examined animals, 166 (44.74%) were found in the lungs and 205 (55.26%) in the liver. Out of the 226 infected cattle, 134 (59.29%) were infected in the liver, 57 (25.22%) in the lungs and 35 (15.49%) in both organs. The range of hydatid cysts in inspected organs was shown in Table (5). The diameter of the cysts ranged from 2.4 to 5.2 cm, the volume ranged from 4.3 to 32.3 ml. Daughter cysts were observed in two fertile hydatid cysts in the liver and lungs.

The biological status of hydatid cysts found in examined animals was shown in Table (6).
Fig. 4. A camel lung infected with hydatid cysts of different size
Table 5: The range of hydatid cysts in affected organs.

<table>
<thead>
<tr>
<th>Animal species</th>
<th>Liver range</th>
<th>Lung range</th>
<th>Spleen range</th>
<th>Heart range</th>
<th>Kidney range</th>
<th>Mesentery range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Camel</td>
<td>1 – 9</td>
<td>2 – 23</td>
<td>1 – 2</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Cattle</td>
<td>1 – 5</td>
<td>1 – 4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sheep</td>
<td>1 – 3</td>
<td>1 – 2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 – 11</td>
</tr>
<tr>
<td>Goat</td>
<td>1 – 2</td>
<td>1 – 2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 – 4</td>
</tr>
</tbody>
</table>
Table 6: The biological status of hydatid cysts encountered in organs of slaughtered animals in Darfur region during October 2001 to September 2003.

<table>
<thead>
<tr>
<th>Species</th>
<th>Cysts status</th>
<th>Liver</th>
<th>Lungs</th>
<th>Spleen</th>
<th>Kidneys</th>
<th>Mesentery</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. found</td>
<td>688</td>
<td>1323</td>
<td>4</td>
<td>3</td>
<td>0</td>
<td>2018</td>
</tr>
<tr>
<td>Camel</td>
<td>% fertile</td>
<td>74.56</td>
<td>73.47</td>
<td>75.00</td>
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<td>% calcified</td>
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<tr>
<td>Cattle</td>
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<td>0.00</td>
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<td>11.22</td>
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<tr>
<td></td>
<td>% caseated</td>
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<td>0.00</td>
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<td>1242</td>
<td>1494</td>
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<tr>
<td>Sheep</td>
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<td>41.30</td>
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<tr>
<td></td>
<td>% caseated</td>
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<td>24.64</td>
<td>26.71</td>
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<td>% calcified</td>
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<td>22</td>
<td>0</td>
<td>0</td>
<td>37</td>
<td>76</td>
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<tr>
<td>Goats</td>
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<td>0.00</td>
<td>0.00</td>
<td>5.88</td>
<td>2.63</td>
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<td>% sterile</td>
<td>20.00</td>
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<td>0.00</td>
<td>0.00</td>
<td>17.65</td>
<td>26.32</td>
</tr>
<tr>
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<td>% caseated</td>
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<td>0.00</td>
<td>33.33</td>
<td>30.26</td>
</tr>
<tr>
<td></td>
<td>% calcified</td>
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<td>45.00</td>
<td>0.00</td>
<td>0.00</td>
<td>43.14</td>
<td>40.79</td>
</tr>
</tbody>
</table>

3.3.1.3 The prevalence in sheep:
Out of 13727 sheep (8296 males and 5431 females) examined, 1494 (946 males and 548 females) were found to be infected with hydatid cysts, with a prevalence rate of 10.88%. The prevalence rates in the three Darfur States were shown in Table (4). Out of the infected animals, 126 (8.43%) were infected in the lungs only, 532 (35.61%) in the liver only, 9 (0.60%) in both organs, 786 (52.61%) in mesentery and omentum, 26 (1.74%) in mesentery, omentum and liver, 13 (0.87%) in mesentery and lungs, and 2 (0.13%) in mesentery, liver and lungs. No daughter cysts were observed in cysts encountered. The intensity of infection with hydatid cysts in inspected organs was shown in Table (5).

Out of 1494 hydatid cysts encountered in examined animals, 77 (5.15%) were found in the liver, 175 (11.71%) in the lungs and 1242 (83.13%) in mesentery. The diameter of the cysts ranged from 1.7 to 6.5 cm, while the volume ranged from 2.4 ml to 47.3 ml.

The biological status of hydatid cysts found in examined animals was shown in Table (6).

3.3.1.4 The prevalence in goats:

Out of a total of 7523 goats (5012 males and 2511 females) slaughtered in Darfur States and examined during the study period, 119 (81 males and 38 females) were found to be infected with hydatid cysts, with a prevalence rate of 1.58%. The prevalence rates in Northern, Southern and Western Darfur State were shown in Table (4). Out of the 119 infected goats, 43 (36.13%) were infected in the lungs only, 15 (12.61%) in the liver only, 18 (15.13%) in both organs, 31 (26.05%) in mesentery and omentum and 12 (10.08%) in mesentery and lungs. No cysts were observed in the heart, kidneys, or
spleen. No daughter cysts were observed in cysts encountered. The total number of hydatid cysts encountered in examined animals was 76, of which 22 (28.95%) were found in the lungs, 17 (22.37%) in the liver and 37 (48.69%) in the mesentery and omentum. The intensity of infection with hydatid cysts in inspected organs was shown in Table (5). The diameter of the cysts ranged from 1.6 to 4.2 cm, while the cyst volume ranged from 2.5 ml to 26 ml. The biological status of hydatid cysts encountered in examined goats was shown in Table (6).

3.3.2 The prevalence in definitive hosts:

3.3.2.1 The prevalence in stray dogs:

Out of 548 dogs (304 males and 244 females) examined, 105 (19.16%) were found infected with *E. granulosus*. 12.07% of infected dogs had fewer than 100 worms, 25.86% of them had 100 – 500 worms, 32.76% of them had 500 – 1000 worms and 29.31% had more than 1000 worms. Most of the worms recovered were found in the proximal half of small intestines. The prevalence of *E. granulosus* infection and average worm burden in Darfur States were shown in Table (7).

3.3.2.2 The prevalence in foxes:

A total of 116 fox specimens collected from all parts of the three Darfur States were examined for *E. granulosus*. Seventy-eight of them bore cestodes of one or more species, but none was found infected with *E. granulosus*. 
Tale 7: Prevalence of *E. granulosus* infection and average worm burden in stray dogs in Darfur States.

<table>
<thead>
<tr>
<th>State</th>
<th>No. dogs examined</th>
<th>No. dogs infected</th>
<th>% infected</th>
<th>Worms recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Range</td>
</tr>
<tr>
<td>Southern Darfur</td>
<td>374</td>
<td>78</td>
<td>20.86</td>
<td>67 - 3524</td>
</tr>
<tr>
<td>Northern Darfur</td>
<td>115</td>
<td>16</td>
<td>13.91</td>
<td>54 – 2116</td>
</tr>
<tr>
<td>Western Darfur</td>
<td>59</td>
<td>11</td>
<td>18.64</td>
<td>93 – 3823</td>
</tr>
<tr>
<td>Total</td>
<td>548</td>
<td>105</td>
<td>19.16</td>
<td>54 – 3823</td>
</tr>
</tbody>
</table>

3.4 Discussion:
Cystic echinococcosis occurs in all continents including circumpolar, temperate, subtropical and tropical zones and ranks in some areas as the leading disease of public health significance (Eckert et al., 2001). The disease is particularly important in developing countries where many rural inhabitants live under poor sanitary conditions and in close proximity to their domestic animals (Andersen et al., 1997). The spectrum of *E. granulosus* host species depends on the regional or local differences in the availability of various host species and the strains of the parasite. The occurrence of the parasite in a specific host reflects the variable degrees of parasite-host adaptation, husbandry practices, environmental conditions, exposure period, and age at slaughter (Macpherson, 1994).

The overall prevalence rate of hydatidosis in Sudan varies with the variations of the number of animals slaughtered in different parts of the country. In the study area, cattle and sheep are slaughtered in all areas investigated. Camels and/or goats are not favoured in most of the areas investigated, camels being mostly slaughtered at slaughterhouses, while goats being mostly slaughtered at rural slaughter slaps. Due to livestock exportation and high price of male animals, most animals locally slaughtered were females except sheep in Nyala town where exported sheep were slaughtered at Nyala abattoir.

Investigation of hydatidosis in animals in Darfur region revealed that 61.42, 5.23, 10.88 and 1.58% of camels, cattle, sheep and goats carcasses examined, respectively were found infected with hydatid cysts. Apparently, there were no differences in the degree of infection in these animal species between the three Darfur States. This
may be due to the local environmental conditions and also to the seasonal migration of nomads within the area. The low prevalence of camel hydatidosis in Western Darfur State could not reflect the true prevalence of the disease among camel population of that State as the number of camels examined was comparatively small.

