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An Epidemiologic Study of Canine Echinococcosis and Livestock Hydatidosis in the Sudan

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Abbreviations

AE	Alveolar Echinococcosis
appr	Approximate
BLAST	Basic Local Alignment Search Tool
bp	Base pair
CA-ELISA	Copro-antigens ELISA
CE	Cystic echinococcosis
CI	Confidence interval
CT	Computed Tomography
°C	Degree centigrade
cox1	Cytochrome c oxidase subunit 1
cox2	Cytochrome c oxidase subunit 2
DALYs	Disability adjusted life years
DNA	Deoxyribonucleic acid
dnTPs	Desoxynucleosidtriphosphate
E. granulosus s.s.	*Echinococcus granulosus* sensu stricto
E. granulosus s.l.	*Echinococcus granulosus* sensu lato
ELISA	Enzyme linked immunosorbent assay
G	Genotype
g	Gram
Ig	Immunoglobulin
IST	Intestinal scraping technique
ITS1	Internal transcribed spacers 1
Kg	Kilogram
mg	Milligram
min	Minute
mm	Millimeter
ml	Millilitre
mPCR	Multiplex PCR
MRI	Magnetic resonance imaging
µM	Micromolar
µm	Micrometer
nad1	NADH dehydrogenase subunit 1
ng	Nanogram
OIE	Office International des Epizooties
PAIR	Puncture-aspiration-injection-reaspiration
PCR	Polymerase chain reaction
p.i	Post infection
PPS	Protein Precipitation Solution
PTA	Percutaneous thermal ablation
p-value	Probability value
RAPD-PCR	Random amplification of polymorphic DNA
RFLP	Restriction fragment length polymorphism
RNA	Ribonucleic acid

rrn S	Small sub- unit of ribosomal RNA
s	Second
SCT	Sedimentation and counting technique
SPP	Species
Sub-mPCR	Sub multiplex PCR
UK	United Kingdom
US	Ultrasonography
USA	United States of Amerika
US$	United States Dollar
WHO	World Health Organization
w/v	Weight/Volume
xg	Times gravity
x-ray	Radiation
x^2	Chi- square test

1 Introduction

Echinococcosis/hydatidosis is a chronic cyst-forming parasitic helminthic disease of humans as well as domestic and wild animals, mainly affecting ungulates. It is caused by the infection with metacestodes of tapeworm of the genus *Echinococcus*. The life cycles of these parasites are maintained through carnivores as definitive hosts, which harbor the adult worm in their intestine and a wide variety of animal species that serve as intermediate hosts. Humans can also become infected and act as aberrant dead-end intermediate hosts.

Based on recent molecular data and phylogenetic analysis, the genus *Echinococcus* includes nine valid species comprising *E. granulosus* sensu stricto (*E. granulosus* s.s., genotypes G1–G3), *E. equinus* (G4), *E. ortleppi* (G5), *E. canadensis* (G6–G10), *E. felidis*, *E. multilocularis*, *E. oligarthrus*, *E. shiquicus*, and *E. vogeli* (Carmena and Cardona, 2014). Genetic variability of the agents of cystic echinococcosis (CE) may influence host specificity as well as morphological, biochemical and biological differences (Alvarez Rojas et al., 2014). *E. granulosus* s.s., particularly the genotype G1, is responsible for most cases of human CE. *E. canadensis* and *E. ortleppi* are also known to be infective to humans (Torgerson and Budke, 2003).

Feeding dogs with raw offal of infected animals contributes to perpetuating the domestic cycle of *E. granulosus*. The infection has a cosmopolitan distribution particularly in poor pastoral communities where people raise livestock and keep dogs for guarding and/ or herding animals. In regions where cystic echinococcosis is endemic, human incidence rates can exceed 50 per 100 000 person-year and prevalences may range between 5-10% in parts of Peru, Argentina, east Africa, central Asia and China (Craig et al., 2007; Alvarez Rojas et al., 2014). Reports from Bulgaria, Kazakhstan and China described human CE as an emerging or reemerging disease in view of the increased numbers (Eckert et al., 2000).

CE therefore remains a serious human and animal health concern and has important economic consequences. In a comprehensive study, Budke et al. (2006) assessed the global socioeconomic impact of cystic echinococcosis. The annual global burden for human CE has been estimated to be approximately 1 million DALYs (disability-adjusted life years) or US $ 763.980.979 possibly losses. Moreover, livestock associated economic losses attributable to cystic echinococcosis include the liver being condemned as unfit for human consumption,

reduction in the weight of carcasses, decrease in hide value, decrease in milk production and decrease fecundity with up to US $ 2 billion annual losses on the global livestock industry.

Although CE is regarded as endemic in sub-Saharan Africa, recent studies revealed large regional variations in the prevalence of the infection within this area. CE has been reported in west African as well as east African countries from humans, domestic animals and many species of wild animals (Wahlers et al., 2012). The disease is a significant health problem among the pastoralists particularly in the Turkana district in Kenya with one of the highest CE incidences worldwide (176-220 cases per 100 000 persons) (Casulli et al., 2010). In the Sudan, CE has been documented in livestock animals from several regions (Elmahdi et al., 2004; Omer et al., 2010; Ibrahim et al., 2011). An ultrasound survey carried out to investigate human hydatid disease in two different areas in central Sudan with 300 and 651 people showed that the prevalence ranged between 0.3% and 0.8%, respectively (Ahmed et al., 2011).

Dogs infected by *E. granulosus* sensu lato (*E. granulosus* s.l.) are the main source of infection for human CE (Carmena and Cardona, 2013). Macpherson and Torgerson (2013) confirmed that identification of infected dogs may be important for diagnostic, epidemiologic or control/ surveillance reasons. The current epidemiological situation of canine echinococcosis is unknown or invalid in many parts of the world specifically in African countries, where prevalence data are scarce with limited molecular studies investigating the genetic diversity of canine isolates (Carmena and Cardona, 2013). This is particularly true in the Sudan, where only a few studies on canine echinococcosis have been conducted so far (Saad and Magzoub, 1986; Njoroge et al., 2001).

Therefore the main objectives of the study were:

1. To assess the prevalence of canine echinococcosis and to identify potential risk factors in owned dogs in different geographical regions in the Sudan.
2. To determine the prevalence of cystic echinococcosis in different animal species in these regions using abattoir data.
3. To type isolates of *E. granulosus* obtained from different hosts and regions of the Sudan at the molecular level (genotyping)

2 Literature review

2.1 Taxonomy

Human echinococcosis refers to the zoonotic infection caused by adult or larval (metacestodes) stages of cestodes belonging to the genus *Echinococcus* (Rudolphi, 1801), which is a member of the family *Taeniidae* in the order Cyclophyllidea, subclass Eucestoda, class Cestoda and phylum Platyhelminthes. The taxonomy of the genus *Echinococcus* has been extensively revised because of the inadequate morphological descriptions and sympatric occurrence of subspecies. Of the 16 species and 13 subspecies originally described within the genus *Echinococcus*, most are regarded as synonyms of *E. granulosus* and subsequent taxonomic revision recognized only four morphospecies (Table 2.1):

E. granulosus Batsch, 1786

E. multilocularis Leuckart, 1863

E. oligarthrus Diesing, 1863

E. vogeli Rausch and Bernstein, 1972

as valid taxa (Rausch and Nelson, 1963; Rausch, 1967; Rausch and Bernstein, 1972; Kumaratilake and Thompson, 1982). On the other hand, *E. equinus, E. ortleppi, E. canadensis, E. shiquicus and E. felidis* have recently been confirmed as valid species by several authors (Thompson and McManus, 2002; Xiao et al., 2005; Nakao et al., 2007; Hüttner et al., 2008) (Table 2.2).

Over the years, considerable phenotypic variations have been largely observed in *E. granulosus* and between isolates of the parasite from different intermediate host species in different geographical areas. The concept of a strain refers in the case of *Echinococcus* to variants, which differ significantly from other groups of the same species in gene frequencies and in one or more characters of actual or potential significance to the epidemiology and control of echinococcosis (Thompson and Lymbery, 1988). The intraspecific variation within the *E. granulosus* complex may affect several characteristics such as life-cycle pattern, host specificity, development rate, pathology, antigenicity, transmission dynamics and sensitivity

to chemotherapeutic agents. These differences may have important implications in epidemiology and control of echinococcosis (Thompson and McManus, 2002)

2.1.1 *E. granulosus*

E. granulosus is the most widely distributed species and occurs as a series of genetically distinct strains/genotypes. Based on morphological, biological, biochemical and molecular characteristics, ten distinct genotypes (G1–G10) have been identified within the *E. granulosus* (Bowles et al., 1992a; Bowles and McManus, 1993; Scott et al., 1997; Thompson and McManus, 2002; Lavikainen et al., 2003). These include two sheep strains (G1 and G2), two bovid strains (G3 and G5), a horse strain (G4), a camel strain (G6), two pig strains (G7 and G9) and cervid strains (G8 and G10). Recent taxonomic revisions suggested that *E. granulosus* genotypes should be split into 4 species: *E. granulosus* sensu stricto (genotypes G1–G3), *E. equinus* (G4), *E. ortleppi* (G5) and *E. canadensis* (G6–G10) (Nakao et al*.,* 2007).

2.1.2 *E. granulosus sensu stricto*

The three known genotypes of the proposed species *E. granulosus sensu stricto* are called the common sheep (G1), the Tasmanian sheep (G2) and the buffalo (G3) strains. All three have broad geographical distributions, a wide range of host specificity and occur sympatrically (Thompson, 2008). The common sheep strain (G1) is the most cosmopolitan form and is associated generally with human CE infection. Highest prevalences of infection are recorded in populations involved in extensive sheep farming and epidemiological studies suggest that this genetic variant is the principal strain infecting humans (Eckert et al., 2001b). The presence of this variant corresponds with areas of high prevalence of human CE such as in Morocco, Tunisia, Kenya, Kazakhstan, western China and Argentina (Jenkins et al., 2005).

The G2 strain has been detected for the first time in sheep from the state of Tasmania in Australia (Bowles et al., 1992a), then in human samples in Argentina (Rosenzvit et al., 1999) and more recently in sheep and cattle from Romania (Bart et al., 2006). For the first time in Africa, Maillard et al. (2007) confirmed the presence of the Tasmania sheep strain (G2) in sheep, camel and human samples in Algeria.

The buffalo G3 strain was initially introduced by Bowles et al. (1992a) as Indian buffalo strain of *E. granulosus*. This strain is not exclusively confined to its typical host, the water

buffalo, but it can be frequently harboured by sheep and cattle and is known to be infective to humans (Busi et al., 2007).

Table 2.1 Characteristics of the four species currently recognized within the genus *Echinococcus* (Adapted from Thompson and McManus, 2001)

Character	*E. granulosus*	*E. multilocularis*	*E. oligarthrus*	*E. vogeli*
Geographic distribution	Cosmopolitan	Central and northern Eurasia, northern North America	Central and South America	Central and South America
Definitive host	Primarily dogs and other canids	Primarily foxes, also other canids and cats	Wild felids	Bush dog
Intermediate and aberrant hosts	Primarily ungulates, also marsupials and primates, humans	Primarily arvicolid rodents, also other small mammals, humans	Rodents; agoutis, paca, spiny rats, humans	Primarily agoutis, also other rodents, humans
Nature of cyst	Unilocular	Multivesicular	Polycystic	Polycystic
Location of cyst	Visceral, primarily liver and lungs	Visceral, primarily liver	Peripheral, primarily muscles	Visceral, primarily liver
Mean length of large hooks of adult (μm)	32.0-42.0 (25.0-49.0)	31.0 (24.9-34.0)	52.0 (43.0-60.0)	53.0 (49.0-57.0)
Mean length of small hooks of adult (μm)	22.6-27.8 (17.0-31.0)	27.0 (20.4-31.0)	39.0 (28.0-45.0)	42.6 (30.0-47.0)
Mean number of segments (range)	3 (2-6)	4-5 (2-6)	3	3
Total length of strobila (mm)	2.0-7.0	1.2-4.5	2.2-2.9	3.9-5.6
Position of genital pore in mature segment	Posterior to middle	Anterior to middle	Anterior to middle	Posterior to middle
Mean number of testes (range)	32-68 (25-80)	18-26 (16-35)	29 (15-46)	56 (50-67)
Form of uterus	Lateral sacculation	Sac-like	Sac-like	Long tubular and sac-like

Egg production (days)	34-53	28-35	80?	?

2.1.3 *E. equinus*

E. equinus represents the horse strain of *E. granulosus* or G4 (Thompson, 2008). The parasite of horse/ dog origin was formally recognized by Williams and Sweatman (1963) from the type locality in Britain as a subspecies named *Echinococcus granulosus equinus*. Because this parasite occurred sympatric with the other subspecies, Rausch (1967) proposed that *E. g. equinus* should be invalidated. In the early 1970s *E. granulosus* of horse origin attracted much attention in the UK due to the high incidence of hydatid disease in horses (Thompson and Smyth, 1974). A subsequent comprehensive research on the epidemiology, biology and molecular genetics on *E. granulosus* of horse and sheep origin revealed that the two parasites differ biologically and genetically and that they are thus distinct from each other as either is from *E. multilocularis* (Le et al., 2002; Thompson and McManus, 2002). The parasite appears to have the dog as the only known definitive hosts and exclusively equines as intermediate hosts. No human infections with the G4 strain have been documented. In UK and Ireland, the horse/ dog cycle seems to be maintained by the feeding of horse offal to foxhounds (Torgerson and Budke, 2003). In south-western Africa, the parasite was recovered from the intestine of a dog that had been fed with a metacestode from a mountain zebra (*Equus zebra*) (Hüttner and Romig, 2009).

2.1.4 *E. ortleppi*

The cattle strain or G5 was first described as the species *E. ortleppi* by Lopez-Neyra and Soler Planas (1943) based on adult worms previously identified (Ortlepp, 1934). Once more the taxonomic status of *E. ortleppi* was questioned (Rausch and Nelson, 1963). According to the following studies on morphology and genetic analysis, there is certainly a form of *Echinococcus* that is adapted to cattle as its intermediate host. This form is quite distinct from *E. granulosus* of horse and sheep origin morphologically, genetically and in developmental characteristics. The cattle form is found in Europe, South Africa, India, Sri Lanka, Nepal, Russian Federation and possibly South America (Thompson and McManus, 2001, 2002). Dinkel et al. (2004) proved its existence in a domestic pig from Kenya and cattle from Sudan and Omer et al. (2010) stated that two hydatid cysts from cattle were identified as *E. ortleppi*.

The circulation of the cattle form in the one-humped camel from Tamboul in the Sudan was reported by Ahmed et al. (2013). Unlike resultant cysts of *E. granulosus* of sheep origin in cattle, the cysts of *E. ortleppi* are fertile and characterized by their well pulmonary development. In addition, this form has unusual strobilar morphology and rapid rate of development of the adult worm (Thompson and McManus, 2002).

In the Netherlands, Bowels et al. (1992b) documented one human case infected with *E. ortleppi* suggesting that this species may be less pathogenic to humans than the sheep strain of *E. granulosus*.

2.1.5 *E. canadensis*

The recommended taxonomic revision to unify the following genotypes: camel (G6), pig (G7), cervid (G8), pig/human (G9) and the Fennoscandian cervid (G10) into a single species called *Echinococcus canadensis* is supported by various molecular studies (Xiao et al.*,* 2005 ; Lavikainen et al.*,* 2006 and Nakao et al.*,* 2007). Whole mitochondrial genome sequences for *E. granulosus* genotype G6 (Kazakhstan)*,* *E. granulosus* genotype G7 (Poland) and *E. granulosus* genotype G8 (USA) were obtained by Nakao et al. (2007) and suggested that G6-G10 should be considered as single species named *E. canadensis*. The phylogenetic relations within this group are still under debate, since Thompson (2008) suggested that the *E. canadensis* cluster should be divided into two species *E. canadensis* (G8/ G10) and *E. intermedius* (G6/ G7).

By means of mitochondrial DNA sequencing, Bowles et al. (1992a) identified the camel strain (G6) in dromedary isolates from Somalia and the Sudan and a goat isolate originating from the Turkana region in Kenya. The camel form is known to principally affect camels and goats and found in the Middle East, Africa, southern Asia and South America (Thompson and McManus, 2001). Several studies have identified *E. canadensis* G6 genotype as the most prevalent genotype of *E. granulosus* in the Sudan, therefore camels seem to play an important role in the transmission cycle of this parasite (Dinkel et al., 2004; Omer et al., 2010; Ibrahim et al., 2011; Ahmed et al., 2013). It was believed that this genotype was less infective for humans or not infective at all, nevertheless cases of human infection are documented from Nepal, Iran, Mauritania, Kenya, Sudan, and Argentina (Thompson and McManus, 2002; Dinkel et al., 2004; Omer et al., 2010).

Table 2.2 Recent taxonomy of *Echinococcus* (Adapted from Thompson and McManus, 2001; Thompson and McManus, 2002; Xiao et al., 2005; Nakao et al., 2007; Thompson, 2008 and Hüttner et al., 2008).

Species	Strain/genotype	Intermediate host	Definitive host	Zoonotic
E. granulosus sensu stricto	G1(Common sheep)	Sheep, cattle, pigs, camels, goats, macropods, man	Dog, fox, dingo, jackal, hyena	Yes
	G2(Tasmanian sheep)	Sheep, cattle?, man	Dog (fox)	Yes
	G3(Buffalo)	Buffalo (cattle?), man	Dog (fox?)	Yes
E. equinus	G4(Horse)	Horses and other equines	Dog	Unknown
E. ortleppi	G5(Cattle)	Cattle, man	Dog	Yes
E. canadensis	G6(Camel)	Camels, goats, cattle?, man	Dog	Yes
	G7(Pig)	Pigs, man	Dog	Yes
	G8 (Cervid)	Cervids, man	Wolf, dog	Yes
	G9(Pig/ human)	Pigs, man	Unknown	Yes
	G10(Fennoscandian cervid)			?
E. multilocularis	Different isolates	Rodents, domestic and wild pig, dog, monkey, horse?, man	Fox, dog, cat, wolf, raccoon-dog	Yes
E. oligarthrus	None reported	Rodents	Wild felids	Yes
E. vogeli	None reported	Rodents	Bush dog	Yes
E. shiquicus	?	Plateau pika	Tibetan fox	Unknown
E. felidis	Lion	Zebra, wildebeest, warthog, bushpig, buffalo, various antelope, giraffe? Hippopotamus?	Lion	Unknown

These findings point out that the G6 strain has a wider distribution than previously thought. In addition to sheep and cattle, other camelids (Guanaco, Llama, and Alpaca) could be good putative intermediate hosts for the G6 genotype (Rosenzvit et al., 1999).

The pig strain (G7) is transmitted by domestic pigs in Europe, Asia and South America (Jenkins et al., 2005) and was also found in *Castor fiber*, wild boar (*Sus scrofa*) and cattle (Bart et al., 2006). A closely related genotype (G9) has been detected in Polish human patients, however the animal reservoir is still unidentified (Scott et al., 1997).

E. granulosus in cervids is primarily maintained by a predator–prey relationship involving wolves (*Canis lupus*) and wild Cervidae, principally elk (*Alces alces*), red deer (*Cervus elephas*) and reindeer (*Rangifer tarandus*), in the arctic and subarctic regions of Europe, Asia and North America (Eckert and Deplazes, 2004; Jenkins et al., 2005). The cervid strain (G8) was genetically characterized by Bowles at al. (1994) in metacestodes of the North American moose, whereas Lavikainen et al. (2006) described the Fennoscandian cervid genotype (G10) in cervid isolates in Finland and Sweden. In the majority of human cases, the clinical course due to cervid form is relatively benign as compared to that of domestic disease caused by other forms of *E. granulosus* (McManus et al., 2002).

2.1.6 *E. shiquicus*

Most recently, the data of mitochondrial DNA sequencing and morphological studies have identified *E. shiquicus* as a new sister species to *E. multilocularis* (Xiao et al., 2005; Nakao et al., 2007). The parasite has been isolated from the Tibetan fox (*Vulpes ferrilata*) in the Qinghai-Tibet plateau region of China. The larval form occurs in the plateau pika (*Ochotona curzoniae*), develops into a unilocular cyst mainly in the liver and its zoonotic transmission potential is unknown (Xiao et al., 2005).

2.1.7 *E. felidis*

Sylvatic cycles involving the lion and a number of intermediate host species counting warthog, zebra, wildebeest, buffalo and antelope have been documented from several African countries (Thompson, 2008). Based on re-examination of the morphology of *Echinococcus* spp., Verster (1965) proposed *E. granulosus felidis* as a subspecies. However, Rausch (1967) considered this as invalid because of the sympatric occurrence of various 'subspecies' of *E. granulosus* in southern Africa. Hüttner et al. (2008) collected taeniid eggs from fecal

samples of lions in Uganda and compared them with the preserved South African material of adult *E. g felidis*, which had been recovered from the lion (*Panthera leo*), identified morphologically 40 years ago and described it as a new species. Molecular analysis revealed that they were genetically identical and considered *E. felidis* is an independent taxon, although it is closely related to *E.g. sensu stricto*. The species is characterized by its unique use of a felid as the definitive host and the maintenance of genetic identity for both isolates from South Africa and isolates from Uganda. Further studies stated that *E. felidis* possibly occurs in hyenas (Hüttner et al., 2009).

Presently, its pathogenicity to humans is still unknown but its public health impact may be considered as minor because lions are largely restricted to national parks and game reserves, where there is little human activity. However, *E. felidis* may have an impact on pastoralists in East Africa who coexist with wildlife (Moro and Schantz, 2009).

2.1.8 *E. multilocularis*

E. multilocularis, commonly known as fox tapeworm, is widely found in areas of central Europe, most of northern and central Eurasia, parts of North America and probably in parts of northern Africa (Torgerson and Budke, 2003). Most human cases of Alveolar Echinococcosis (AE) in Europe were recorded in France, Austria, Germany and Switzerland. A major endemic area was spotted in south Gansu province in central-northwest China (Eckert et al., 2001b). In Central Europe, the life cycle of *E. multilocularis* is principally sylvatic, based on the predator-prey relationship between the red foxes (*Vulpes vulpes*) as the main definitive hosts and small rodents such as the common vole (*Microtus arvalis)* and the water vole (*Arvicola terrestris*) as intermediate hosts (Romig, 2003). Recent data from some parts of Europe revealed the presence of sylvatic cycle in urban areas due to growing fox populations after the introduction of anti-rabies vaccination and invasion of urban areas by these foxes. These data provide indication that there is ecological overlap to humans and the occurrence of such an urban cycle of *E. multilocularis* has to be regarded as a potential infection risk for humans (Eckert et al., 2000).

Together with the red fox, the arctic fox (*Alopex lagopus*), the coyote (*Canis latrans*), the wolf (*Canis lupus*), the raccoon-dog (*Nyctereutes procyanoides*), the sand fox (*Vulpes corsac*), and the Tibetan fox (*Vulpes ferrilata*) are known definitive hosts of *E. multilocularis*, depending on the geographic location (Torgerson and Budke, 2003).

Domestic dogs and cats can also act as definitive hosts after acquiring the infection from wild rodents infected with metacestodes and may be involved in a synanthropic cycle (Eckert and Deplazes, 1999; Craig et al., 2000; Petavy et al., 2000). Cats are much less susceptible to infection with the parasite as canids with a low or negligible egg excretion (Goodfellow et al., 2006).

Many species of small mammals are susceptible to *E. multilocularis* including members of the families *Arvicolidae*, *Sciuridae*, *Cricetidae*, *Dipodidae* and *Muridae*. Lagomorphs of the family *Ochotonidae* are commonly infected in parts of China. There have been also occasional reports of infections in insectivores such as the *Soricidae* and *Talpida* (Torgerson and Budke, 2003).

2.1.9 *E. vogeli*

E. vogeli was first recognized by Rausch and Bernstein (1972) after a routine fecal examination from a captured Ecuadorian bush dog, *Speothos venaticus*. The two neotropical species, *E. vogeli* and *E. oligarthrus* are confined to Central and South America (D'Alessandro, 1997). Human Neotropical Echinococcosis due to *E. vogeli* has been reported in Brazil, Colombia, Ecuador, Panama, Suriname and Venezuela (Rausch and D'Alessandro, 2002). The life cycle of *E. vogeli* is perpetuated with the bush dog and domestic dog as natural definitive hosts and rodents as intermediate hosts such as paca (*Cuniculus paca*) and agouti (*Dasyprocta* spp.). Since the bush dog is a timid canid and rarely observed, it may play a limited role in infecting man. It is assumed that domestic dogs may become infected with metacestodes when fed viscera of pacas by the hunters and appear to be the source of infection to humans (D'Alessandro and Rausch, 2008).

2.1.10 *E. oligarthrus*

Definitive hosts of *E. oligarthrus* are wild felids. In Central and South America the infection has been recorded naturally in the yaguarundi (*Felis yagouaroundi*), the puma (*F. concolor*), the jaguar (*F. onca*), the ocelot (*F. pardalis*), the pampa cat *(F. colocolo*), and the Geoffroyi cat (*F. geoffroyi*). The South American rodents; Agoutis (*Dasyprocta spp.*), spiny rats (*Proechimys spp.*) and pacas are the known intermediate hosts (Basset et al., 1998; Tappe et al., 2008). Domestic cats could be experimentally infected, therefore they may represent a potential risk to humans (Tappe et al., 2008). Hydatid disease caused by *E. oligarthrus* was reported in Venezuela, Brazil and Suriname (D'Alessandro, 1997).

2.2 Global distribution of *E. granulosus*

Echinococcus granulosus has a worldwide geographical distribution (Figure 2.1). It occurs in all continents, with highest prevalence in parts of Eurasia (particularly Mediterranean region, Russian Federation, adjacent independent states and China), north and east Africa, Australia and South America. Only a few areas for instance Iceland and Greenland believed to be free of *E. granulosus* (Eckert et al., 2001b).

In Africa, cystic echinococcosis (CE) is endemic in all North African countries including Morocco, Algeria, Tunisia and Libya. The disease has been also recorded from most of Sub-Saharan states such as Sudan, Ethiopia, Kenya and Uganda, and most of Western, Central and Southern Africa (Torgerson and Budke, 2003; Cardona and Carmena, 2013). CE is a major public health problem among transhumant pastoralists of eastern Africa (Karamajong, Maasai, Nyangatom, Toposa and Turkana peoples) with the highest worldwide incidence in man in the Turkana communities in the northwest of Kenya (Casulli et al., 2010).

Sheep, goats, cattle, camels and pigs are most commonly domestic intermediate hosts in different parts of Africa. Furthermore CE is also recorded from a wide range of wild herbivores similar to zebra and buffalo. *E. granulosus sensu stricto.* (G1- G3), *E. ortleppi* (G5) and *E. canadensis* (G6-G10) have been described in production animals in different regions in Africa. For instance, *E. granulosus* G1 (sheep strain) has been reported from Algeria, Tunisia, Ethiopia and Kenya. Moreover, *E. ortleppi* (G5) (cattle strain) has been documented in the Sudan and Ethiopia, whereas G6 (camel strain) has been identified in Egypt, Sudan, Mauritania, Somalia and Kenya (Romig et al., 2011; Cardona and Carmena, 2013).

Cystic echinococcosis has been investigated in the Sudan in all domestic livestock species. Spatial variations in CE prevalence estimate are reported in abattoir-based surveys from central, western and southern Sudan. From central Sudan, prevalences from 2.7-20% in cattle, 29.7-55.6% in camels and 0.6-6.9% in sheep were reported (Elmahdi et al., 2004; Ibrahim et al., 2011; Wahlers et al., 2012). In western Sudan, the highest prevalences of CE were described among camels (61.4%), sheep (11.9%), cattle (5.2%) and goats (1.9%) (Omer et al., 2010). In southern Sudan, infection rates of 7.1%, 2.7% and 4.3-7.1% were found in cattle, sheep and goats respectively (Njoroge et al., 2000; Omer et al., 2010).

In the Sudan, the basic data on the prevalence of *E. granulosus* in dogs is still lacking. However, Saad and Magzoub (1986) reported that 25 dogs out of 49 (51%) were infected with *E. granulosus* in the Tamboul area in central Sudan. In another necropsy study conducted in 2001, 54.2% of the dogs in southern Sudan were recorded positive for the presence of *E. granulosus* infection indicating that the prevalence of CE in this area may be higher than in other parts of the world (Njoroge et al., 2001).