Despite that the camel is considered a browsing animal, the high infection rate in Darfur region may be explained by the fact that during most of the dry season camels graze on grasses subjected to contamination with dog faeces. Another reason justifying this high infection is that nomads after slaughtering a camel throw infected offals to their dogs, thus helping in the transmission of the infection. Camels are usually slaughtered at advanced age, when they no longer have value for transportation. This high rate of infection may also be due to the parasite strain characteristics. This high prevalence rate in camel is in agreement with previous surveys conducted by Mohammed and Elmalik (2000) in Nyala area and also in line with Saad et al. (1989) who reported it to be 67.74% in camels in El Obied and Saad and Magzoub (1989a) in the Sudan. Elmahdi (2003) reported that the highest rate of hydatidosis was recorded in camels (47.31%) in Tampool area.

The prevalence rate of hydatidosis in cattle reported in this study (5.23%) was in accordance with findings of Mohammed and Elmalik (2000) who reported it to be 6.42% in Nyala area. Similarly Elkhawad et al. (1976) reported prevalence rates of 6.2 and 7.6% in cattle examined in Equatoria and Bahr-el-Ghazal provinces respectively. In contrast, Eisa et al. (1962) reported a high prevalence rate (25%) in cattle examined in Equatoria Province. They attributed
this high infection rate to the high infection rate in dogs (86.5%) in the area. However, Saad and Magzoub (1989a) reported a low prevalence rate (3.64%) of bovine hydatidosis in the Sudan. Similarly Elmahdi (2003) reported a prevalence rate of bovine hydatidosis of 2.99% in the Central Sudan.

The prevalence rates of hydatidosis in sheep and goats reported in this study were 10.88 and 1.58% respectively. These low rates of infection in these animal species may be due to their immunity or parasite strains characteristics, although high prevalence rates were recorded in other areas or countries (Molan, 1993). Elansary and Hamad (1997) reported a 30.5% prevalence rate in sheep slaughtered at Kassala Slaughterhouse. Goats feed mainly by browsing, rather than grazing therefore, they usually exhibit low rates of infection.

The results of this study are in agreement with those of previous studies conducted in different parts of the Sudan by Saad and Magzoub (1989b), El Sawi (1994) and Elmahdi (2003). Rausch (1997) stated that the prevalence of larval cestode in sheep and goats in Africa had been comparatively low.

As far as the biological status of cysts encountered in this study is concerned, the fertility rate encountered in cysts from different animals showed the camel cysts to have the highest rate (73.84%) followed by cattle cysts (27.49%) and sheep cysts (9.24%), while goat cysts have the lowest fertility rate (2.63%). These higher fertility rates in camels and cattle were in agreement with those reported by Mohammed and Elmalik (2000) in Nyala area. This descending order of cysts fertility rates in camels and cattle were previously documented by Saad and Magzoub (1989a) and Elmahdi (2003). High
fertility rates of 59.8 and 94.5% were also reported in camels by Tola (1987) and Dada et al. (1980) respectively. The low fertility rates of cysts in sheep and goats reported in this study confirmed the previous findings of Saad and Magzoub (1989b) who reported only two fertile cysts in sheep. All other cysts detected were calcified or under calcification. Similarly, Elmahdi (2003) reported only 1.32% of 907 cysts from sheep in Central Sudan to be fertile. These results are in contrast with findings of Elansary and Hamad (1997) who reported fertility rate of 26.2% in sheep slaughtered in Kassala. Our findings were also in contrast with findings in other parts of Africa where sheep are heavily involved in the transmission of *E. granulosus* (Andersen et al., 1997). This discrepancy may be due to occurrence of strains which are known to differ in their host preferences or may be due to immunity exerted by indigenous sheep and goats. Elansary and Hamad (1997) suggested the active role of sheep as intermediate host of the parasite in Kassala area.

In this study, the camel cysts were mostly found in the lungs (65.56%) followed by the liver (34.09%) and other organs (0.35%). In cattle hydatid cysts were observed in the liver (55.26%) and the lungs (44.74%). Most hydatid cysts encountered in sheep and goats were in the mesentery and omentum (83.13% in sheep and 67.11% in goats) followed by lungs (11.71% in sheep and 26.32% in goats) then the liver (5.15% in sheep and 6.58% in goats). Our results in camels and cattle were similar to those reported by Mohammed and Elmalik (2000), Eisa et al. (1962) and Saad and Magzoub (1989a). The fertility rate of cysts in the liver was higher than in the lungs in camels, cattle and sheep. This is in agreement with Elkhawad et al. (1979) but differ
from Saad and Magzoub (1989a). This contradiction may be due to the number of cysts encountered. In goats, the cyst fertility was higher in the mesentery than in other organs. This is in contrast with Saad and Magzoub (1989b). The high presence of cysts in the mesentery reported in this study was in agreement with the findings of Elansary and Hamad (1997) in slaughtered sheep in Kassala area.

From our findings, it is apparent that camels and cattle are commonly infected with hydatid cysts and that as far as the data collected is concerned, they are the major intermediate hosts of the parasite and their role is significant in the transmission of the disease, while the role of sheep and goats as intermediate hosts for the parasite is insignificant or negligible in the area.

As far as the definitive host is concerned, the dog is undoubtedly the final host in most parts of the world. However, other carnivores such as hyaena, jackal, cape hunting dog and fox are known to harbour the adult stage of the parasite in some areas of the world especially in Africa (Macpherson, 1986).

In this study a prevalence of 19.16% in stray dogs was recorded for *E. granulosus*. The prevalence rate was highest in Southern Darfur and lowest in Northern Darfur State. The average worm burden ranged between 972 to 1319. This high prevalence seems to bear true epidemiological relationship to the level of cystic hydatidosis observed in slaughtered livestock in the area, especially camels. The high prevalence in the area may be due to the attitude of the people that camel lungs are unfit for human consumption and therefore thrown to dogs regardless of its condition. Improper disposal of condemned affected organs, unauthorized slaughter of meat animals
and poor meat hygiene practices were probably behind the high prevalence of the disease reported in this area. Similar results were reported by Mohammed and Elmalik (2000) in Nyala area. Saad and Magzoub (1986) reported a prevalence rate of 51% and high worm count (28400 worms in one dog) in Tampool area. Washira et al. (1994) reported a prevalence rate of 72.4% in dogs caught near abattoirs, while none of the dogs collected from veterinary clinics and other residential areas of Nairobi town showed *E. granulosus* infection. They concluded that people likely to be most exposed to risk of infection are those who live near abattoirs and are associated with meat industry. The number of worms recovered from dogs examined in this study was within the range that reported by Eisa et al. (1962) but lower than those reported by Saad and Magzoub (1986) and Mohammed and Elmalik (2000).

Out of 116 foxes examined, 78 of foxes bore cestodes of one or more species, yet, none of them was found infected with *E. granulosus*. Data on prevalence of fox echinococcosis in the Sudan were not available. This is the first time to investigate the prevalence of *E. granulosus* infection in foxes in the Sudan. The absence of *E. granulosus* in the current study could mean that foxes no longer have the opportunity to ingest viable cysts from dead livestock or prey on sheep and goats due to advances in animal husbandry. Previous studies on prevalence of hydatidosis in sheep and goats revealed the low significance of these animal species in the transmission of the parasite.

Experimental transmission of infection showed that if by chance foxes gain access to infective material, they may develop the adult
sexually mature stage of the parasite. Our findings are consistent with Coman (1973) who examined 1320 fox specimens in Australia and found no specimen of *E. granulosus*. Clarkson and Walters (1991) concluded that foxes were poor final hosts for *E. granulosus*.

CHAPTER FOUR

Experimental transmission of *Echinococcus granulosus*
4.1 Introduction:

*Echinococcus granulosus* is primarily maintained in a synanthropic transmission cycle with the dog as final host and domesticated ungulates as intermediate hosts. Variants of the cycle (sylvatic cycle) may involve wild canid carnivores as final hosts and wild ungulates as intermediate hosts (Rausch, 1995). These cycles may be independent or inter-linked (Eckert *et al.* 2001).

Studies on experimental transmission *E. granulosus* to dogs in the Sudan were first conducted by Slepnev *et al.* (1977). They infected four puppies with cysts originating from camels and cattle with variable harvest of adult worms from infected dogs. They reported 46 – 50 days duration of the cycle in dogs. Saad (1985) and Mohammed (1997) reported that the prepatent period was between 37 and 63 days. Slepnev and Zenkov (1974) showed that the time required for *E. granulosus* to reach maturity in intestines of dogs varies from 54 to 97 days.

The first record of wild carnivores as definitive hosts of *E. granulosus* in Africa was made by Ortlepp (1937). He found the parasite in cape hunting dog and lion. Foxes infected naturally and experimentally with *E. granulosus* of sheep origin in various African, European and American countries showed variable degrees of susceptibility (Macpherson, 1986; Cook, 1989; Jones and Walters, 1992).

Experimental infection of sheep and sometimes goats were conducted by several workers (Dada *et al.*, 1981; Gusbi and Awan, 1991). The difference in the outcome of these studies were attributed, among other factors, to variation in strains in the same species.
Dada et al. (1981) investigated the infectivity of a camel strain of E. granulosus experimentally raised in dogs for domesticated food and burden animals. They found that hydatid cysts develop poorly in sheep and goats. In the Sudan, experimental transmission of E. granulosus to sheep and goats were carried out by El Sawi (1994).

Wild intermediate hosts were reported in Africa for the first time by Verster (1962) who reported hydatid cysts in the cape molerat, warthog and wildebeest. Subsequently other wild intermediate hosts were reported (Dinnik and Sachs, 1969; Macpherson et al., 1983). Both susceptibility to infection and cyst fertility rate are essential factors in determining the importance of different intermediate hosts to local maintenance of E. granulosus.

From the work done in the Sudan, the role of foxes and gazelles in the host-parasite relationship of E. granulosus was not determined.

In this work experimental attempts were conducted to:

1. Compare the suitability of dogs and foxes as definitive hosts for E. granulosus cysts originating from camels, cattle and sheep.

2. Investigate the infectivity of E. granulosus of the camel/dog strain to local sheep and goats and to wild gazelles to monitor the possibility of their respective role in maintenance of the cycle.

**4.2 Materials and Methods:**

The laboratory examination of specimens was carried out at Department of Preventive Medicine and Public Health, Faculty of Veterinary Science, University of Nyala.
4.2.1 A. Experimental transmission to final hosts:

4.2.1.A1 Experimental animals:

Four puppies of 4 – 5 weeks of age (Fig. 5) and 10 approximately 4 weeks old foxes (Fig. 6) were used. Permission to capture foxes and gazelles was obtained from Wildlife Conservation Administration, Southern Darfur State and transported in cages. The foxes were captured by baited traps at their burrows. To exclude any naturally acquired infection, each animal received 5 mg/kg body weight of Praziquantel prior to infection. All experimental animals were confirmed to be free of parasites after three faecal examinations prior to infection. All animals were kept in cages at Faculty of Veterinary Science, University of Nyala and fed on milk and sterilized meat obtained at Nyala Abattoir twice a day and water was given ad libitum.

4.2.1.A2 Experimental design:

The dogs were numbered and split into two groups consisting of two animals each. The animals were starved 18 hours before they were infected. Each dog in group one received 35000 protoscolices of camel hydatid cysts removed from liver and lungs, while dogs in group two (control) received sterile minced meat mixed with normal saline. Clinical observations were recorded daily throughout the period of the experiment. From five weeks post infection, faecal samples from all animals were examined weekly for worms until
Fig. 5. Local breed of dogs experimentally infected with *Echinococcus granulosus*

Fig. 6. Foxes (*Vulpes pallida*) experimentally infected with *Echinococcus granulosus*
autopsied at 7 to 8 weeks post infection. The worms were harvested following the same procedures used for examination of stray dogs for *E. granulosus*. Samples of adult worms with gravid segments were washed in normal saline three times and kept in bottles containing 0.9% NaCl solution for oral inoculation of sheep, goats and gazelles.

Foxes were numbered and divided into five groups, each consisting of two animals. Each experimental fox in groups one, two three and four received aliquots of approximately 35000 protoscolices in sterile minced meat after fasting for 16 hours. Group one received protoscolices of camel origin that used to infect experimental dogs. Groups two and three received protoscolices of cattle and sheep origins respectively. Group four received protoscolices of camel and sheep origins (mixed), while foxes in group five (control) received sterile minced meat mixed with normal saline. Clinical observations were recorded daily throughout the period of the experiment. From five weeks post infection, faecal samples from all animals were examined weekly until autopsied at 10 to 11 weeks post infection. All animals were starved for 18 hours before being autopsied. The autopsy, removal and examination of small intestines, and collection of worms recovered were followed the same techniques used for examination of dogs for *E. granulosus*. Worms collected were counted, evaluated for development and sexual maturity and then preserved in 70% ethanol. Worms harvested from foxes in group one were compared morphologically and developmentally with those harvested from experimental dogs. Samples of worms from groups one to four were transferred to Institute of Parasitology (University of Hohenheim, Stuttgart, Germany) for strains characterization by molecular techniques.
4.2.1.A3 Infective material:

The experimental design, preparation of infective material and administration of protoscolices were as described in Chapter Two.

4.2.1.A4 Postmortem examination:

The autopsy procedures, removal and collection of intestines, and transportation to the laboratory for examination were as described previously in Chapter Two.

4.2.1.A5 Laboratory examination:

Opening of intestines, harvesting of worms, their identification, counting, evaluation for sexual maturity and preservation were as described in Chapter Two. For morphological studies, *E. granulosus* worms were allowed to relax in physiological saline at room temperature for about 30 minutes, fixed in 10% formaldehyde solution and subsequently stained with eosin. Strobila measurements were made on formalin-fixed material. Hook measurements were done as described by Thompson *et al.* (1984) and Kumaratilake *et al.* (1986), under oil immersion using a calibrated eyepiece micrometer. Statistical methods were used as previously described by Kumaratilake *et al.* (1986).

Samples of worms with gravid segments were washed in normal saline three times and kept for oral inoculation of sheep, goats and gazelles. Samples of worms from all experimental groups were preserved in 70% ethanol for molecular characterization of strains.

4.2.1B. Experimental transmission to intermediate hosts:

4.2.1.B1 Experimental animals:

Six lambs and 6 kids of one month old and 4 gazelles (*Gazella dorcas*) approximately two months old were used in the experiment.
Sheep and goats were kept in separate pens at Nyala University Farm. They were fed grasses and concentrates twice daily and water was available *ad libitum*. The pens were cleaned every week and the drinking troughs were cleaned with each change of water. Gazelles were kept in a pen in Nyala Zoo (Fig. 7). They were managed in a similar manner to sheep and goats.

4.2.1.B2 Infective material:

*Echinococcus granulosus* worms harvested from experimentally infected puppies described previously in this Chapter were used to infect the animals. The number of *E. granulosus* worms with gravid segments per one ml of worm suspension in saline was determined with the aid of a hand lens. Approximate number of eggs in gravid segments per one ml of the worm suspension was determined microscopically.

4.2.1.B3 Administration of eggs to the animals:

Sheep and goats were numbered and then each species was divided into three groups, each consisted of two animals. Gazelles were also numbered and divided into two equal groups.

Group one in each animal species were inoculated orally with 40 *E. granulosus* gravid segments (approximately 2000 infective eggs) in 5 ml of saline solution per animal. Each animal in group two in sheep and goats received 80 gravid segments (approximately 4000 eggs) in 5 ml saline solution, while each animal in the corresponding control groups in the three animal species received 5 ml saline solution.
Fig. 7. Gazelles (Gazella dorcas) used for experimental transmission of hydatid cysts
4.2.1.B4 Postmortem examination:

All animals were kept for 10 to 11 months, after which they were slaughtered and organs of the thorax and abdomen were examined for hydatid cysts. The procedures used for examination of slaughtered animals and collection of hydatid cysts encountered were the same as described in Chapter Two.

4.2.1.B5 Laboratory examination:

The procedures for examination of hydatid cyst volume, size and biological status were as previously described in Chapter Two.

4.3 Results:

4.3.1A Experimental transmission to final hosts:

4.3.1.A1 Transmission to dogs:

As shown in Table (8), *E. granulosus* infection established in all the dogs that received protoscolices. The total numbers of worms recovered were regarded as approximate values due to the possible loss of some worms during the processing of material. The worm recovery ranged between 27460 (78.46%) and 30154 (86.15%). In all animals, 82% to 93% of the parasites were found in the proximal two thirds of the small intestines. No clinical signs were observed in infected dogs. The prepatent period was between 46 and 55 Days as determined from faecal examination.

*Echinococcus granulosus* recovered from dogs at day 47 post infection had reached an average total length of 2.2 mm. By this time, approximately 20% of the parasites had developed three segments (Fig. 8) and the uterus of 37% of the total worm population was full of developing eggs in the terminal segment. At day 57 post infection, the length of worms had increased (ranged from 1.8 to 4.6 mm). Seventy one percent of the parasites had three segments and up to 56% of them
Table 8: Worm burdens and intestinal distribution of *E. granulosus* of camel origin in experimentally infected dogs.