Figure 2.1

Distribution of *Echinococcus granulosus* and cystic echinococcosis (hydatidosis), worldwide, 2009
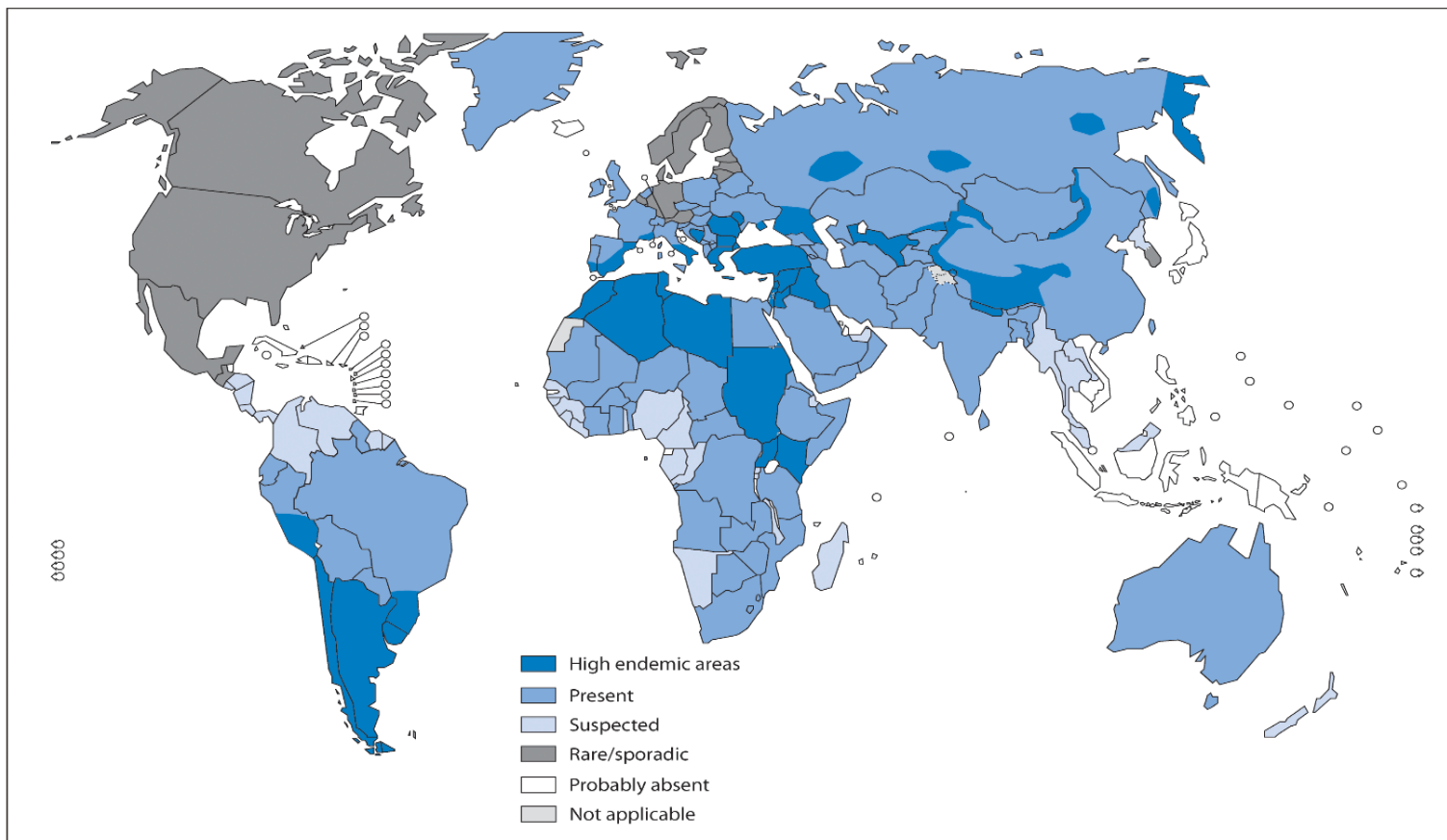
Legend:
- High endemic areas
- Present
- Suspected
- Rare/sporadic
- Probably absent
- Not applicable

Data Source: World Health Organization
Map Production: Control of Neglected Tropical Diseases (NTD)
World Health Organization

World Health Organization

2.3 Biology and life cycle

The life cycles of *E. granulosus* strains can be classified as domestic, involving dogs and farm livestock or as sylvatic, involving wild carnivores and ungulates. The latter transmission cycle is globally the most widespread and poses the greatest threat to humans (Torgerson and Budke, 2003). In many areas of endemic infection, both life cycles coexist or overlap. For example, the common sheep strain operates between dingoes and macropods marsupials in Australia. Another life cycle involving dogs and domesticated reindeer operate in the higher latitudes in northern North America and Eurasia. Complex situations of coexisting or overlapping domestic and sylvatic cycles complicate the control efforts of echinococcosis (McManus et al., 2003) (Figure 2.2).

E. granulosus characteristically uses Canidae, principally domestic dog (*Canis familiaris*), and other wild carnivores such as hunting dogs (*Lycaon pictus*), jackals (*Canis mesomelas* and *C. aureus*), wolves (*Canis lupus*), coyotes (*Canis latrans*), dingoes (*Canis dingo*) and foxes (*Vulpes vulpes*) as definitive hosts. A large number of mammalian species can be intermediate hosts, including domestic and wild ungulates belonging to various groups for instances, Bovidae, Suidae, Equidae, Camelidae and Cervidae. Infection with the metacestodes stage of *E. granulosus* occurs also in hosts, which do not play a role in the perpetuation of the cycle (aberrant or accidental intermediate hosts) similar to humans and other mammals (Eckert et al., 2001a).

Definitive host acquires the infection by ingestion of offal containing hydatid cysts with viable protoscolices (McManus et al., 2003). After the ingestion of protoscolices, they evaginate in the upper duodenum following exposure to pepsin in the stomach, bile and an increased temperature. Later they develop to the sexually mature adult worm nearly 4-6 weeks after infection depending on the species and strain and on susceptibility of the host (Thompson and McManus, 2001). The adult tape worm varies between 2-7 mm, usually has three or four segments and resides in the small intestine of the definitive host, where gravid proglottids release eggs that are passed in the feces. The eggs of *Echinococcus* spp. are 30-40 μm in diameter and morphologically indistinguishable from other tapeworm eggs of the genus Taenia. Following ingestion by a suitable intermediate host, the eggs hatch, thus releasing the oncospheres, which penetrate the intestinal wall assisted by the hook movements and are transported through the circulatory system to the liver, lungs and other

organs like kidneys, spleen, muscles and brain where they develop in several months into a cyst (hydatid cyst) that contains numerous tiny tapeworm heads called protoscolices. The larval stage (metacestode) is a bladder consists of two parasite-derived layers: an outer protective acellular layer (laminated layer) and an inner cellular layer (germinal layer) from which the protoscolices are produced through a sexual budding (McManus et al., 2003) (Figure 2.3).
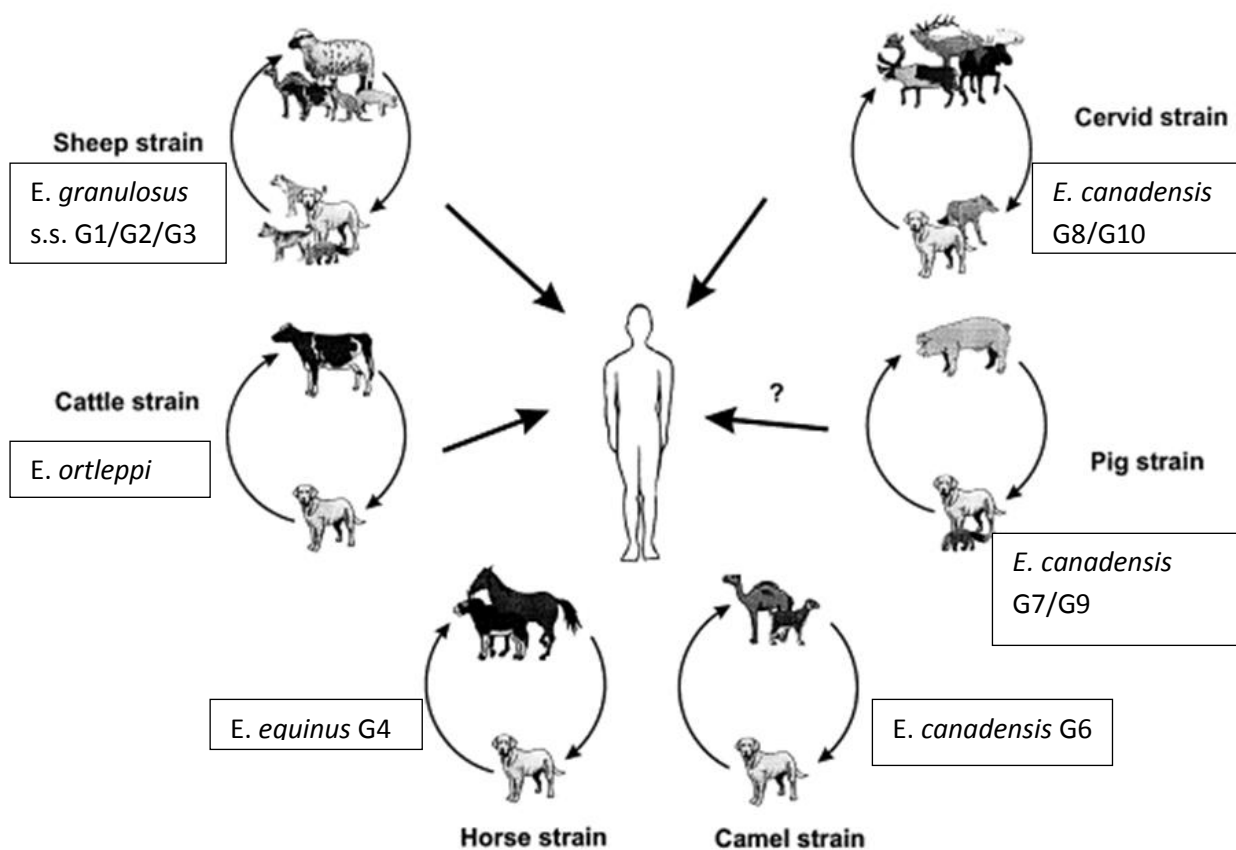
Figure 2.2 Life cycle patterns of *Echinococcus granulosus* represent distinct strains (adapted and modified from Thompson and Lymbery, 1990)

Figure 2.3 life cycles of *Echinococcus granulosus*

(Modified from http://www.cdc.gov/parasites/echinococcosis/biology.html)
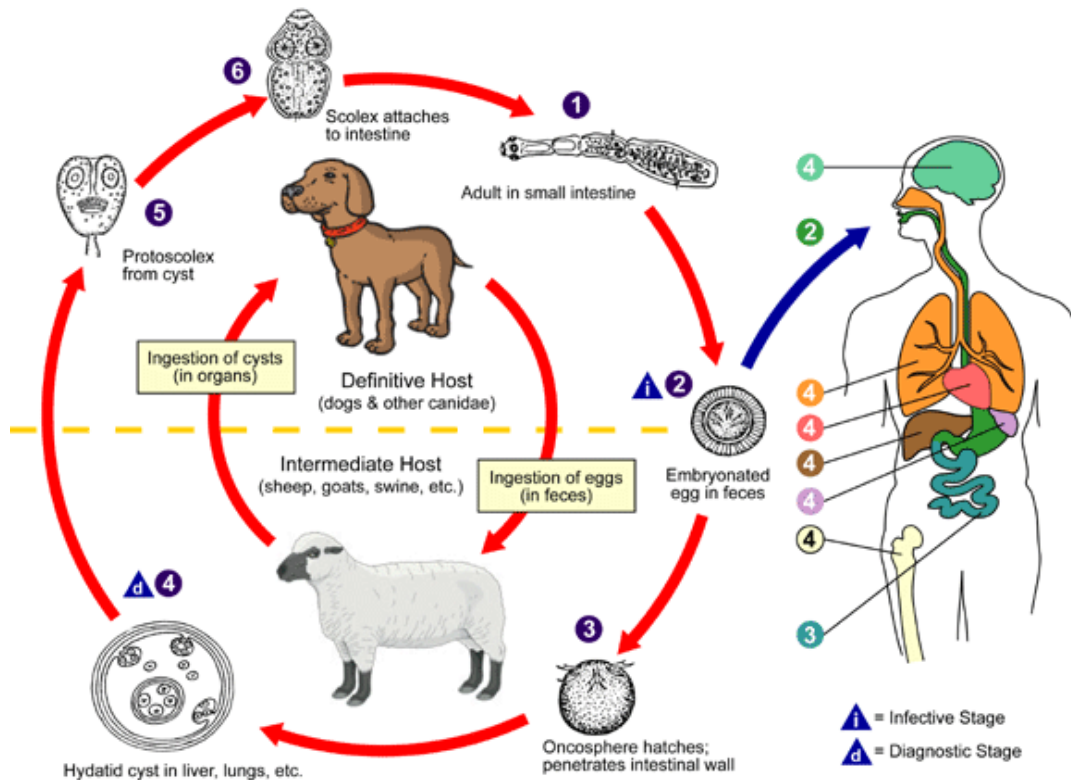
❶ The adult *Echinococcus granulosus* (3 to 6 mm long) resides in the small bowel of the definitive hosts, dogs or other canids.

❷ Gravid proglottids release eggs that are passed in the feces.

❸ After ingestion by a suitable intermediate host, the egg hatches in the small bowel and releases an oncosphere that penetrates the intestinal wall and migrates through the circulatory system into various organs, especially the liver and lungs.

❹ In these organs, the oncosphere develops into a cyst that enlarges gradually, producing protoscolices and daughter cysts that fill the cyst interior.

❺ The definitive host becomes infected by ingesting the cyst-containing organs of the infected intermediate host.

❻ After ingestion, the protoscolices evaginate, attach to the intestinal mucosa, and develop into adult stages ❶in 32 to 80 days.

❷ Humans become infected by ingesting eggs, with resulting release of oncospheres ❸in the intestine and the development of cysts④, ④, ④, ④, ④, ④in various organs.

2.4 Epidemiology and transmission dynamics of *E. granulosus*

In spite of the fact that very limited information is currently available about canine echinococcosis in the Sudan, the domestic dog serves as the only known reservoir for the *E. granulosus* adult worm. In the Tamboul area in central Sudan, Saad and Magzoub (1986) confirmed the presence of the parasite in the intestine of 25(51%) necropsied stray dogs and the range of worm recovery was 7-28.400. In the absence of recent records on the situation of CE in wild animals in Sudan, the domestic cycle involving dogs and livestock (sheep, goats, cattle and camels) was the only transmission cycle has been reported (Elmahdi et al., 2004; Omer et al., 2010 Ibrahim et al., 2011; Ahmed et al., 2013). For that reason, dogs play a recognized role as the main source of infection for both humans and livestock animals in the country.

One of the most important factors influencing the spread, persistence and reemergence of *E. granulosus* infection is the presence of large numbers of stray and free-roaming dogs with a high prevalence of the parasite (Todorov and Boeva, 1999). This situation may also be relevant to the Sudan because of the high prevalence of the disease previously reported by Saad and Magzoub (1986) combined with lack of information and statistics regarding the true number of stray dogs. Therefore, these circumstances could lead to a high contamination of the environment with *E. granulosus* eggs and consequently imply a potential risk for both humans and food animals. Likewise human behavior helps to perpetuate the domestic cycle of *E. granulosus* through feeding of infected livestock viscera to dogs (both owned and ownerless), which is the principal source of infection with *E. granulosus* (Eckert and Deplazes, 2004; Macpherson, 2005). A further significant risk factor is dogs having easy access to organs of livestock infected with *E. granulosus* cysts specifically in the rural areas where inefficient animal slaughtering and meat inspection are present (Todorov and Boeva, 1999). Dogs allowed scavenging or roaming freely has also been identified as a risk factor in endemic areas (Buishi et al., 2005a).