<table>
<thead>
<tr>
<th>Dog number</th>
<th>Necropsy (days post Infection)</th>
<th>Total number of worms recovered</th>
<th>Percent of worms in each third of small intestine</th>
<th>Percentage of worms with gravid segments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>47</td>
<td>27460</td>
<td>4 89 7</td>
<td>37</td>
</tr>
<tr>
<td>2</td>
<td>57</td>
<td>30154</td>
<td>6 76 18</td>
<td>56</td>
</tr>
</tbody>
</table>
Fig. 8. Adult *Echinococcus granulosus* recovered from experimentally infected dogs.

Fig. 9. *Echinococcus granulosus* worms at different developmental stages collected from experimentally infected foxes on day 76 post infection.
contained embryonated eggs with a fully developed embryophore (thick-shelled eggs). Rostellar hook characteristics and morphological measurements were shown in Table (9). The genital pore in gravid segment was found at about the middle of the segment or slightly anterior to the middle of segment in 60% of worms. The length of terminal segment ranged between 0.6 and 2.8 mm.

4.3.1.A2 Transmission to foxes:

As shown in Table (10), all foxes, which received protoscolices were found to be infected with *E. granulosus* at autopsy (Fig. 9). The majority of worms were recovered from the anterior two thirds of the small intestines. The prepatent period was between 69 and 79 days. The worm recovery ranged from 5400 (15.43%) to 23705 (67.73%). No clinical signs were observed other than the bloody diarrhoea in foxes No. 3 and No. 7 which died on days 13 and 22 post infection respectively. Early developmental stages of the parasite were observed on postmortem in intestines of foxes No. 3 and No. 7. Worm burdens of camel origin were higher than those of sheep and cattle origins. The number of worms recovered and percentages of gravid segments in infected groups were shown in Table (10).

The morphological development of *E. granulosus* of different intermediate host origin raised in foxes was shown in Table (11).

A comparative study of worms development in dogs and foxes experimentally infected with hydatid material of the same source (camel) was conducted. Substantial worm burdens were harboured by all animals, and the majority of worms in both animal species were recovered from the anterior portion of the small intestines. However, the worm burdens in foxes were less than in dogs. The prepatent
Table 9: Rostellar hook and morphological characteristics of adult *E. granulosus* of camel origin in experimentally infected dogs.

<table>
<thead>
<tr>
<th>Worm characteristics</th>
<th>Mean ± SD (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Large hooks</strong></td>
<td></td>
</tr>
<tr>
<td>TL (mm)</td>
<td>34.9 ± 1.2 (30.4 – 37.0)</td>
</tr>
<tr>
<td>BL (mm)</td>
<td>14.2 ± 0.8 (13.6 – 17.3)</td>
</tr>
<tr>
<td><strong>Small hooks</strong></td>
<td></td>
</tr>
<tr>
<td>TL (mm)</td>
<td>27.6 ± 1.8 (24.4 – 33.0)</td>
</tr>
<tr>
<td>BL (mm)</td>
<td>9.5 ± 0.6 (8.5 – 11.2)</td>
</tr>
<tr>
<td><strong>Total number of hooks</strong></td>
<td>43.0 ± 2.3 (28.0 – 36.0)</td>
</tr>
<tr>
<td><strong>Total worm length</strong></td>
<td>3.2 ± 0.5 (1.2 – 4.6)</td>
</tr>
<tr>
<td><strong>Length of terminal segment (mm)</strong></td>
<td>1.6 ± 0.4 (0.6 – 2.8)</td>
</tr>
<tr>
<td><strong>Maximal number of segments</strong></td>
<td>3</td>
</tr>
</tbody>
</table>

TL = Total length  
BL = Blade length
Table 10: Results of *E. granulosus* in experimentally infected foxes with protoscolices from different intermediate hosts.

<table>
<thead>
<tr>
<th>Group</th>
<th>Origin of protoscolices</th>
<th>No. of foxes infected</th>
<th>Dose of protoscolices/animal</th>
<th>Necropsy post-infection (days)</th>
<th>No. of worm recovered range (mean)</th>
<th>Percentage of gravid segments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Camel</td>
<td>2</td>
<td>35000</td>
<td>76</td>
<td>15400-23705 (19550)</td>
<td>24.5 – 31</td>
</tr>
<tr>
<td>2</td>
<td>Cattle</td>
<td>2</td>
<td>35000</td>
<td>78</td>
<td>5800-14960 (10380)</td>
<td>19 – 21.3</td>
</tr>
<tr>
<td>3</td>
<td>Sheep</td>
<td>2</td>
<td>35000</td>
<td>81</td>
<td>5400-9720 (7560)</td>
<td>9.7 – 13.2</td>
</tr>
<tr>
<td>4</td>
<td>Camel + Sheep</td>
<td>2</td>
<td>35000</td>
<td>77</td>
<td>11290-21650 (16470)</td>
<td>16 – 23.6</td>
</tr>
</tbody>
</table>
Table 11: Morphological characteristics of *E. granulosus* of camel, cattle and sheep origins experimentally raised in foxes.

<table>
<thead>
<tr>
<th>Worm characteristics</th>
<th>Source of parasite material, mean ± SD (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Camel</td>
</tr>
<tr>
<td>Total length (mm)</td>
<td>1.45±0.20 (1.0–3.8)</td>
</tr>
<tr>
<td>Length of terminal segment (mm)</td>
<td>0.9±0.3 (0.8–1.7)</td>
</tr>
<tr>
<td>Small hooks</td>
<td></td>
</tr>
<tr>
<td>TL (mm)</td>
<td>22.30±1.58 (20.10–27.30)</td>
</tr>
<tr>
<td>BL (mm)</td>
<td>9.90±0.60 (8.80–11.40)</td>
</tr>
<tr>
<td>Large hooks</td>
<td></td>
</tr>
<tr>
<td>TL (mm)</td>
<td>28.33±0.61 (27.5–31.10)</td>
</tr>
<tr>
<td>BL (mm)</td>
<td>14.50±1.5 (9.5–17.2)</td>
</tr>
<tr>
<td>Total No. of hooks</td>
<td>35±2.8 (28–36)</td>
</tr>
</tbody>
</table>

TL = Total length
BL = Blade length
period was longer in foxes (69 – 79 days) than in dogs (46 – 55 days). No significant differences in total worm length were found between worms from dogs and foxes (P> 0.05). Similarly, no significant differences were apparent in dimensions of scolex (P> 0.05) or hooks (P> 0.05) of worms harvested from dogs and foxes (Table 12).

4.3.1.B. Transmission to intermediate hosts:

Out of four experimentally infected sheep, one in group 1 and two in group 2 were found to develop hydatid cysts. Three sterile hydatid cysts in mesentery, one in the liver and one calcified cyst in the lung were encountered.

Out of four experimentally infected goats, only one showed two caseated hydatid cysts in the mesentery and one sterile cyst in the lung.

None of the two experimentally infected gazelles was found to be infected with hydatid cysts.

The numbers, size, biological status and location of hydatid cysts encountered in experimentally infected small ruminants were shown in Table (13).
Table 12: Comparative development of *E. granulosus* of camel origin in dogs and foxes.

<table>
<thead>
<tr>
<th>Animal species</th>
<th>Dose of protoscolices/animal</th>
<th>Exposure period (days)</th>
<th>Prepatent period (days)</th>
<th>Total length (mm) Mean + SD (range)</th>
<th>Scolex width (mm) Mean + SD (range)</th>
<th>Rostellar width (mm) Mean + SD (range)</th>
<th>Sucker diameter (mm) Mean + SD (range)</th>
<th>Hook length (mm) Mean + SD (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog</td>
<td>35000</td>
<td>47 - 57</td>
<td>46 - 55</td>
<td>27460-30154 (78.46-86.15)</td>
<td>3.2±0.5 (1.2-4.6)</td>
<td>0.25±0.025 (0.2-0.29)</td>
<td>0.10±0.01 (0.09-0.14)</td>
<td>0.10±0.006 (0.08-0.10)</td>
</tr>
<tr>
<td>Fox</td>
<td>35000</td>
<td>76</td>
<td>69 - 79</td>
<td>15400 -23705 (44.0 - 67.73)</td>
<td>1.45±0.20 (1.0 - 3.8)</td>
<td>0.25±0.019 (0.20-0.28)</td>
<td>0.12±0.01 (0.10-0.13)</td>
<td>0.10±0.007 (0.08-0.11)</td>
</tr>
</tbody>
</table>
Table 13: Hydatid cysts found in experimentally infected small ruminants with *E. granulosus* of camel origin.

<table>
<thead>
<tr>
<th>Animals species</th>
<th>Cyst characteristics</th>
<th>Liver</th>
<th>Lung</th>
<th>Mesentery</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep</td>
<td>No. of cysts</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Size (cm)</td>
<td>2.2</td>
<td>1.8</td>
<td>2-3.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Biological status</td>
<td>Sterile</td>
<td>Calcified</td>
<td>sterile</td>
<td></td>
</tr>
<tr>
<td>Goat</td>
<td>No. of cysts</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Size (cm)</td>
<td>-</td>
<td>1.9</td>
<td>2.3-2.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Biological status</td>
<td>-</td>
<td>Sterile</td>
<td>Caseated</td>
<td></td>
</tr>
<tr>
<td>Gazelle</td>
<td>No. of cysts</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Size (cm)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Biological status</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>
**4.4 Discussion:**

Protoscolices of *E. granulosus* of camel origin were found to be readily infective to dogs. Saad (1985), Saad and Magzoub (1988) and Mohammed (1997) found that puppies are readily susceptible to infection with *E. granulosus* of camel origin. The prepatent period reported in dogs in this study was between 46 and 55 days. This result confirmed the observations of Saad and Magzoub (1988), Mohammed (1997) and Slepnev *et al.* (1977) who reported similar results. The worm recovery rate ranged between 78.46 and 86.15%. This recovery rate is similar to that recorded by Saad (1985) who reported up to 81.5% recovery rate of adult worms of camel origin in dogs, but much higher than that reported by Mohammed (1997) and Slepnev *et al.* (1977). This variation may be attributed to differences in the viability rates of protoscolices in different cysts. It may also be attributed to a variable degree of resistance to infection showed by the different breeds of dogs. Gemmell (1962) mentioned that weak acquired resistance which develops slowly may affect both the number of worms that establish themselves in the host and their size.

Slepnev *et al.* (1977) reported a very low rate of recovery in dogs (0.13%). They attributed the low worm recovery to the storage of the cysts in the refrigerator for 3 days before administration to dogs. In the present experiment, the dogs were fed protoscolices 3 – 4 hours after their collection from the slaughterhouse. This may explain the high recovery rate of adult worms from dogs fed with protoscolices of camel origin. In natural circumstances dogs get access to infected organs immediately after slaughter.
Foxes were fed protoscolices of camel, cattle and sheep origins. They were found to be readily susceptible to infection with *E. granulosus*. Similarly results were obtained by Thompson (1983) who demonstrated comparable growth and morphological development of *E. granulosus* of Australian sheep origin in dogs and European red foxes. The recovery rate of adult worms in this study was higher (55.86%) in foxes fed protoscolices of camel origin, followed by those fed mixed material of camel and sheep origins (47.06%) and those fed material of cattle origin (29.66%) but lower (21.60%) in foxes fed material of sheep origin. These variations in recovery rate may be attributed to genetic characteristics of the foxes that affect the ability of the parasites to establish themselves and develop normally in the final host or it may be due to the variability rates of protoscolices from different hosts. Similarly in Great Britain, Clarkson and Walters (1991) found that foxes were poor final hosts for the cestode, and they observed as well that breeds of dogs differed in the degree of susceptibility to infection. The prepatent period in foxes reported in this study ranged between 69 and 79 days. This period is longer than that reported in dogs. Gemmell (1959) reported that no ova developed in foxes kept for 112 days post infection with protoscolices of sheep origin.

The growth and development of *E. granulosus* of camel origin in dogs and foxes were compared. All animals were found to be infected with the parasite at autopsy, but the worm burden in foxes were lower than in dogs. Similar results were reported by Thompson (1983) who studied the development of *E. granulosus* of sheep origin in dogs and foxes. Smyth and Smyth (1968) referred this to
differences in the microtopography of the gut between dogs and foxes which may influence initial establishment of *E. granulosus* in the definitive host, although such differences may not appear to affect the ability of the parasite to reach sexual maturity once it is established. The susceptibility of foxes to infection with *E. granulosus* may also be influenced by the immunological status of the hosts. Concerning growth and morphological development of *E. granulosus*, no significant differences in total worm length or hooks dimensions were observed between worms harvested from experimental dogs and foxes. Similar findings had been recorded by Thompson (1983).

The findings of this study confirmed the principal role played by dogs as being the major final host of the camel strain of the parasite in the Sudan. This is the first record of experimental transmission of *E. granulosus* to foxes in the Sudan. From the results of the present experiments, it is evident that foxes are potential definitive hosts of the camel strain of *E. granulosus*, in the Sudan although their role in the epidemiology of hydatidosis is yet to be confirmed as none was found naturally infected.

Although the number of intermediate hosts used in the experiment was small to allow establishment of a general conclusion, the result obtained in this study illustrated that hydatid cysts of camel origin can develop in sheep and to a lesser extent in goats. The results were in accordance with those recorded by El Sawi (1994). Dada *et al.* (1981) investigated the infectivity of a camel strain of *E. granulosus*, experimentally raised in dogs, for domesticated food and burden animals. They found that hydatid cyst developed poorly in sheep and goats. No fertile cysts were observed in this study. This is in
agreement with Gusbi *et al.* (1991) who studied the growth rate of hydatid cysts in Libyan sheep dosed orally with gravid segments of *E. granulosus*, taken from stray town dogs. They reported that sheep dosed with 4000 – 200000 eggs had developed only sterile cysts when examined 358 days after dosing. These findings were previously documented by Saad and Magzoub (1989b) and Elmahdi (2003) who found only calcified, under calcified or caseated cysts in sheep and goats naturally infected with hydatid cysts in most parts of the Sudan. The smaller-sized cysts encountered in this study can be attributed to the fact that animals were not allowed ample time to develop large-sized cysts or may be due to the host immunity. The localization of cysts in the mesentery may increase the rate of transmission to dogs especially at weekly outdoor markets in rural areas where meat animals were slaughtered without veterinary supervision and the infected viscera are likely discarded to ever-present stray dogs, which wander aimlessly about, even into killing areas, during and after slaughter. Although some fertile cysts were recorded previously in naturally infected sheep and goats in the area, no fertile cysts were recorded in this study. This suggests that sheep and goats may have minor or no role in maintaining the life-cycle of the parasite. This is in contrast to the situation in other African countries where goats have been found to be good intermediate hosts for the camel strain of *E. granulosus*, but Wachira *et al.* (1993b) reported that their role in maintenance of the cycle is doubtful.

This is the first time to conduct experimental transmission of *E. granulosus*, to gazelles. No hydatid cysts were detected in the two gazelles inoculated orally with infective eggs of *E. granulosus*, of
camel origin raised in dogs. This non-susceptibility of gazelles to infection may be due to host immunity or to the parasite characteristics. This result suggests that gazelles (Gazella dorcas) are not suitable intermediate hosts for a camel strain of *E. granulosus*, but could not be regarded as a general rule as the number of animals used was small.
5.1 Introduction:

Epidemiological studies and strain identification are basic requirements for effective control of echinococcosis/hydatidosis (Eckert and Thompson, 1997). Strain identification is based on a combination of criteria notably morphological, biological, biochemical, epidemiological features and recently on characteristics of the genome using molecular techniques for DNA analysis (Eckert and Thompson, 1997; Kamenetzky et al., 2002, Dinkel et al., 2004; Obwaller et al., 2004). The criteria used in identification of strains from various animal hosts take into consideration the infectivity to different intermediate hosts and humans, developmental features in vivo and in vitro, biochemical comparisons, pathological and serological differences, and DNA analysis (Thompson, 1995; Lymbery, 1995). Morphological and biological studies have provided extremely useful information for strain identification but they may be influenced by host and environmental factors and may not necessarily reflect distinctness at the genetic level (Bowles and McManus, 1993c). Several molecular techniques are now available. They allow a direct characterization of the genome of the parasite using genetic markers and are not confounded by variability induced by the host or the environment or associated with life cycle stage (Bowles and McManus, 1993c).

Fresh, frozen or ethanol preserved material of adult or metacestode stages is required for such examination. Genetic variation in *E. granulosus* can be investigated in either the mitochondria or
nuclear genome. Recent molecular techniques involved include: Restriction Fragment Length Polymorphism (RFLP) of ribosomal DNA or linked with PCR (RFLP-PCR), Gene sequencing (GS) of the mitochondrial cytochrome c oxidase subunit 1 (CO1) and of the NADH dehydrogenase-1 (ND1) genes determined by PCR, the random amplified polymorphic DNA-PCR (RAPD-PCR), and more recently, specific and sensitive PCR system for rapid diagnosis of *E. granulosus* genotype G1, *E. granulosus* genotypes G5/6/7 (Dinkel *et al.*, 2004). These genetic studies have principally confirmed the concept of strain diversity within species of *E. granulosus*, previously based on morphological and biological features. Currently, 9 genetically distinct strains (G1 – 9) had been identified which all appear to be adapted to a particular life-cycle pattern and host assemblage (Bowles and McManus, 1993b and 1993c; Thompson and McManus, 2001). At least 5 of these strains were thought to exist in Sub-Saharan Africa (Thompson, 1995). In the central Sudan, the camel strain (G6) was reported in camel, sheep and cattle, while cattle strain (G5) in cattle (Elmahdi, 2003). Data on *E. granulosus* strains in other parts of the Sudan are not available.