Home slaughter, e.g. during religious and local festivities, accompanied by inadequate disposal of viscera is a common practice cited as a factor associated with the presence of the parasite in domestic dogs (Torgerson and Budke, 2003; Seimenis, 2003). Previous studies

have proven that being a farm dog is a risk factor for *E. granulosus* infection due to the higher contact with livestock and consequently great access to infected carcasses. Male and younger dogs have been found carrying higher worm burdens than female, older dogs. (Budke et al., 2005; Buishi et al., 2005a; Moro et al., 2005; Acosta-Jamett et al., 2010; Inangolet et al., 2010). Numerous studies stated the increasing risk for canine infection and socio-economic factors such as dog owner's lack of knowledge about parasite transmission and shortages in the anthelmintic treatment of dogs (Buishi et al., 2005a; Buishi et al., 2005b; Acosta-Jamett et al., 2010).

Improper disposal of infected viscera, backyard slaughtering under unhygienic conditions, lack of dog population control measures, absence of any anthelmintic treatment of dogs and poor public awareness about the disease are also factors associated with high risk of canine echinococcosis in African dogs as reviewed by several authors (Buishi et al., 2005a; Azlaf et al., 2007; Kebede et al., 2009; Inangolet et al., 2010; Jones et al., 2011). These determinants increase the access of dogs to uncooked and infected offal and therefore maintain the transmission of *E. granulosus* in African countries.

The biotic potential of the parasite, acquired immunity by the intermediate hosts and environmental factors in the free-living egg phase were identified as parameters contributing to the regulation and stability of the of populations of *E. granulosus*. An understanding of such factors is an essential basis for the planning of control measures. The biotic potential is a major contribution to the transmission dynamics of the parasite and can be defined as the potential number of viable cysts, which can be established in an intermediate host by an individual definitive host per day. In contrast to the other *Taenia* species, the biotic potential of *E. granulosus* is relatively low with a generally reported mean worm burden of about 200-400 (Gemmell, et al., 2001b). In highly endemic areas, however, dogs may be infected with heavier worm burden. In Tunisia, infected dogs had a mean worm burden of 2,543 adult worms (Lahmar et al., 2001). Previous studies indicated that there is no evidence of parasite-induced immunity in the intermediate host. Immunity against *E. granulosus* can be experimentally acquired or induced but it requires large numbers of eggs. Together environmental temperature and humidity influence egg survival and infectivity. Increasing the temperature gradually decreases the life-span of the eggs to 2-14 day at 37-39°C and in the same way lower humidity decreased the life-span dramatically (Torgerson and Heath, 2003).

2.5 Human echinococcosis

Human cystic echinococcosis occurs when humans accidentally ingest eggs of *E. granulosus*, which have been shed in the feces of the definitive host. Fecal-oral transmission occurs by handling infected definitive hosts particularly in the course of playful and intimate contact with dogs. It has been shown that *Echinococcus* eggs adhere to the hairs around the anus, muzzle and paws. Contaminated water and food and secondary contamination via flies and other arthropods are potential sources of infection. Metacestodes develop slowly in various anatomic sites of the human body and can get a volume of several liters and may contain thousands of protoscolices. The liver and the lungs are the most frequently affected organs and approximately 20-40% of patients have multiple cysts or multiple organ involvement (Moro and Schantz, 2009). The initial phase of CE is asymptomatic with small well-encapsulated cysts and it has been reported that up to 60 % of the CE cases may be asymptomatic. After several months or years, the infection may become symptomatic due to the pressure of the cyst on the adjacent tissues (Pawlowski et al., 2001). The induction of morbidity depends on numerous factors including; number, size and developmental status of the cysts, activity or inactivity, the organ, the position of the cysts within the organ, the pressure of cysts on surrounding tissues and the defense mechanisms of the infected individual (Eckert and Deplazes, 2004).

E. granulosus infection in humans has re-emerged in several countries. In Bulgaria, the annual incidence of human CE increased to 3.3 per 100 000 between the 1970s and the mid-1990s (Todorov and Boeva, 1999). A similar situation has also been reported from Kazakhstan, China and South America (Eckert et al., 2000).

Diagnosis of human echinococcosis is highly dependent on imaging techniques such as ultrasonography (US), computed tomography (CT), standard radiology (X-ray) and magnetic resonance imaging (MRI). An international classification system of ultrasound images for hepatic cysts has been released by the World Health Organization (WHO). Ultrasonography-guided fine-needle puncture has been used in recent years as diagnostic procedure in doubtful cases of human CE (Pawlowski et al.*,* 2001; McManus et al.*,* 2003). Immunodiagnostic tools are useful in primary diagnosis and also for follow-up patients after surgical or pharmacological treatment (McManus et al.*,* 2003). IgG- ELISA is used in the diagnosis of CE by detecting serum antibodies but false-negative and false-positive results may occur. Following puncture or surgical approach, hydatid cyst fluid samples can also be detected by

ELISA for the presence or absence of *Echinococcus* antigen (Pawlowski et al., 2001). The standard double antibody sandwich ELISA is commonly used for measuring the concentration of circulating parasite antigens (Zhang et al., 2012). The immunoblot assays confirm the infection by using specific echinococcal antigens (Moro and Schantz, 2009).

For treatment of human CE, surgery has the potential to remove the cysts. However, it may be impractical in patients with multiple cysts or patients with high surgical risk. Chemotherapy with benzimidazole compounds, more recently treatment with PAIR (puncture-aspiration-injection-reaspiration) and PTA (percutaneous thermal ablation) are other options for CE treatment (Eckert and Deplazes, 2004).

2.6 Diagnosis

As the life cycle of *Echinococcus* spp. involve two mammalian hosts, the diagnosis of infection for epidemiological studies must address both metacestodes in the intermediate host and the adult worm in the definitive host using different diagnostic approaches due to different parasite locations in these hosts (Craig et al., 2003).

2.6.1 Diagnosis of *E. granulosus* in the definitive host

2.6.1.1 Detection of eggs and proglottids

In contrast to many helminth infections, reliable intra vitam diagnosis of taeniid tapeworm infections cannot be achieved by routine coproscopic techniques (flotation method). Straightforward microscopical detection of worm eggs in fecal samples, may reveal the presence of taeniid eggs, but will not allow distinguishing infections with *Echinococcus* spp. from those with *Taenia* spp. (Mathis and Deplazes, 2006). Additionally, due to irregularity of egg excretion, the sensitivity of this methodology is limited by the variable intensity of egg shedding over time (Deplazes et al., 2003). *E. granulosus* proglottids spontaneously discharged with feces may be detected and morphologically identified if they are in good condition (Eckert et al., 2001a).

2.6.1.2 Arecoline purgation

Arecoline purgation is a standard method for ante-mortem diagnosis of *E. granulosus* infection in dog populations (Craig et al., 1995). Arecoline surveillance was used during eradication campaign in New Zealand and in many epidemiological studies worldwide (Torgerson and Deplazes, 2009). Application of the parasympathomimetic drug arecoline

hydrobromide (4 mg/kg) to dogs results in purgation of the entire intestinal contents after 30 min (OIE, 2008). The drug also paralyses tapeworms which can then be collected and identified morphologically (Torgerson and Budke, 2003). This technique has several serious limitations: it is time consuming, can be hazardous to the operator and can also cause serious reactions in dogs. Furthermore, not every dog will purge and the method has a highly variable sensitivity (Craig et al., 1995). A recent study indicates that the sensitivity of arecoline purgation might be as low as 38% for *E. granulosus* detection in dogs (Ziadinov et al., 2008). With 100% specificity, purgation remains the only quantitative technique that can be used in the living dog and can also be employed when evaluating other diagnostic procedures such as copro-antigen or copro-DNA tests, because it can prove the presence of infection (Torgerson and Deplazes, 2009).

2.6.1.3 Necropsy

The most reliable means of diagnosis of canine echinococcosis is by necropsy, as the worm burden can be estimated and parasites collected for identification (Eckert, 1997). Straight forward coproscopical examination may reveal the presence of Taeniid eggs, but will not distinguish infection with *Echinococcus* species (Torgerson and Budke, 2003). Necropsy of dogs followed by the sedimentation and counting technique (SCT) can be applied after worms are released, retrieved and counted from the sediment using a binocular microscope. The intestinal scraping technique (IST) is a less laborious method for mass screening and has a sensitivity of about 78% (Torgerson and Deplazes, 2009). These methods are time-consuming and require special safety precautions due to the infection risk for the investigator. Moreover, they can be applied to dead animals only and accordingly they are unsuitable for diagnosis in pet animals (Deplazes et al., 2003). Consequently immunological and molecular approaches have been developed.

2.6.1.4 Serum antibodies detection

Serological diagnosis of canine echinococcosis due to *E. granulosus* has been based on the detection of antibodies (IgG, IgA and IgE) by ELISA using an *E. granulosus* protoscolex antigen. Experimental infection of dogs in Australia indicated that IgA antibody levels were detectable after one week post infection (p.i.), while IgG levels peaked after three weeks p.i. and an increase in IgE antibodies was observed after two weeks (Gasser et al., 1993). The application of this method in endemic areas of Kenya and Uruguay indicated that the overall specificity ranged from 70–97% and sensitivity varied (40–61%) when compared to positive

worm burden at necropsy (Jenkins et al., 1990) or after arecoline purgation (Gasser et al., 1994). The high specificity of these tests supports the useful application in screening of *Echinococcus* in dog populations (Eckert et al., 2001a).

2.6.1.5 Coproantigen detection by ELISA

Recently, two alternative approaches, the detection of parasitic copro-antigens by ELISA (CA-ELISA) and copro-DNA molecules by PCR (Copro-PCR) in animal fecal samples have been successfully developed and evaluated (Hartnack et al., 2013). The application of Taeniid-specific coproantigen was first reported for *E. granulosus* in infected dogs. In this study, *Echinococcus* antigens were demonstrated in feces prior to patency, and therefore independent of egg production. Additionally cross-reaction with antigens in human Taenia-infected feces was detected (Allan and Craig, 2006).

CA-ELISAs using antibodies against somatic or excretory-secretory antigens of adult *E. granulosus* have been developed for the diagnosis of intestinal *Echinococcus* infections in dog populations (Allan et al., 1992; Deplazes et al., 1992). The sensitivity of such tests is strongly dependent on the worm burden. Deplazes et al. (1992) described a diagnostic sensitivity and specificity of 87% and 98%, respectively, for animals harboring more than 200 worms. Copro-antigen tests have the advantage that they may detect prepatent infections (10-20 days post infection) and disappear within a few days after the treatment of infected dogs. Another important advantage is that they can also be applied to environmental samples, which facilitates investigation of parasite transmission dynamics without interacting with the populations of wild or domesticated definitive hosts. They also allow the reliable diagnosis of *Echinococcus* spp. from both necropsied and living definitive hosts (Deplazes et al., 2003).

Coproantigen ELISAs have been successfully used in a number of studies in Uruguay (Malgor et al., 1997), Cyprus (Christofi et al., 2002) and Libya (Buishi et al., 2005a), demonstrating their usefulness for epidemiological investigations.

2.6.1.6 CoproDNA detection by PCR

The CoproDNA PCR approach is based on detection of parasite DNA directly from fecal samples. Parasite DNA excreted together with eggs, proglottids or parasite cells (Mathis and Deplazes, 2006). Chemical treatments for the disruption of the keratin embryosphere or physical means by freezing and thawing the eggs followed by chemical method have been

described form several groups to enable the extraction of the oncospheres (Cabrera et al., 2002a). Bretagne et al. (1993) developed a novel procedure for DNA isolation from feces using alkaline lysis step and an organosolvent extraction followed by a step of purification with a commercial DNA purification kit.

PCR was introduced as a coprodiagnosis tool in the search for alternative methods for the diagnosis of infection with *Echinococcus* spp. in definitive hosts (Dinkel et al., 1998). PCR-based techniques for diagnosis of intestinal infections are mainly used as confirmatory tools of copro-antigen positive samples or a method of choice to identify taeniid eggs recovered from fecal specimens or from environmental samples (Mathis and Deplazes, 2006).

Recently species specific or strain specific PCR based tests have been developed for directly use on feces or on egg isolated from feces to confirm the presence of *Echinococcus* infection (Barnes et al., 2012). First, Bretagne et al. (1993) developed a species-specific coproDNA PCR for the detection of intestinal *E. multilocularis* infections in fecal samples of foxes targeting the U1 snRNA gene. The test reportedly has 100% specificity and an analytical sensitivity of 1 egg per 4 g of feces. *E. multilocularis* mitochondrial 12S rRNA gene has been targeted by Dinkel et al. (1998) in a nested PCR with a specificity of 100%. A PCR test was established by Cabrera et al. (2002a) for the specific diagnosis of six strains of *E. granulosus* (G1, G2, G4, G5, G6 and G7) eggs collected from the environment using a set of primers that was designed from aligned mitochondrial sequences. Cabrera et al. (2002a) concluded that the primer set showed high sensitivity and specificity for the identification of *E. granulosus* eggs and the test displayed no cross reactivity with *E. multilocularis* but shared similar genetic sequences to *E. oligarthrus* and *E. vogeli*, so it will only behave as species-specific for samples from areas other than South and Central America.

Different PCR systems have so far been reported by at least three research groups (Abbasi et al., 2003; Stefanic et al., 2004; Dinkel et al., 2004). Abbasi et al. (2003) optimized the first PCR that amplified a newly repeated sequence of sheep strain of *E. granulosus* (Eg G1 Hae III). A 100% analytical sensitivity and 100% analytical specificity were reported but the test also amplified DNA from horse, camel cattle and goat metacestodes. The other PCR assays have been developed to amplify regions of the 12S rRNA gene for the identification of *E. granulosus*. Stefanic et al. (2004) described also a specificity of 100% and no cross-reaction with *E. multilocularis* and *E. vogeli*.

Boufana et al. (2008) assessed the Abbasi, Stefanic and Dinkel primers using tissue and canid samples. The overall sensitivities of the three PCR tests were 52.6%, 73.7% and 100% for the Abbasi, Dinkel and Stefanic tests, respectively, for DNA amplification from feces of naturally infected dogs with *E. granulosus* and worm burdens ranging from 4 to > 4000 adult parasites. According to Boufana et al. (2008) the Abbasi PCR primers were the most species-specific for detecting *E. granulosus* and therefore useful in confirmation of *E. granulosus* infection in dogs. In addition, the Dinkel primers appeared to be strain specific for *E. granulosus* G1 but they show evidence of cross-reaction with other canid tapeworm species. Although, the Stefanic primers was the most sensitive of the three assays but had the lowest specificity for *E. granulosus*.

For the diagnosis of parasitic helminth infection, multiplex PCR (mPCR) was first demonstrated for the differential diagnosis of taeniasis and cysticercosis from human fecal materials (Yamasaki et al., 2004). For the identification of morphologically indistinguishable eggs of taeniid tapeworms from carnivores, Trachsel et al. (2007) developed a multiplex PCR using primers that were designed targeting the mitochondrial genes: NADH dehydrogenase subunit 1 (nad1) and the small subunit of ribosomal RNA (rrnS). This mPCR allows the differentiation among *E. granulosus* (all genetic variants), *E. multilocularis* and *Taenia* spp. infections. Additional analysis of the amplicons by sequencing or restriction fragment length polymorphism (RFLP) could identify most of the described *E. granulosus* genotypes and *Taenia* species. Using DNA from 55 fecal samples positive for taeniid eggs, validation of his PCR revealed the high sensitivity of the approach as a single taeniid egg could be detected, in accordance with other single-target PCR-based assays described by Dinkel et al. (1998) and Abbasi et al. (2003). Although the primers for *E. multilocularis* were species-specific, both primers for identifying *Taenia* spp. or *E. granulosus* were not strictly specific for their proposed target organisms. *E. granulosus* primers also amplified DNA from *E. vogeli*, whereas the *Taenia* spp. primers also detected some non-taeniid cestodes including *Mesocestoides* spp., *Dipylidium caninum* and *Diphyllobothrium latum.* Nevertheless, combination of this approach with the initial isolation of taeniid eggs by the sequential sieving method developed by Mathis et al. (1996) and subsequent mPCR only for taeniid egg-positive samples would enhance the removal of eggs which would be positive by the assay but do not represent taeniid eggs. Otherwise, in samples containing taeniid as well as other cestodes eggs, a definitive diagnosis to the species level can be accomplished by further sequencing or RFLP. In the current study, this mPCR assay is used to differentiate between

the *E. granulosus* and *Taenia* spp. infections and the availability of co-infection. Since *E. vogeli* is confined to Central and South America and the samples used in the present study originate from Africa, there is no possibility for cross-reaction between *E. granulosus* and *E. vogeli*.

2.6.2 Diagnosis of *E. granulosus* in the intermediate host

2.6.2.1 Cyst detection

Since the infections with *E. granulosus* cysts in intermediate hosts are typically asymptomatic, cyst detection during routine meat inspection of slaughtered animals at abattoirs or at post-mortem examination remains the most reliable diagnostic method. Consequently, the method most frequently used to estimate the CE prevalence and intensity of *E. granulosus* infection in livestock animals (Cardona and Carmena, 2013). However, the accuracy of this procedure may be affected by the occurrence of false positive cases due to other cyst-like lesions and false negatives due to small developing cysts (Cabrera et al., 1996; Bardonnet et al., 2002; Gatti et al., 2007). As the prevalence of *E. granulosus* cysts increases with age (Torgerson et al., 1998), abattoir surveys may be potentially biased, when certain age groups of animals, which vary with countries and cultures, are sent to the slaughterhouse, leading to over-estimation of the real CE prevalence if older animals are slaughtered or under-estimation if younger animals are slaughtered (Barnes et al., 2012).

E. granulosus cysts can be observed in many organs, but the principal sites of predilection are the lungs and liver. Great care is needed to differentiate between *E. granulosus* metacestodes and the larval stage of *Taenia hydatigena* (Eckert et al., 2001a; OIE, 2008).

Hydatid cysts have been also identified by ultrasonography in small animals such as sheep and goats (Maxson et al., 1996; Njoroge et al., 2000), but it has also been used in the horse (Hermann et al., 1988). In Kenya, ultrasound examination of the lung and liver detected hydatid cysts in sheep and goats with a sensitivity and specificity of 54% and 97%, respectively. This is basically because pulmonary cysts cannot be detected if they are deep in the pulmonary parenchyma (Sage et al., 1998). Maxson et al. (1996) provided an estimation of the CE prevalence in livestock in Turkana, Kenya, where mass slaughter is not possible due to the dependence of Turkana people on livestock milk and blood.

2.6.2.2 Serum antibodies detection

Currently, there is no suitable sensitive and specific serological test available for individual diagnosis of *E. granulosus* infection in livestock species (Eckert et al., 2001a). Previous studies have shown that natural infections in sheep produce relatively poor antibody responses compared to high levels of *Echinococcus*-specific antibodies produced in human cases. Enzyme-linked immunosorbent assay (ELISA) has been carried out for detection of exposure to *E. granulosus* using hydatid cyst fluid and crude parasite antigens at the herd or flock level. This tool may be useful in hydatid screening and surveillance programs (Kittelberger et al., 2002). Reports of > 90% sensitivity have been documented using antigen B-enriched hydatid fluid extracts. However, there are cross-reactions with other taeniid species (mainly *Taenia hydatigena* and *Taenia ovis*) (Carmena et al., 2006).

2.6.2.3 Molecular typing methods

Several DNA approaches are now available for genotyping genetic variants of *E. granulosus* using metacestode material from intermediate hosts (Eckert et al., 2001a). Methods used previously for molecular strain typing include partial sequencing of mitochondrial cytochrome c oxidase subunit 1 (*cox1*) and of NADH dehydrogenase 1 (*nad1*) genes (Bowles et al., 1992a; Bowles and McManus, 1993), analysis of ribosomal DNA region (ITS1) by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) (Bowles and McManus, 1993) and random amplification of polymorphic DNA (RAPD-PCR) (Scott and McManus, 1994). Although these methods are useful for characterizing *E. granulosus* isolates, they are costive, time consuming and require a high DNA quality. Consequently, they are not suitable for screening large numbers of samples (Dinkel et al., 2004). Currently, PCR-based methods that simplify genotyping have been designed. Dinkel et al. (2004) developed PCR/semi-nested PCR system for a rapid discrimination of *E. granulosus* (G1, G5, G6/G7). Schneider et al. (2008) described a new PCR protocol that allows discrimination between *E. multilocularis* and *E. granulosus* (G1, G6/G7). Based on the identification of discriminating polymorphic sites in nuclear and mitochondrial genes of the *Echinococcus* genus, Boubaker et al. (2013) established a single-tube multiplex PCR (mPCR) approach that allows a rapid and simultaneous detection and discrimination of the different members of the *E. granulosus* complex. The mPCR allows three levels of discrimination: (i) *Echinococcus* genus, (ii) *E. granulosus* complex, and (iii) genetic variants within the *E. granulosus* complex. A set of 22 primers was used in the mPCR, which allowed the amplification of 11

different size-specific PCR products. Due to strain variability from region to region, only a locally adapted primer combination can be utilized instead of inserting the whole set of primers in the final mPCR mix. The analytical sensitivity of the mPCR was found to be 5 ng *Echinococcus* DNA and the analytical specificity was 100% detecting all members of the *E. granulosus* complex with no cross reaction to closely related species such as other members of the genera *Echinococcus* or *Taenia*. Unfortunately, the sensitivity of this technique was only less than 40% for the detection of *Echinococcus* eggs in fecal samples of infected dogs. This approach is an easy, rapid and inexpensive one-step detection method for the *E. granulosus* complex and subsequently has the potential for worldwide application in large-scale molecular epidemiological studies on the genus *Echinococcus*.

2.7 Control and prevention

Echinococcosis control programmes can be divided into four phases; planning, attack, consolidation and maintenance (Gemmell et al., 2001a). During the planning phase, base-line data such as cost and methods to use in control are collected to aid as references for measuring progress (Economides et al., 1998; Eckert and Deplazes, 2004). The attack phase involves non-discriminatory application of control measures to the entire host populations at risk while the consolidation phase is targeting areas of risk only.

On the other hand, options for control of echinococcosis consist of horizontal and vertical approaches. The first emphasizes long term measures of public health education via introduction of posters and pamphlets and enhancement of veterinary public health activities such as sanitation measures, dog registration and improvement of slaughter hygiene and meat inspection. However, experience from two countries (New Zealand and Uruguay) has shown that this option alone has no effect on the prevalence of echinococcosis in humans and animals. The vertical approach targets directly the interruption of the parasite transmission which is based on legislation, control of dog population and dog dosing joined with a base-line surveillance of intermediate host for progress monitoring (Gemmell et al., 2001a).

The disease has been successfully controlled only in a few islands including Iceland, New Zealand, Tasmania and southern Cyprus, where long-term highly effective and sustained control programmes have been undertaken (McManus et al., 2003). Other circumstances on these islands such as possible border control and the absence of wild life reservoirs support the activities for control (Barnes et al., 2012). The Arecoline purge testing program in the attack phase followed by anthelmintic dosing (praziquentel) were implemented in New

Zealand and Tasmania for more than 30 years, where dog-owners and government bodies work together effectively and this resulted nearly in the elimination of *E. granulosus* (Craig et al., 2007). In both campaigns, transmission to humans almost ceased within about 10-12 years (Gemmell et al., 2001a). In contrast to New Zealand and Tasmania, the Cyprus control program was adopted, where large numbers of wandering dogs were killed irrespective of owner permission and registration. Consequently people started to hide or translocate dogs preventing them from killing (Gemmell et al., 2001a; Barnes et al., 2012). In continental regions including Argentina, Chile, Uruguay, Spain, Bulgaria, Australia and some north African countries, control measures have been applied with only limited effect (Eckert et al., 2000).

The prepatent period of *E. granulosus* is approximately 6 weeks and therefore it has been usually the recommended treatment interval. Although six-weekly application is highly effective, it is expensive and subsequently less suitable for use in poor countries. Praziquentel is currently the most effective anthelmintic used in dogs (Torgerson and Budke, 2003).

Lightowlers et al. 1996 developed a recombinant vaccine, designated EG95, against *E. granulosus* in sheep with protection level close to 100%. The vaccine will provide a new tool that could be used in the future for control of hydatid disease. Moreover, it has the potential to be used directly in humans particularly in areas of high endemicity for human cystic echinococcosis or where sylvatic life cycles are present. Field trials in New Zealand, Australia and Argentina demonstrated a high level of protection (96-100%) for at least 1 year against challenge with different geographical isolates indicating that it could have widespread applicability in hydatid control campaigns (Lightowlers et al., 1999). The vaccine EG95 prevents new infections but does not eliminate cysts already present (Torgerson and Budke, 2003).

New interventions strategies for control and prevention of hydatidosis were described including an effective vaccine against *E. granulosus* in dogs, development of better diagnostics for both human beings and definitive hosts mainly dog coproantigen detection, more active anthelmintic treatment and the use of mathematical models to simulate best possible cost-effective means of control (Craig et al., 2007). In low income countries, where the control of CE is a great challenge, economic models can be combined with simulation models to determine the most effective use of resources to reduce the disease in animals and humans (Torgerson and Budke, 2003).

Because of economic or political reasons, no specific control measures have been initiated in many endemic countries in Eurasia, Africa and other regions in the world. Accordingly, it is

predictable that *E. granulosus* and human CE will persist or re-emerge in these areas (Eckert et al., 2000).

Due to the fact that echinococcosis is a complex disease with variety of factors contributing to the maintenance of the infection, only a few countries have been able to reduce the disease through control and/ or elimination programmes (Seimens, 2003). Recognition of the strains of *E. granulosus* that are locally present and the hosts supporting the continuation of the life cycle are prerequisites for successful control campaigns (Thompson and McManus, 2001).

3 Materials and methods

3.1 Study of Cystic Echinococcosis in different slaughtered animal species in the Sudan

3.1.1 Study design

A cross-sectional study was conducted to update the CE prevalence and to investigate epidemiological factors associated with the infection in sheep, goats, cattle and camels slaughtered at urban and rural abattoirs of five regions in the Sudan (Figure 3.1). The study areas were selected due to their geographical locations and the highest concentration of livestock production. Tamboul and Elobeid were purposively chosen because camels are harboured in large numbers. In Madani, Tamboul, Senga and Elobeid, the only governmental slaughterhouse was visited. The sample size to estimate the prevalence in each species of animals was calculated according to Thrusfield (2007) based on previous infection rates of CE in livestock animals in the country (Table 3.1) with a 95% confidence interval and 5% desired absolute precision.

A stratified random sampling method was used to carry out this study. Between July 2011and January 2012, a local abattoir in each study area was visited on daily basis for two weeks. At every single visit, all slaughtered concerned species were examined for the presence of hydatid cysts.

Table 3.1 Animal populations per species in the Sudan, the prevalence estimates from previous studies, the calculated sample size and the true numbers of samples taken per species.

Animal species	Estimated livestock population in the Sudan (2011)*	Prevalence of *E.granulosus* from previous studies	Estimated sample size	Sample size taken
Cattle	29,618,000	6.1% (Omer et al., 2010)	87	735
Camels	4,715,000	59.9% (Omer et al., 2010)	371	430
Sheep	39,296,000	11.3% (Omer et al., 2010)	150	535
Goats	30,649,000	1.9% (Omer et al., 2010)	30	291

3.1.2 Study area

3.1.2.1 Khartoum

Khartoum, the national capital of the Republic of Sudan and the capital of Khartoum State, is located at the confluence of the White Nile and the Blue Nile between latitude 15° 32.799′N and longitude 32° 32.0166′ E in an area about 22.122 square kilometers, bordered by River Nile State to the north and the east side, the Northern State to the northwest and States of Kassala, Gedaref and Gezira to the east and south. The population is estimated to be over five million people (2008). Khartoum features a semi-desert climate with average annual temperature ranging between 22.7° C and 37.1° C with a mean annual rainfall of 156.8 mm. The livestock in Khartoum State comprises of 250,566 cattle, 442,672 sheep, 642,927 goats and 6,472 camels (Sudan Ministry of Animal Resources and Fisheries, 2011). The study was carried out in three abattoirs (Elkadro (15.74°′N, 32.58°′E), Elsalam (15.64°′N, 32.39°′E) and Elsabaloga (15.70°′N, 32.36°′E), where certain livestock species was slaughtered.

3.1.2.2 Wad Madani

The capital of Al Gezira State in east-central Sudan, lies on the west bank of the Blue Nile (14° 24′N, 33° 31′E), 136 Km southeast of Khartoum. It has average annual temperatures between 19.7° C and 36.4° C with average annual precipitations of 373 mm. The human population of the area is approximately 345,290 inhabitants (2008). The livestock comprises of 2,463,899 cattle, 296,850 sheep, 149,611 goats and 156,858 camels.

3.1.2.3 Tamboul

Tamboul is a small village located in the Al Gezira State in east-central Sudan. The area is one of the most important ones for camels raising in the country. At the time of the study, the main local slaughterhouse (14.92°′N, 33.43°′E) was visited; it represents one of the main abattoirs of camel in the Sudan.

3.1.2.4 Elobeid

The capital of North Kurdufan State in western Sudan (13° 11′N, 30° 13′E), has a human population of about 340,940 inhabitants (2008). The area is occupied by nomads and

pastoralists. The mean annual minimum and maximum temperatures are 19.5° C and 34.6° C, respectively, the mean annual rainfall is 418 mm. North Kurdufan State has a well-developed livestock industry including 960,503 cattle, 7,223,357 sheep, 3,605,603 goats and 1,212,613 camels (Sudan Ministry of Animal Resources and Fisheries, 2011).