This part of the study aims at the characterization of the parasite strains from different host species in Darfur region by molecular techniques as a complement to the epidemiological and biological studies.

### 5.2 Materials and Methods:

The laboratory work of this part of the study was done by Ribah Ali under supervision of Dr. Thomas Romig at Institute of Parasitology (University of Hohenheim, Stuttgart, Germany).
A total of 56 fertile hydatid cysts collected from different localities of Darfur States and from different intermediate hosts (camels, cattle and sheep) were examined. Hydatid cyst fluid was aspirated and centrifuged at 1000 g for 30 minutes. Protoscolices were kept in 70% ethanol until used. Samples were transferred to Institute of Parasitology (University of Hohenheim, Stuttgart, Germany) where Analysis of the samples was done. DNA from previously typed *E. granulosus* common sheep strain (G1 genotype), camel strain (G6 genotype and cattle strain (G5 genotype) were used for comparative purpose. Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) and sequencing of 366 base pair of cytochrome c oxidase (CO1) genes were used to characterise the isolates of *E. granulosus* (Bowles and Manus, 1993a, Bowles, et al., 1992). DNA was extracted from ethanol preserved samples using standard protocol of phenol-chloroform extraction and ethanol precipitation method (Sambrook et al., 1989). Samples of adult worms from experimentally infected dogs and foxes were preserved in 70% ethanol and sent to Institute of Parasitology (University of Hohenhiem, Stuttgart, Germany) where analysis of the samples was done by the same techniques used for Protoscolices.

**5.2.1 DNA extraction:**

DNA was extracted from ethanol preserved samples using standard protocol of phenol-chloroform extraction and ethanol precipitation method (Sambrook *et al*., 1989). The protoscolices samples were placed in Eppendorf tube and centrifuged at 13000 g for 1.5 min. The supernatant was discarded and 500 µl of the digestion buffer was added and centrifuged at 13000 g for 1.5 min. The
supernatant was discarded and 500 µl of digestion buffer, 600 mAU/ml proteinase K were added and the sample was vortexed and incubated at 56°C overnight. The sample was centrifuged at 13500 g for 1.5 min. The supernatant was transferred into a new tube and the pellet was discarded. The same amount of phenol/chloroform was added to the tube (under the hood) and then vortexed and centrifuged at 13500 g for 10 minutes. 500 µl of the upper water phase was transferred into a new tube and 50 µl 3 M Na-acetate (pH= 4.8 – 5.2) was added at 1:10 ratio, 1000 µl absolute ethanol was also added and then vortexed and incubated at –20°C overnight or –80°C for 2 hour. The sample was centrifuged at 13500 g for 30 minutes at 4°C and the supernatant was discarded. The sample was centrifuged shortly and DNA was dried at 55 – 60°C and then 200 µl of TE buffer (pH= 7.6) was added, then the tube was inverted and incubated at 4°C overnight. For DNA measurements, the cold solution was heated for 10 minutes at 50°C. For optical Density Measurements (OD), 10 µl of the samples was diluted with 200 µl TE buffer [10 mM Tris-HCl (pH 8.0), 1 mM EDTA]. DNA concentration was measured by spectrophotometer at 260 wave length.

5.2.2 Polymerase Chain Reaction – Restriction Fragments length Polymorphism (PCR-RFLP):

Internal Transcript Spacer 1 – Polymerase Chain Reaction (ITS-1 – PCR was done as previously described by Bowles and McManus (1993a). When BD1 and 4S was used in amplification reactions, the product spans internal transcribed spacer 1 (ITS-1) of the rDNA repeat unit and includes most of the 5.8S gene (Fig.10).
Fig. 10 the structure of a single rDNA repeat unit showing the positions where primers were designed to anneal during PCR.
Primers sequences as shown BD1, \(5'\)GTCGTAACTAGGTTTCCGTA\(3'\) (Ampe gene, ROTH) and 4S primer \(5'\)TCTAGTTGTGTTGAA (AG) TGTCGATG\(3'\) (ROTH). PCR-RFLP analysis of ribosomal DNA internal transcribed spacer 1 (ITS-1) region (Bowles and McManus, 1993a) using the restriction enzymes Msp1, Cfo1 and Rsa1 was done. The site of each endonucleases cutting (Roche diagnostic GmbH) are shown below:

\[
\begin{align*}
\text{Rsa1} & \quad \text{GT} \uparrow \text{AC}, \quad \text{Msp1} \uparrow \text{CGG}, \quad \text{Cfo1} \uparrow \text{GCG} \\
\text{CA} & \quad \text{TG} \quad \text{GGC} \quad \text{C} \quad \text{CG} \\
\end{align*}
\]

PCR-linked RFLP of \(E.\) granulosus origin were analyzed. PCR with the primer pair BD1 and 4S was performed as described by Bowles and McManus (1993c) in a 100 µl volume with the following modifications: The reaction mixture consisted of 400 – 500 ng DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM MgCl₂, and deoxynucleoside triphosphate at a concentration of 200 µM, 50 pmol of each primer and 1.5 units Ampli-Taq Polymerase (Perkin Elmer). The PCR conditions were 30 cycles, denaturation for 30 seconds at 95°C, annealing for 30 seconds at 55°C and elongation for 30 seconds at 72°C. PCR products were digested for 5 hours with the 4-base cutting restriction endonucleases Msp1, Cfo1 and Rsa1. After inactivation of the enzymes at 65°C for 15 minutes, the resulting restriction fragments were separated in a 3% agarose gel containing 1 µg of ethidium bromide. The electrophoresis apparatus was run at 100 voltage for 45 min. The gel was exposed to UV light and photographs of the pattern were obtained using a digital camera.

5.2.3 Mitochondrial DNA sequencing (Cytochrome C Oxidase-1):

The sequence of a region of the mitochondrial cytochrome c oxidase subunit 1 (CO1) was obtained. Primers designated by Bowles
et al. (1992) were used for PCR and sequencing of cytochrome c oxidase 1 gene. The sequence of the primers used was as follows; Forward 5’> TTTTTTGGGCATCCTGA GGTAT<3’ and Reverse 5> TAAAGAAAGACATAATGAAAATG <3’.

5.2.3.1 PCR for cytochrome c oxidase 1 sequence:

RCR was performed as described previously (Bowles et al., 1992; Bowles and McManus, 1993b). DNA sample (100 – 200 ngm) was mixed with 10 µl PCR buffer, 10 µl MgCl₂, 2 µl dNTPs, 50 Pmol CO1 Primer forward, 50 Pmol CO1 primer reverse, and 2.5 units Ampli-Taq polymerase (Perkin Elmer). The volume was completed to 100 µl with DNA free water. The condition of PCR reaction was completed in 30 cycles (94°C for 1 minute, 59°C for 1 minute and 72°C for 40 seconds). PCR product were electrophoresed through 1.5 (W/V Seakem ME (FMC Bioproducts), Tris-borate/EDTA (TBE) agarose gel and stained with ethidium bromide.

5.2.3.2 PCR product purification:

PCR products were purified with Q1A quick ™ columns to remove dNTPs and Primers. Fragments ranging from 100 bp – 10 Kb were purified from primers, nucleotides, polymerases and salts using Q1A spin columns (Q1A quick ™ GmbH, Germany) in a micro-centrifuge. The steps of purification of products of PCR - CO1 were as follows:

150 µl of phosphate buffer was added to 30 µl of PCR product and placed in Q1A quick spin column in a provided 2 ml collection tube. To bind DNA, the sample was added to the Q1A quick column and centrifuged for 30 – 60 seconds. Flow through was discarded, Q1A quick column was placed back to the collection tube. The column was then washed by 750 µl of phosphate buffer and
centrifuged for 30 – 60 seconds, flow through was discarded and the Q1A column was placed back in the same tube and the column was centrifuged for an additional 1 minute at maximum speed. Q1A quick column was placed in clear 1.5 ml microcentrifuge tube. The elute DNA, 50 µl of elution buffer (10 mM Tri-HCL, pH:8.5) or H₂O added to the centre of Q1A quick membrane and the column was centrifuged for 1 minute. To obtain high concentration of DNA, 30 µl of elution buffer was added to the centre of Q1A quick membrane and the column was left to stand for 1 minute and then centrifuged again. Elution buffer was dispensed directly into Q1A quick membrane for complete elution of DNA.

5.2.3.3 Cycle sequencing:

Cycle sequencing was performed on the Gene Amp 2400 (Perkin Elmer) with the AB1 Prism Big Dye Terminator Cycle sequencing Ready Reaction Kit (Applied Biosystems). The spin column was vortexed gently to resuspend the resin. The cap of the column was loosen quarter turn. The button closure of the spin column was snapped off and the spin column was placed in 2-ml collection tube. The sample was centrifuged at 3000 g for 3 minutes and the spin column was transferred carefully to another clean centrifuge tube and the sequencing reaction was applied slowly (10 – 20 µl) to the gel bed. Cycle conditions of 25 cycles were denaturation for 10 seconds at 94°C and annealing for 4 minutes at 60°C. Sample electrophoresis was done on the AB1 Prism 310 Genetic Analyzer (Applied Biosystems).

5.3 Results:

In this study a total of 56 . *E. granulosus* isolates (28 camels, 14 cattle and 14 sheep) and 4 samples of adult parasite (from a dog and 3
foxes were genotyped by PCR-RFLP and gene sequencing (CO1). The data indicated that the camel/dog strain (G6) occurs in Darfur region in camels, cattle and sheep as intermediate hosts and in dogs and foxes as final hosts.

5.3.1 PCR-RFLP:

Out of 56 isolates of camel, cattle and sheep examined, 42 were detected as G5/6 genotype with PCR-RFLP but 14 isolates could not be amplified giving a sensitivity of 75%. With the PCR-RFLP, 5 out of the 28 camel isolates could not be amplified, whereas the other 23 isolates were detected as the G6 (camel strain) genotype. Out of 14 cattle isolates examined, 4 could not be amplified, whereas the other 10 isolates were detected as G5 and G6 genotypes. Out of 14 sheep isolates examined, 5 could not be amplified whereas the other 9 isolates were detected as G6 genotype (Table 14).

Using PCR-RFLP we could not discriminate between the G5 (cattle) and G6 (camel) genotypes from cattle. Although of diverse geographical origin and intermediate hosts of camel, cattle and sheep isolates examined, they gave similar RFLPs pattern in Msp1, Cfo1 and Rsa1 restriction enzymes (Fig. 11).

5.3.2 Cytochrome c oxidase-1 gene sequence (CO1):

Cytochrome c oxidase-1 gene sequence (CO1) was obtained for the camel, cattle and sheep isolates examined above by PCR-RFLP. The sequences obtained by CO1 were aligned with published sequences (366 bp) for camel strain (G6) (Bowles et al., 1992).
Table 14: Genotyping of *E. granulosus* isolates analyzed by PCR-RFLP and CO1 sequencing.

<table>
<thead>
<tr>
<th>Host species</th>
<th>No. of Isolates</th>
<th>PCR-RFLP results (MSP1, Cfo1, Rsa1)</th>
<th>Gene sequencing results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Genotype</td>
<td>No. isolates</td>
</tr>
<tr>
<td>Camel</td>
<td>28</td>
<td>G6</td>
<td>23</td>
</tr>
<tr>
<td>Cattle</td>
<td>14</td>
<td>G5/6</td>
<td>10</td>
</tr>
<tr>
<td>Sheep</td>
<td>14</td>
<td>G6</td>
<td>9</td>
</tr>
</tbody>
</table>
Fig. 11. PCR-RFLP analysis of *E. granulosus* isolates using restriction endonuclease RsaI. Lane 1, cattle strain (G5); lane 2, sheep strain (G1); lanes 3 cattle isolate, lane 4, sheep isolate; lane 5 and 6 camel isolates; lane 7, camel strain (G6). Size of the molecular weight markers (ladder) indicated are: 100, 200, 300, 400, 500, 600, 700 and 800bp.
All the 56 *E. granulosus* isolates examined by (CO1) produced identical sequences to that of camel strain (G6 genotype) (Fig. 12).

The genotyping of the 4 samples of adult parasite from three foxes and one dog experimentally infected with protoscolices of camel, cattle and sheep origins using PCR-RFLP and gene sequencing (CO1) were detected as G6 (camel) genotype.
Fig. 12. PCR for CO1 sequencing of *E. granulosus* isolates. Lane 1, cattle strain (G5); lane 2, sheep strain (G1); lanes 3 and 4, camel isolates; lane 5 cattle isolate; lane 6, camel strain (G6); lanes 7 and 8, sheep isolates. Size of the molecular weight markers (ladder) indicated are: 100, 200, 300, 400, 500, 600, 700 and 800bp.
5.4 Discussion:

Several molecular techniques are now available. They allow a direct characterization of the genome of the parasite using genetic markers. Genetic variation in *E. granulosus* can be investigated in either the mitochondrial or the nuclear genome (Obwaller *et al.*, 2004).

In this study using RFLP-PCR and CO1 sequencing approach the occurrence of camel strain (G6) was confirmed in camels, cattle and sheep as intermediate hosts in the study area.

RFLP-PCR pattern of *E. granulosus* isolates of camel and cattle examined in this study demonstrated characteristic band patterns previously referred to as camel strain (G6) by Bowles and McManus (1993a). Using RFLP-PCR, amplification products suitable for RFLP were obtained from only 42 of 56 *E. granulosus* isolates. In addition, the band patterns obtained from reference material of cattle strain (G5) could not be distinguished from band patterns obtained from the G6 genotype of *E. granulosus*.

Gene sequencing of a part of the mitochondrial CO1 (Bowles *et al.*, 1992) that is highly specific revealed the occurrence of camel strain in camels, cattle and sheep. However, gene sequencing (CO1) is not suitable for screening of large numbers of samples due to high costs and time consumption.

The low sensitivity obtained by RFLP-PCR compared with the results obtained by CO1 sequencing may be explained by differences in stability between mitochondrial and nuclear DNA. The target of CO1 sequencing, is substantially more stable than nuclear DNA, the
target of the RFLP-PCR, due to its annular molecular structure presenting fewer targets for destructive DNAses.

Similar findings had been reported by Elmahdi (2003) in central Sudan where he also detected cattle strain (G5) for the first time in the Sudan. The presence of camel strain in the Sudan was previously described by McManus and Rishi (1989) who reported that the Sudanese, Somalian and Kenyan camel isolates all produce common patterns of hybridization by southern blot technique.

The absence of common sheep strain (G1) in the study area confirm the results of Elmahdi (2003) in central Sudan. Moreover, the very low fertility rate of cysts encountered in sheep in this study supported the previous findings by Saad and Magzoub (1989b) and Elmahdi (2003). However, the common sheep strain which is highly fertile in sheep, is prevalent in some neighbouring countries (Kenya and Libya). Since all host species (dogs and sheep) required for transmission of G1 are present, the accidental introduction of this strain from elsewhere may occur.

For the first time in the Sudan, *E. granulosus* was developed experimentally up to sexual maturity in foxes. RFLP-PCR and gene sequencing of mitochondrial (CO1) conducted on adult *E. granulosus* isolates of camel origin raised in dogs and foxes, and of cattle and sheep origins raised in foxes detected camel strain (G6) genotype in these hosts. These findings indicated that foxes could be suitable final hosts as dogs for the camel strain. This would complicate the epidemiological situation of the disease in the Sudan.
CHAPTER SIX
GENERAL DISCUSSION

Despite all the studies on human and animal echinococcosis prevalent in the Sudan (Eisa et al., 1962; Saad and Magzoub, 1989a and 1989b, Mohammed and Elmalik, 2000; Magambo et al, 1996), yet the real magnitude of the disease in domestic and wild animals and in man is still in need of further studies. The financial losses incurred by the disease have not been taken into consideration. Control measures and use of effective mass chemotherapy against both larval and adult stages of the parasite have not yet officially been adopted. This situation is often aggravated by the close association of dogs with domesticated animals and humans. It can also be influenced by human behaviour, cultural practices and social structures, land-use, life-styles and traditions in various communities (Watson – Jones and Macpherson, 1988). On the other hand, wild animals may break into the synanthropic cycle of the parasite due to their preying action on domestic animals. Eisa et al. (1962) pointed out to the possibility of wild carnivores harbouring the parasite in the Southern Region of the Sudan.