Figure 3.1 Map of the Sudan with locations of study areas

3.1.2.5 Senga

Senga is the capital city of Sennar State. It lies in southeastern Sudan between latitude 12.5°-14.7° and longitude 32.58°-35.42° on the west bank of the Blue Nile, bordered by Ethiopia and Gedaref State in the East, White Nile State and South Sudan in the West, Gazira State in the North and Blue Nile State in the South. The mean annual rainfall varies from 350 to 800 mm, whereas the annual mean temperatures range between 20° C and 40° C. Most inhabitants are involved in agricultural and animal husbandry and the livestock population consists of 46,750 cattle, 207,734 sheep, 40,321 goats and 8,310 camels. The area was selected purposively because of its neighborhood to a wildlife zone (Dinder National Park).

3.1.3 Hydatid cysts collection

A total of 1,991 animals (535 sheep, 291 goats, 735 cattle and 430 camels) were post-mortem surveyed in abattoirs for the presence of *Echinococcus* cysts (hydatid cysts). For each carcass, lungs, liver, heart and kidneys were examined carefully by visual inspection, palpation and incision. Data related to the origin, sex, breed, anatomic site and number of cysts per animal were recorded for every single slaughtered animal (see appendices). The age of each animal was determined by questioning the owner of the animal, but due to difficulties in estimating the exact age of some animals or obtaining reliable information from the owners, we finally used two discrete age groups (young and adult).

Table 3.2 Definition of animal ages in months

Animal Species	Young	Adults
Sheep	≤12 months	>12 months
Goats	≤12 months	>12 months
Cattle	≤24 months	>24 months
Camels	≤36 months	>36 months

Hydatid cysts were cautiously removed from the organs, counted and collected in plastic bags. Each bag was used for cysts obtained from one animal, labeled and transported to the laboratory.

3.1.4 Fertility and viability

Cysts were individually examined for fertility and viability after dissection with a sterile scalpel blade and hydatid fluid aspiration (Capuano et al., 2006; Scala et al., 2006) (Figure 3.2 and 3.3). To determine cyst fertility, aspirated fluid was examined under a light microscope (Olympus CHBS, Tokyo, Japan) (40x) in a Petri dish by demonstration of protoscolices. The viability was assessed by the morphology of protoscolices and the motility of their flame cells. Hydatid cysts were classified as fertile (containing live protoscolices), sterile (with hydatid fluid but without protoscolices) and cysts with degenerative changes (cysts with caseous necrosis and/or calcified cysts). Only cysts with clean and transparent fluid and a whitish germinal membrane were collected for molecular analysis and stored at -20° C until DNA extraction.

3.1.5 DNA extraction from hydatid cyst

Genomic DNA was extracted from protoscolices and/or germinal membranes obtained from 69 individual hydatid cysts using the High Pure PCR Template Preparation Kit (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions with slight modifications. 25-50 mg of each sample were digested with 40 μl of proteinase K stock solution and 200 μl of tissue lysis buffer.

Figure 3.2 Hydatid cyst in the lung of a cow
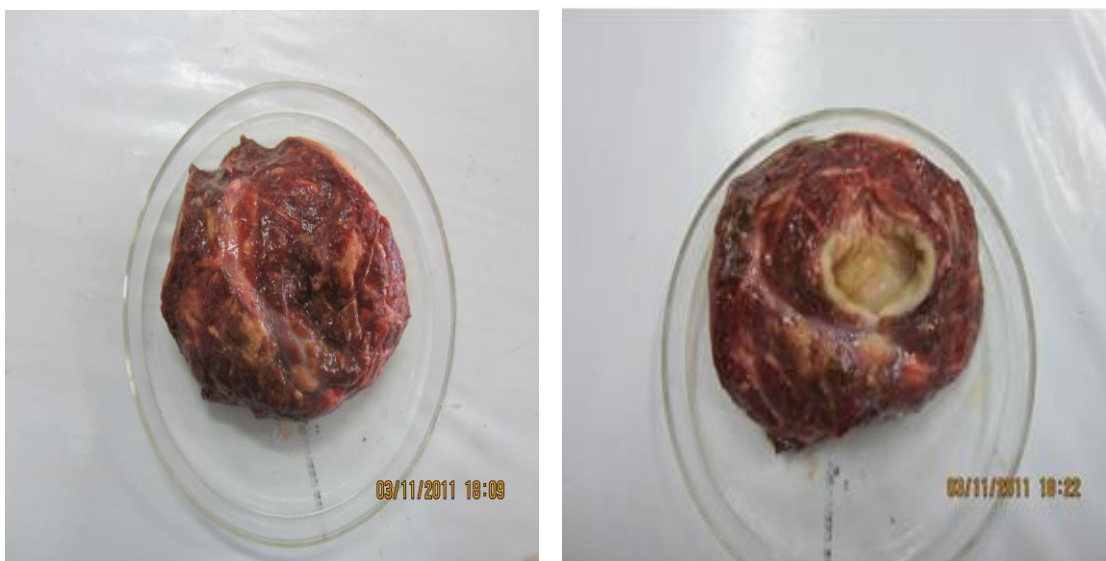
Figure 3.3 Hydatid cyst in the liver of a cow
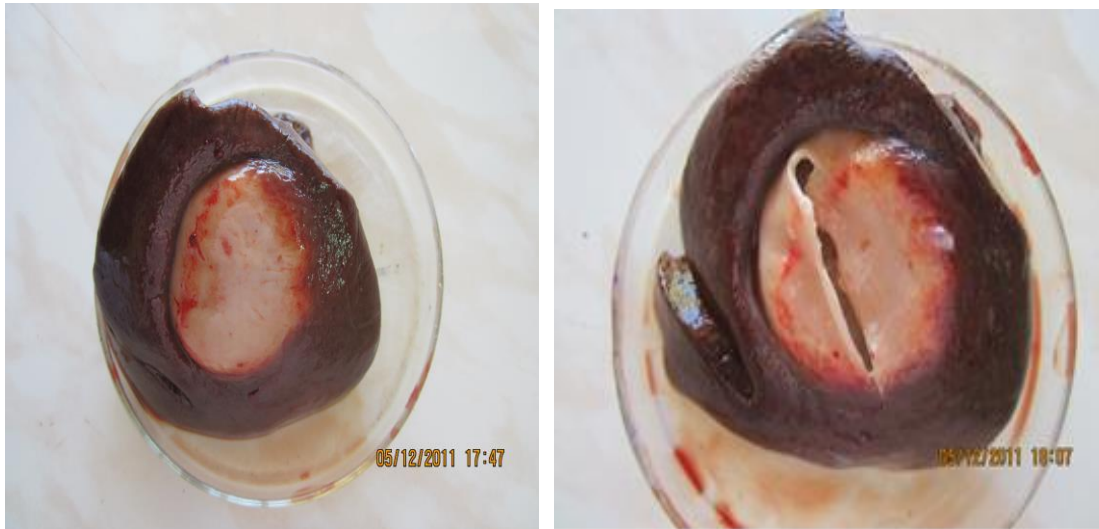
The mixture was immediately vortexed and samples were incubated overnight at 55° C. 200 μl binding buffer were added, the mixture was vortexed and then incubated at 70° C for 10 minutes. To each sample, 100 μl of isopropanol were added and the vial vortexed thoroughly. The mixture was pipetted into a High Filter Tube placed in a 2 ml collection tube, while insoluble tissue was removed. Samples were centrifuged at 8,000 x g in a 5424 centrifuge (Eppendorf AG, Hamburg, Germany) for 1 minute. Flow-through and collection tubes were discarded and the filter tubes placed into new collection tubes. 500 μl inhibitor removal buffer were added and the vials centrifuged at 8,000 x g for 1 minute. Flow-through and collection tubes were again discarded and the filter tubes placed into new collection tubes. Filter tubes were washed twice with 500 μl wash buffer and centrifuged at 8,000 x g for 1 minute. Flow-through and collection tubes were discarded and the filter tubes finally placed into clean 1.5 ml centrifuge tubes, where the DNA was eluted with 200 μl Elution buffer prewarmed to 70° C. DNA was collected after centrifugation at 8,000 x g for 1 minute and stored at 4° C until analysis.

3.1.6 Sub-multiplex PCR (Sub-mPCR) for detection and differentiation between *E. ortleppi* (G5) and *E. canadensis* (G6)

Recently, a multiplex PCR for detection and genotyping of entire *Echinococcus granulosus* complex has been developed and revealed a great potential for further application in large-scale molecular epidemiological studies (Boubaker et al., 2013). Using mitochondrial and nuclear DNA sequences, a set of 11 primer pairs was designed allowing thus three levels of discrimination: (i) the genus *Echinococcus*, (ii) *E. granulosus* complex, and (iii) the specific genotype within the *E. granulosus* complex. Figures 3.4 illustrate the genotype-specific amplicon profile of the *E.granulosus* complex by multiplex PCR.

Previous reports about the molecular epidemiology of circulating *E. granulosus* genotypes/strains indicated the predominance of *E. canadensis* (G6) followed by *E. ortleppi* (G5) in the Sudan (Omer et al., 2010). Thus, we selected a specific combination of primer combinations from list of Boubaker et al. (2013) adapted to the expected situation in the Sudan, reflecting current knowledge on the epidemiology and the strain characteristics of *Echinococcus granulosus* in this country. Three different primers pairs were used, i.e. *E.g* complex F/R, *E. ortp* CoxI F/R and *E. cnd* G6/G7 NDI F/R to amplify a 110, 250 and 339 base pair (bp) PCR products from all *E. granulosus* complex members, *E. ortleppi* (G5) and *E. canadensis* (G6), respectively. Table 3.3 shows the primers used for the mPCR, the final concentration of each primer in the mix, product size, specificity (genotype), the corresponding primer sequences containing polymorphic sites and related target genes of the parasite.

The DNA targets were simultaneously amplified in a 20-µl reaction mixture containing; 100 µM dNTPs, 0.05 units µl−1 GoTaq DNA polymerase in 1× PCR buffer (all Promega) and 6 primers in the molarities shown in Table 3.3. From each *E. granulosus* DNA sample, 2 µl were added into the sub-mPCR mix. Negative controls (PCR grade water) and a positive control (DNA extract from *E. granulosus sensu stricto* (G1) were included in each test run to confirm the results of multiplex PCR and to verify also contamination.

The thermal cycling conditions were as follows: an initial denaturation step of 94° C for 3 min followed by 25 cycles each of denaturation (94° C for 30 s), annealing (56° C for 30 s), and extension (72° C for 1 min) and a final extension at 72° C for 5 min using Gene Amp 9700 Thermocycler (Applied Biosystems, Rotkreuz, Switzerland).

Amplicons were detected after electrophoresis in 2% (w/v) agarose gels by staining the gel with ethidium bromide (10 mg/ ml) and subsequent ultraviolet (UV) illumination.
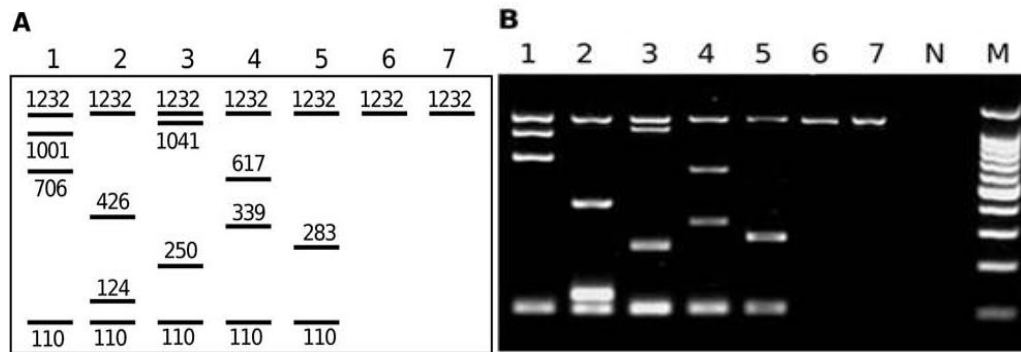
Figure 3.4 Genotype profile of the *E. granulosus* complex by mPCR (Boubaker et al., 2013).

(A) Schematic representation of the genotype-specific banding patterns in bp amplified by mPCR: (lane 1) *E. granulosus s.s.* (G1/G2/G3), (lane 2) *E. equinus* (G4), (lane 3) *E. ortleppi* (G5), (lane 4) *E. canadensis* (G6/G7), (lane 5) *E. canadensis* (G8/G10), (lane 6) *E. multilocularis* and (lane 7) *E. vogeli*. **(B)** Result of the mPCR of the *Echinococcus* species described above (lanes 1–7) visualized on a 2 % agarose gel. The target of 1232 bp is specific for the genus *Echinococcus* and is also amplified for *E. multilocularis* (lane 6) and *E. vogeli* (lane 7). The 110 bp band allows specific detection of members of the *E. granulosus* complex (lanes 1–5). All bands between 1232 bp and 110 bp specifically detected one *E. granulosus* complex species/genotype and showed no cross-reactivity with other members of the complex.

Table 3.3 Primers used in mPCR for the detection of *Echinococcus granulosus* (Boubaker et al., 2013)

Primer name	Conc. in mPCR	Product size (bp)	Specificity	Sequence 5'–3'[a]	Gene marker
E.g complex F	0.15 μM	110	*E. granulosus* complex	TGGTCGTCTTAATCATTTG	cox2
E.g complex R	0.15 μM	110	*E. granulosus* complex	CCACAACAATAGGCATAA	cox2
E.ortp CoxI F	0.2 μM	250	*E. ortleppi* (G5)	GGTT**t**TATGGGTTGTT**A**	cox1
E.ortp CoxI R	0.2 μM	250	*E. ortleppi* (G5)	AC**ACC**a**CCAAACGTG**	cox1
E.cnd G6/G7 NDI F	0.3 μM	339	*E. canadensis* (G6/G7)	**c**TGCAGAGGTTTGC**C**	nad1
E.cnd G6/G7 NDI R	0.3 μM	339	*E. canadensis* (G6/G7)	**c**ACAAC**a**G**C**At**AAAGCG**	nad1

[a]) Strict specific bases in each primer are written in bold. Tiny characters mark additional polymorphic sites (but not strict).

3.1.7 EgCO1⹁PCR and sequencing

Samples of genomic DNA of *Echinococcus granulosus* were further analyzed by PCR-sequencing of the mitochondrial marker *coxI* (Bart et al., 2006). PCR was performed in a 50 µl volume containing 4 µl genomic DNA, 100 µM dNTPs, 0.05 units µl−1 GoTaq DNA polymerase in 1× PCR buffer and 0.5 µM of each of the primers (EgCOI1: 5′-TTT TTT ggC CAT CCT gAg gTT TAT-3′; EgCOI 2:5′-TAACgACATAACATAAtgAAAATG-3′) (Bart et al., 2006).