Investigation of climate and system of animal husbandry in the area revealed that in dry seasons crowding of animals during grazing on contaminated pasture enhances the parasite transmission to intermediate hosts. The moisture provided by the vegetation cover of soil during the rainy season creates a suitable microclimate for parasite eggs deposited on pasture, hence favour the survival and longevity of free-existing eggs.
As far as the definitive hosts are concerned in this study, a prevalence rate of 19.16% was recorded for *E. granulosus* infection in adult stray dogs, while none of the 116 foxes necropsied was found infected with *E. granulosus* although other species of large cestodes were encountered. This high prevalence in dogs and the large number of worms recovered from dogs examined (54 – 3823 worms) seem to bear true epidemiological relationship to the level of cystic hydatidosis observed in slaughtered camels (61.42%) and cattle (5.23%) in the area. This high prevalence in dogs may be due to the attitude of the people that camel lungs are unfit for human consumption and therefore thrown to dogs regardless of it condition. Improper disposal of condemned affected organs, illegal or unsupervised slaughtering in the community, low sanitary state of abattoirs, improper meat inspection, unawareness of the people and increased numbers of stray dog population were also behind the high prevalence observed in this study. In rural areas, packs of stray dogs were routinely seen in weekly market places (*Souks*) where large number of meat animals were slaughtered without meat inspection and affected organs and viscera were routinely discarded in the open or given directly to dogs. This practice naturally increases the parasite biomass in dogs and subsequently increases the risk of infection to people.

Although *E. granulosus* was not encountered in foxes examined in this study, the experimental transmission of the parasite of camel origin revealed comparable development of the parasite up to sexual maturity in foxes and dogs, with slightly fewer worm counts and relatively longer prepatent period in foxes. Also parasites of cattle and
sheep origins did develop in foxes. It is the first time to document the susceptibility of foxes to *E. granulosus* in the Sudan. The nonexistence of *E. granulosus* in wild caught foxes could be due to inaccessibility of cysts to foxes and that foxes usually prey on sheep and goats, which were proved by Saad and Magzoub (1989b), Elmahdi (2003) and in this study to play a minor role in the transmission and maintenance of the parasite. It may also be due to individual immunity of foxes examined. Sedentary farmers and nomadic pastoralists use the same source of surface water for drinking with their animals and for other purposes, such sources of water may be contaminated with faeces of dogs and foxes. Foxes are considered as crop pests and were seen in big numbers at groundnut farms at night, so farmers who usually eat uncooked groundnut during harvesting may be subjected to the risk of infection. Foxes are usually killed by hunters and village farmers. Their hides are used for making shoes and their fats as medicines, such practices will increase the risk to local human population.

From the findings in this study, it is obvious that hydatidosis is highly prevalent in camels and to a lesser extent in cattle. In areas where camels are slaughtered in relatively great numbers (Nyala), this may lead to a continuous source of infection to stray as well as owned dogs and subsequently a source of infection to camels and other domestic animals as well as man in the area. The importance of camels in the epidemiology of hydatid disease is also reflected by the high fertility rate in cysts examined (73.84%), the presence of larger cysts (up to 8.3 cm) and the occurrence of many cysts in the lungs of some animals.
Despite the low prevalence rate of hydatidosis among cattle (5.23%), cattle may still play an important role in the cycle as the fertility rate of the cysts recovered was found to be high (27.49%). This finding is in agreement with Saad and Magzoub (1989a), and Mohammed and Elmalik (2000) but differed from Dada (1980) who found a lower rate of fertility (7.4%) in Nigerian cattle. This contradiction may be attributed to the strain differences of *E. granulosus*.

In many countries, sheep and goats are always involved in the cycle of *E. granulosus*. Prevalence rates recorded in this study were 10.88% and 1.58% in sheep and goats respectively. The fertility rates of cysts encountered were 9.24 and 2.63% in sheep and goats respectively. The majority of cysts were calcified. An experiment was conducted to determine the role of sheep, goats and gazelles (*Gazella dorcas*) as true intermediate hosts for camel strain of *E. granulosus*. The results of the experiment had shown that these animal species play a minor or no role in the cycle of the parasite. As has been shown in the results, sterile, calcified and caseated cysts were recorded. These cysts may be of importance when evaluating the prevalence quantitatively but, as far as the transmission of the disease is concerned, one may be interested in the proportion of the infected population that carry fertile cysts and can therefore play an important role in the epidemiology of the disease.

The concept of strain diversity within the species *E. granulosus*, previously based on morphological, biological and biochemical and other features, has been principally confirmed by modern genetic studies (Eckert *et al.*, 2001). The modern molecular techniques will
allow a relatively rapid clarification of the epidemiological situation in a
given area, for example the identification of spill-over situations between
domestic and sylvatic cycles. Such studies are important for effective
control.

In this study, RFLP-PCR and CO1 sequencing techniques were
used. The results obtained in this work showed the existence of camel
strain (G6) in camels, cattle and sheep in the area. The results also
confirmed the absence of other *E. granulosus* strains. These are in
agreement with Elmahdi (2003) who reported the existence of camel
strain in camels, cattle and sheep and absence of sheep strain (G1) in
central Sudan. He also reported the presence of cattle strain (G5) in
cattle. This variation may be attributed to the small size of samples
examined, or the limited mix up of camel and cattle herds in the study
area. Because intra-specific variation in *E. granulosus* can influence the
antigenicity, pathogenicity and sensitivity to chemotherapeutic drugs
(Thompson and McMnus, 2001), it is suggested that strain variability
and the reservoirs of the parasite in each endemic region should be taken
into account to design control programme.

It is likely that the most important epidemiological determinants
for cystic echinococcosis in the Sudan include existence of
agricultural/animal husbandry management system with high numbers of
intermediate hosts (principally camels and cattle) and definitive hosts
(principally owned and stray dogs), most inhabitants living in close
proximity to their domestic animals, a low level of knowledge
concerning the basic life cycle and transmission routes of the parasite
and a prevailing lifestyle with poor sanitation. In addition, the role
of weekly outdoor market places (*souks*) in rural areas can not be
overlooked as an important factor in transmitting hydatid disease. Animals are slaughtered, meat inspection is not carried out and viscera are sold or discarded with infected material to ever-present stray dogs.

In conclusion, it is appropriate to refer to the following noticeable epidemiological facts.

In the sequential studies carried by several workers and confirmed in this study, camels in the Sudan can be considered as the main intermediate hosts for cystic echinococcosis and molecular characterization studies of *E. granulosus* strains indicated that camel strain (G6) was identified in camels, cattle and sheep.

Despite the low prevalence in cattle, the high fertility rate of cysts encountered during the investigation indicates their important role in the parasite cycle.

The role of sheep and goats as intermediate hosts for camel strain of *E. granulosus* was suggested to be insignificant in Darfur region.

The high prevalence of hydatidosis in camels and to a lesser extent in cattle, the presence of large number of infected stray dogs and improper disposal of infected offals suggest an on-going cycle of dog-camel-dog and dog-cattle-dog in the study area and that humans are at a high risk in this area.

Experimentally, foxes fed cysts of camel, cattle and sheep origins have proved to be susceptible to *E. granulosus* infection. This suggests the existence of spill-over situation between domestic and sylvatic cycles, but gazelles (*Gazella dorcas*) experimentally infected
with gravid segments of camel-dog origin were found non susceptible to infection.

The factors of transmission are totally uncontrolled and completely ignored by nearly all rural inhabitants who are most exposed to contamination risks. Basic hygiene practices and rigid control of animal slaughtering are not emphasized or enforced. Butchers in rural areas routinely feed hydatid cysts from slaughtered animals to their dogs and nomads leave carcasses of dead animals accessible to dogs.

In effect of the available information in this and other studies it is recommended that:

1. Further investigations of the existence of sylvatic cycles and cross transmissions and molecular characterization of cycling strains are necessary.

2. Initiation of control campaign in the area and concentration on mass educational programmes.
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Craig, P.S. (1986). Detection of specific circulating antigen, immune complexes and antibodies in human hydatidosis from Turkana (Kenya) and Great Britain, by enzyme – immunoassay. Parasite Immunology, 8: 171 – 188.


١٥٥
لا يمكن قراءة النص بشكل طبيعي من الصورة المقدمة.
لدى حالة باثرة تجربة أجريت لمدة ٥٦ يومًا: اكتشفت نتيجة التجربة أن عدد الغزالين، والذين تم التعرف على بعضهم البعض، كان منخفضًا في نسبة المخاطية.preferred

تتضمن تقنيات الاستخدام الحيوية للأERTICALITICといった الاهتمام، حيث تتم دراسة العدد الجزيئي الأحشاء، وتتضمن مناطق PCR - RFLP. وقد أجرت دراسة إحدى مناطق الغاز، تمكن من تحليل RNA و السلسلة الوراثية من في الغاز، حيث تبين أن الغازين (١٠٨) يمثلون معدل ليخت، و يحتوي على RNA بقية الغاز، بالإضافة إلى الغاز أخر.