Reaction conditions for the amplification of the *coxI* gene were as follows: an initial denaturation step at 94° C for 3 min was followed by 35 cycles of 30 s at 94° C, 30 s at 58° C and 30 s at 72° C and a final extension step at 72° C for 7 min. A negative control (PCR grade water) and a positive control (DNA extract from *E. granulosus* s.s (G1) were included in each PCR run.

The genotypes of EgCOI-PCR positive samples were determined on the basis of the DNA sequences of the PCR products. Prior to these molecular analyses, the specificity and the size of PCR products (443 bp) of individual aliquots from amplicons (5 µl) were verified following electrophoresis in ethidium bromide-stained 1.5 % agarose gel under UV transillumination.

Then PCR products were purified with High Pure PCR Product Purification Kit (Roche Applied Science, Switzerland) according to the manufacturer's instructions. After addition of 500 µl binding buffer to the PCR products, the mixture was applied to a High Pure Filter Tube and centrifuged at maximum speed for 60 s in a 5415D centrifuge (Vaudaux-Eppendorf AG, Schoenenbuch, Switzerland). The flow-through was discarded and 500 µl wash buffer were added. Samples were centrifuged at 13,000 x g for 1 min and the flow-through discarded. After other washing step with 200 µl, centrifugation was performed as above, and the flow-through again discarded. PCR products were eluted from filter tubes using 50 µl elution buffer after centrifugation at 13,000 x g for 1 min.

Subsequently PCR products were sequenced with the primers employed in the previous PCR in both directions using an ABI Prism 3130 DNA sequencer (Applied Biosystems, Rotkreuz, Switzerland) through BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Warrington,UK). The thermal profile was: 10 s at 96° C, 5 s at 50° C and 2 min at 60° C for 25 cycles.

The nucleotide sequences obtained for 69 hydatid cysts isolates were analyzed and corrected by visual inspection using BioEdit software7.0.9 (Hall, 1999).

To determine the *E. granulosus* genotype of each isolate, the *coxI* nucleotide sequences were compared to reference sequences of different *E. granulosus* genotypes using BLAST (Basic Local Alignment Search Tool) in the GeneBank database (http://www.ncbi.nlm.nih.gov).

3.2 Investigation of echinococcosis in dogs in the Sudan

3.2.1 Canine sampling

A cross-sectional survey was carried out over a period of seven months from July 2011 to January 2012 to determine the prevalence of canine echinococcosis in owned domestic and in stray dogs and data collected to identify potential risk factors relevant for the transmission of *E. granulosus*. The study was conducted in the same sites, where also the status of slaughtered intermediate hosts was analyzed, with the exception of Wad Madani, where unsuccessful trials were made to obtain fecal sampling. Sample sizes were calculated as described above by taking an expected prevalence of 54.2% (Njoroge et al., 2001) into account.

Table 3.4 Estimation of dogs sample size and the true numbers taken

Animal species	Prevalence of echinococcosis	Estimated sample size	Sample size taken
Dog	54.2%	381	143

3.2.2 Fecal samples collection

Fecal samples were collected from dogs routinely submitted to veterinary hospitals, household dogs as well as stray dogs. A total of 143 (63 domestic and 80 stray dogs) were examined for *E. granulosus* infection. Whole household and veterinary hospital fecal samples analyzed in this study originated from the area of Khartoum. Similar attempts were made in the other study areas; however lower numbers of household dogs, absence of veterinary clinics, lack of knowledge of referring dogs to veterinary hospitals, inaccessibility of dogs or failure of dogs to pass feces at the time of sampling, resulted in this situation. Stray dogs were shot in the study locations in cooperation with the local authorities.

A fresh fecal sample was taken from each dog rectally using a wooden spatula. The samples were collected in plastic bags, sealed and labeled with name of the dog, date and sampling area. The fecal samples were then transported to the laboratory and stored at -20° C until examination.

Figure 3.5 Owned dogs (hunting dogs) sampled for canine echinococcosis in the area of Khartoum

Figure 3.6

A: Tamboul abattoir in central Sudan

B: Stray dogs roam freely near the abattoir of Tamboul

3.2.3 Questionnaire

A questionnaire was developed to identify possible risk factors for canine echinococcosis with the aid of some questions that had already been asked in previous studies (Moro et al., 2005, Ziadinov et al., 2008). To obtain accurate informations, closed questions with multiple choices were structured (see appendices).

The questionnaire, written in English, was completed for each dog in an interview with the owner when the fecal samples were taken. The questionnaire consisted of three parts. The first part contained general information about the dog owner's occupation, the name of the sampling area and date of sampling. Data relating to the age, sex, breed, and type of use (classified into pet dog, guard dog, hunting dog or shepherd dog) of each dog were obtained in the second part of the questionnaires along with information on dog keeping practices, access of the dog to viscera of slaughtered animals and details on the nature of contacts of the dog to humans. Dog owners were also asked, how their dogs were restrained, and if they were treated with anthelminthics. If so, information on the deworming strategy was recorded (frequency of treatments and kind of anthelmintics used). The questionnaire concluded with the third part that focused on periodical home slaughter of livestock by the dog owner, disposal of viscera of slaughtered animals and on the owner's knowledge about echinococcosis in dogs and the infection risk for humans.

3.2.4 Isolation of taeniid eggs

Taeniid tape worm eggs are morphologically indistinguishable, i.e. it is not possible to distinguish infections with *Taenia* spp. from those with *Echinococcus* spp.. It was therefore necessary to use a molecular approach for differentiation.

Fecal samples were inactivated by freezing at -80° C for 7 days to minimize the risk of human exposure to infective eggs or proglottids of *Echinococcus* species.

Fecal materials were subjected to combined flotation and sedimentation method. Approximately 10 g fecal matter from each sample was transferred into a 50 ml Falcon tube. Tap water was added to a volume of appr. 25 ml, the suspension mixed with glass rod until it was completely dispersed and the suspension was passed through a sieve into a conical flask. Water was added to the top of the flask. The sample was then allowed to sedimentate for 30 min and the supernatant discarded. Roughly 3 ml sediment from each sample was transferred into a 15 ml falcon tube, filled with concentrated sugar solution (specific gravity 1.3) to a

final volume of 15 ml. The suspension was then homogenized by shaking the tube vigorously and the sample centrifuged for 10 min at 2028 x g.

5-7 drops were obtained from the floated upper part of the mix using a sterilized and cooled inoculation loop, placed on a glass slide and covered with a coverglass. The specimen was examined for the presence of taeniid eggs under a microscope (Olympus CX40, Tokyo, Japan) (magnification 40-100 x) and then eggs were counted.

In addition, 3 ml of sediment were taken from each sample for DNA extraction.

3.2.5 Extraction of DNA from fecal samples

All fecal samples were examined for the presence of taeniid eggs and positive samples consequently analyzed by PCR. DNA from taeniid eggs was obtained using a commercial kit (FastDNA® SPIN Kit for Soil, MP Biomedicals, Germany) according to the manufacturer's recommendations with some modifications.

978 µl Sodium Phosphate Buffer and 122 µl MT Buffer were added to 400 µl sediment sample in a lysing matrix E tube. Samples were homogenized by means of a SpeedMill P12 Homogenizer (Analytik Jena AG, Germany) at a speed setting of 5.5 for 12 min. Samples were then centrifuged at 14,000 x g for 10 min to eliminate excessive fecal debris and supernatants transferred into new 2 ml microcentrifuge tubes. To each tube, 250 µl PPS (Protein Precipitation Solution) were added and the tubes shaken by hand for 10 min. After centrifugation at 14,000 x g for 5 min, supernatants were transferred into new 15 ml tubes, to which 1 ml binding matrix suspension was added and the sample was resuspended. Tubes were several times inverted by hand for 2 min to allow the binding of DNA before they were placed in a rack for 3 min at ambient temperature. 500 µl of the supernatant were removed and discarded. The binding matrix was then resuspended in the remaining amount of supernatant. 600 µl of the mixture were transferred to a SPIN Filter tube and centrifuged at 14,000 x g for 1 min. Catch tubes were cleared and the remaining mixture of the binding matrix added to the SPIN Filter, centrifuged and cleared as before. For washing, 500 µl SEWS_M solution was added to each SPIN Filter, the pellet gently resuspended using the force of the pipet tip, the sample centrifuged for 1 min at 14,000 x g and catch tubes cleared. A further washing step was performed as above and after the catch tubes had been cleared, each SPIN filter was placed in clean centrifuge tube. To dry the matrix, i.e. remove residual wash solution, the tubes were centrifuged for 2 min at 14,000 x g and the SPIN filters transferred into clean centrifuge tubes. The SPIN filters were then dried for 5 min at room

temperature. 50 µl DES (DNase / Pyrogen‑ Free Water) was gently added and the binding matrix resuspended. After 1 min centrifugation at 14,000 x g, the eluted DNA solution was recovered and stored in a fresh tube at 4° C until use.

3.2.6 Multiplex PCR for fecal samples

Multiplex PCR of canine fecal samples was performed as described by Trachsel et al. (2007). The mitochondrial mPCR reaction was designed to amplify a 117 bp and 267 bp of a small subunit of ribosomal RNA (rrnS) of *E. granulosus* and *Taenia* spp., respectively. The mPCR was conducted using primers and condition listed in Table 3.5. The amplification reaction mixture (25 µl) consisted of 2.5 µl buffer 10x, 0.2 µl dNTPs (12.5 mM), $MgCl_2$ (50 mM) 0.2 µl platinum Taq polymerase (5 U/µl) , 5 µl of primer mix (2µM of primer Cest3, Cest4 and 16 µM of primer Cest5 in H_2O) and 2.5 µl of template DNA. Initially, a denaturation step was performed at 94° C for 3 min followed by 40 cycles of 30 sec at 94° C, 90 sec at 58° C and 10 sec at 72° C. Amplicons were detected on 2% agarose gel, stained with ethidium bromide and visualized by electrophoresis.

Table 3.5 Primer sequences (Trachsel et al., 2007)

Target species	Gene	primer	Sequence 5'–3'	Product size
E. granulosus	*rrnS*	Cest4	GTTTTTGTGTGTTACATTAATAAGGGTG	
		Cest5	GCGGTGTGTACMTGAGCTAAAC	117 bp
Taenia spp.	*rrnS*	Cest3	YGAYTCTTTTTAGGGGAAGGTGTG	
		Cest5	GCGGTGTGTACMTGAGCTAAAC	267 bp

3.3 Statistical analysis

Collected data were entered into an Excel spreadsheet (Microsoft Excel, 2010). Differences in prevalence estimates between different hosts and different studied areas were achieved using chi-squared test. The exact binomial 95% confidence intervals (CI) for prevalence values were calculated using the Epi Tools epidemiological calculators in Epi Tools. x^2 or Fisher's exact test were used as appropriate to determine whether the dog-copro PCR and hydatid cyst prevalence differed significantly between the levels of selected possible risk factors associated with the acquisition of canine echinococcosis and cystic hydatidosis,

respectively. A *p*-value of <0.05 was considered statistically significant. All analyses were performed using R Project for Statistical Computing (http://www.r-project.org/).

4 Results

4.1 Hydatidosis in slaughtered animals

A total of 1,991 animals were examined for the presence of cystic echinococcosis at slaughter in five study areas in the period from July 2011to January 2012. These included 535 sheep, 291 goats, 735 cattle and 430 camels. Cattle were the most commonly slaughtered animals in studied areas (735/1,991, 36.9%) and Khartoum State had a significantly higher number of animals surveyed (634/1,991, 31.8%) than any other area. Of 1,991 examined carcasses, 123 (6.2 %) animals were infected with *E. granulosus* larvae. The CE prevalence of camels, which is 25.34%, is higher than those observed in the other animal species. The lowest prevalence was found in sheep and goats with 0.37 and 0.34, respectively, while CE prevalence in cattle was 1.49% (Table 4.1) (Figure 4.1). The infection of cystic echinococcosis was significantly different between camels and cattle ($x^2 = 167.048$; $P = 0.0001$), cattle and small ruminants (if we considered sheep and goats together as small ruminants) ($x^2 = 5.621$; $P = 0.017$) and camels and small ruminants ($x^2 = 217.35$; $P = 0.0001$).

Table 4.1 CE infection rates for the different animal species

Animals	Examined	Infected	Prevalence (%)	95% exact binomial CI (%)
Cattle	735	11	1.50	0.74-2.67
Sheep	535	2	0.37	0.04-1.34
Goats	291	1	0.34	0.00-1.90
Camels	430	109	25.34	21.30-29.74

As shown in Table 4.2, the prevalence of cystic echinococcosis in camels was higher in Elobeid (western Sudan) with 28.57% (95% CI 17.9-41.3) than in Tamboul (eastern Sudan) with 24.8% (95% CI 20.5-29.5). Similarly, Elobeid had a much higher prevalence of the disease in cattle with 4.14 % (95% CI 1.5-8.8) than Madani and Khartoum State with 2.25% (95% CI 0.3-7.9) and 0.69% (95% CI 0.1-2.0), respectively. On the other hand, no CE infection has been recorded in both sheep and goats in all studied areas except in Khartoum State. Statistical analysis of the obtained results indicated that there is a significant difference

in the prevalence rates of cystic echinococcosis between the studied areas (x^2 =165.401; $P <$ 0.05).

Of the 430 camels examined for CE, 16.7% (72/430) were young and 83.3% (358/430) adult while 18.6% (80/430) were males and 81.4% (350/430) females originating from western Sudan (37%), eastern Sudan (53.5%), central Sudan (7.9%) and 7 (1.6%) camels were origin unidentified. A Univariate analysis was carried out to detect potential associations between CE infection in slaughtered camels and the possible risk factors (Table 4.3). No association was found between the CE infection and the origin of the examined camels (x^2 = 4.940; p = 0.176). Camels CE prevalence did not show any significant difference when comparisons between regions one by one were done (western Sudan versus eastern Sudan x^2 = 0.132; p = 1.00; western Sudan versus central Sudan x^2 = 1.152; p = 0.50 and eastern Sudan versus central Sudan x^2 = 0.830; p = 0.40). Hydatid cyst prevalence was significantly higher in camels more than 3 years old compared with those aged 3 or less (x^2 =11.160; p = 0.001) indicating that the probability of infection increased with the age. There was significant variation in CE infection between male and female (x^2 =5.563; p = 0.018), i.e. female camels are more likely (27.7%) to be infected than males (15%). Camel breed was not included in the statistical analysis because all camel owners failed to subdivide their Arab camels to the sub breeds.

Table 4.2 Prevalence estimates of CE for the different animal species in the different studied areas

Region	Animal species	Examined	Infected		95% exact binomial CI (%)	
			Number	Percentage		
Khartoum	Cattle	434	3	**0.69**	0.1	2.0
	Sheep	100	2	2.00	0.2	7.0
	Goats	100	1	1.00	0.00	5.4
Tamboul	Cattle	23	0	0.00	0.00	14.8
	Sheep	92	0	0.00	0.00	3.9
	Goats	39	0	0.00	0.00	9.0
	Camels	367	91	**24.8**	20.5	29.5
Madani	Cattle	89	2	**2.25**	0.3	7.9
	Sheep	55	0	0.00	0.00	6.5
	Goats	40	0	0.00	0.00	8.8
Senga	Cattle	44	0	0.00	0.00	8.0
	Sheep	76	0	0.00	0.00	4.7
	Goats	57	0	0.00	0.00	6.3
Elobeid	Cattle	145	6	**4.14**	1.5	8.8
	Sheep	212	0	0.00	0.00	1.7
	Goats	55	0	0.00	0.00	6.5
	Camels	63	18	**28.57**	17.9	41.3

Table 4.3 Univariate analysis of possible risk factors associated with CE in camels

	No. examined	No. infected	Prevalence (%)	95% CI	x^2	P-value
Origin					**4.940**	**0.176**
Western Sudan	159	42	26.4	1.97-34		
Eastern Sudan	230	57	24.8	19.3-30.7		
Central Sudan	34	6	17.6	6.8-34.5		
Unknown	7	4	57.1	18.4-90.1		
Age					**11.160**	**0.001**
Young	72	7	9.7	4-19		
Adult	358	102	28.5	23.9-33.5		
Sex					**5.563**	**0. 018**
Male	80	12	15	8-24.4		
Female	350	97	27.7	23.1-33.7		

Overall, 256 hydatid cysts were collected from a total of 109 infected camels. 92.7% of the camels were infected only in the lungs and 2.7% only in the liver. Concurrent infection of both liver and lungs was more common than infections of liver alone (4.6%). In majority of examined camels, cysts had a tendency to be located more in the lungs than the liver. The prevalence of infection was 86.3%, 1.6% and 12.1% in the lungs, liver and both liver and lungs, respectively. Of the total cysts recovered, 121 (47.3%) were fertile, 50 (19.5%) sterile and 53(20.7%) calcified and the highest percentage of fertile cysts was recorded in the lungs. All fertile cysts were viable. The average number of cysts per infected animal was 2.3 (ranged from 1-10). The number and percentage of fertile, sterile and calcified cysts was summarized and presented in Table 4.4.

Table 4.4 Frequencies and percentages of hydatid cysts recovered from different organs of camels

Organ	Number of cysts (total positive = 256)	Percentage	95% CI	cysts		
				Fertile	sterile	Calcified
Lung	221	86.3	81.5-90.3	105	45	42
Liver	4	1.6	0.4-4	-	1	-
Lung + liver	31	12.1	8.4-16.7	16	4	11

* The status of 34 cysts was not detected.

735 cattle were post-mortem surveyed for the presence of *Echinococcus* cysts. 56 (7.6%) and 679 (92.4%) were young and adult, respectively whereas 634 (86.3%) were males and 101 (13.7%) females. Examined cattle belonged to 5 origin categories: western Sudan (79.3%); eastern Sudan (3.4%); central Sudan (5.4%); south eastern Sudan (3.9%) and 7.9% unknown origin.
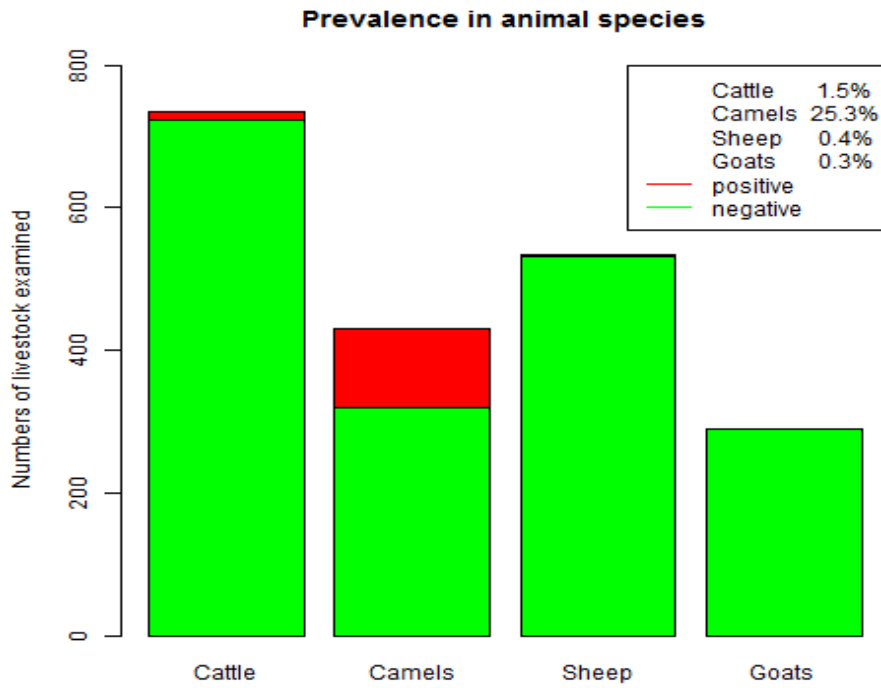
Figure 4.1 Prevalence of cystic echinococcosis in livestock animals
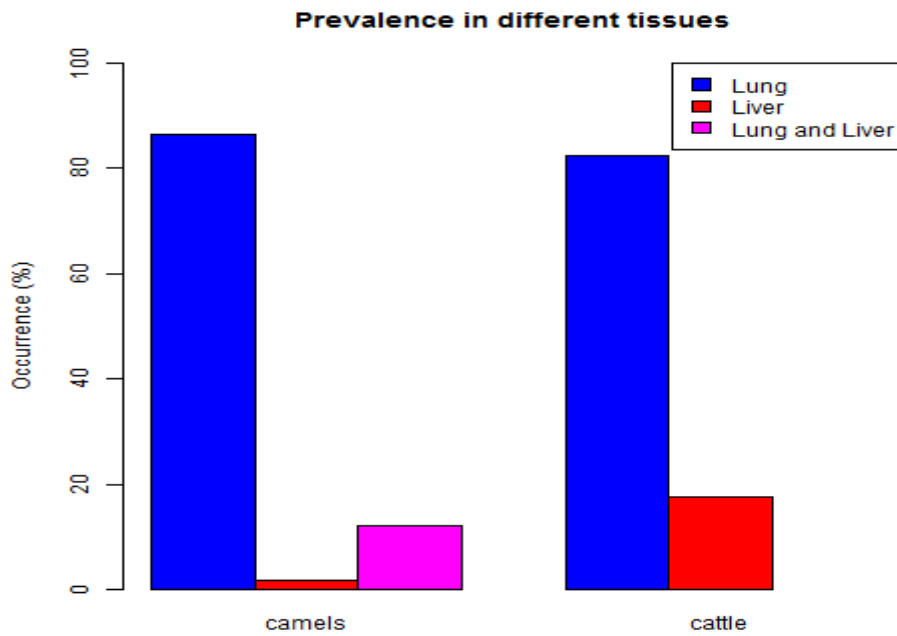
Figure 4.2 Occurrence of hydatid cysts in different organs in camels and cattle

Chi-square analysis showed that there was no significant difference between cattle origin and CE infection ($x^2 = 9.111$; $p = 0.058$) although comparison of proportions of infected cattle between western Sudan versus eastern Sudan revealed that there was a suggestive difference ($x^2 = 5.6248$; $p = 0.074$). Like analysis was not done due to the absence of infection in the other regions. In addition, no association was found between the prevalence of the disease and both breed ($x^2 = 0.073$; $p = 1.00$) and age ($x^2 = 0.921$; $p = 0.69$) of the slaughtered cattle. Cyst prevalence was significantly higher in female cattle compared with males ($x^2 = 9.475$; $p = 0.002$) (Table 4.5).

Of the total 11 cattle harboring one or more hydatid cysts in their internal organs, 72.7% (8/11) had cysts only in the lungs and 27.3% (3/11) only in their liver. Of 17 cysts recovered from infected cows, 12 (70.6%) were fertile, 2 (11.8%) sterile and 1(5.9%) calcified. The greatest proportion of fertile cysts occurred in the lungs of infected cattle and the viability of the protoscolices was 100%. The mean number of cysts per cattle was 1.6 (minimum1, maximum 5). The number and percentage of different cysts are presented in Table 4.6.

Table 4.5 Univariate analysis of possible risk factors associated with CE in cattle

	No. examined	No. infected	Prevalence (%)	95% CI	x^2	p-value
Origin					9.111	0.05
Western Sudan	583	9	1.5	0.7-2.9		
Eastern Sudan	25	2	8	1-26		
Central Sudan	40	0	0	0-8.8		
South eastern Sudan	29	0	0	0-11.9		
Unknown	58	0	0	0-6.2		
Breed					0.073	1.00
Bagara	577	9	1.6	0.7-2.9		
Other	158	2	1.3	0.2-4.5		
Age					0.921	0.69
Young	56	0	0	0-6.4		
Adult	679	11	1.6	0.8-2.9		
Sex					9.475	.002
Male	634	6	0.9	0.3-2		
Female	101	5	4.9	1.611.2		

Table 4.6 Frequencies and percentages of hydatid cysts recovered from different organs of cattle

Organ	Number of cysts (total positive=17)	Percentage	95% CI	cysts		
				Fertile	Sterile	Calcified
Lung	14	82.4	56.6-96.2	12	1	1
Liver	3	17.6	3.8-43.4	-	1	-

* The status of 2 cysts was not detected
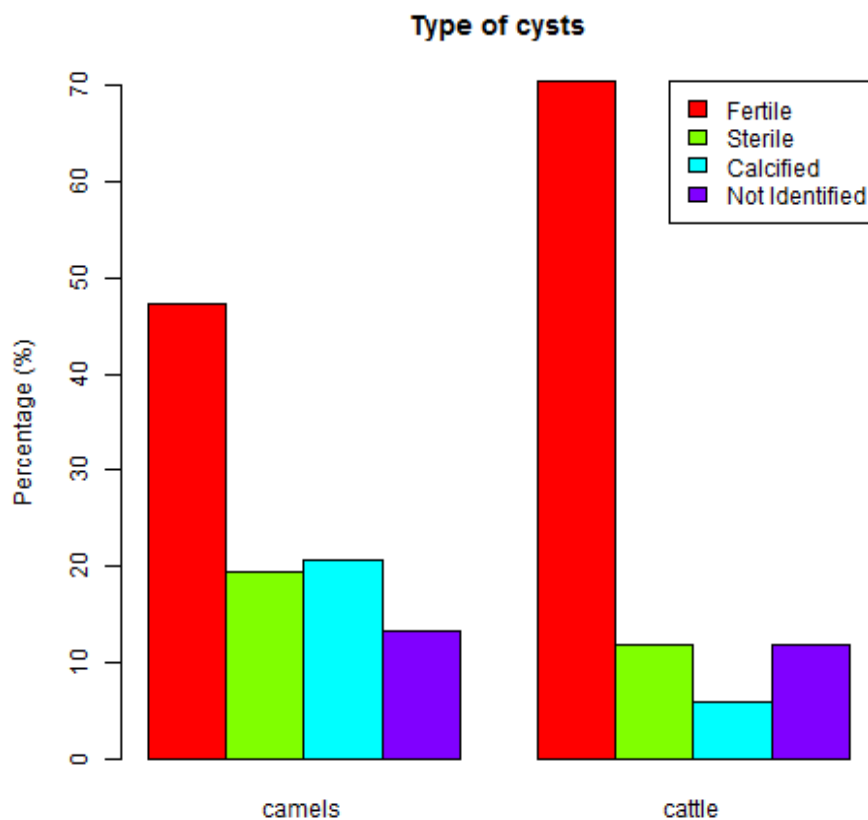
Figure 4.3 Prevalence of different hydatid cysts in camels and cattle

Prevalence of pulmonary hydatid cysts (86.3% and 82.4%, respectively for camels and cattle) was higher than that of hepatic cysts (1.6% and 17.6%, respectively). In addition, concurrent infection of both liver and lungs was higher in camels than infection of liver alone while no co-infection was recorded in cattle (Figure 4.2). A significant difference was found between camels and cattle in infection prevalence among different locations (lungs, liver and together lungs and liver) of hydatid cysts ($x^2 = 18.172$; $p = 0.0001$). Cysts from cattle were more fertile (70.6%) than those from camels (47.2%) while prevalence of sterile and calcified cysts in camels was more related to those in cattle (Figure 4.3). There was no significant difference in cyst types between camels and cattle ($x^2 = 4.024$; $p = 0.133$).

The overall prevalence of hydatid cyst was 0.4% (2/535) in sheep and 0.3% (1/291) in goats. All infected sheep and goats were young and the 3 cysts recovered from each animal were located in the liver and were sterile. The frequencies of the examined sheep and goats were listed in Table 4.7 and 4.8. No further analysis was performed due to the few numbers of animals infected.

Table 4.7 Frequencies of total sheep examined for CE

Variable	No. examined	Percentage (%)
Origin		
western Sudan	212	39.6
central Sudan	129	24.1
Eastern Sudan	56	10.5
South eastern Sudan	62	11.6
Unknown	76	14.2
Breed		
Hamari	245	45.8
Wateesh	66	12.3
Baladi	51	9.5
Dubasi	5	0.9
Ashgar	34	6.4
Kabashi	1	0.2
Unknown	133	24.9
Age		
Young	354	66.2
Adult	181	33.8
Sex		
Male	341	63.7
Female	194	36.3

Table 4.8 Frequencies of total goats examined for CE

Variable	No. examined	Percentage (%)
Origin		
western Sudan	93	32
central Sudan	97	33.3
Butana	7	2.4
south eastern Sudan	18	6.2
unknown	76	26.1
Breed		
Local	118	40.5
Cross	4	1.4
Nubian	78	26.8
Unknown	91	31.3
Age		
Young	208	71.5
Adult	83	28.5
Sex		
Male	214	73.5
Female	77	26.5

4.1.1 Molecular analysis of cyst material

Sub-multiplex PCR (sub-mPCR) was carried out on 69 isolates from 62 camels and 7 cattle. The sets of primers that were used for the sub-mPCR generated highly specific and clearly distinguishable banding patterns. 59 (85.5%) isolates displayed specific pattern consisting of two bands of 100 and 339 bp representing *E. canadensis* (G6/G7). 2 (2.9%) isolates from a cow and a camel showed a different pattern including 100 and 250bp demonstrating *E. ortleppi* (G5) (Figure 4.4). A total of 61(88.4%) of the camel and cattle isolates were genetically identified by the sub-mPCR, whereas 8 (11.6%) were not typed.

Isolates were further analyzed by PCR-sequencing of the mitochondrial marker *cox1*. The amplification of the mitochondrial *cox1* gene with EgCO1/2 primers yielded amplification product of 443 bp (Figure 4.5). Nucleotide sequence for 42 *E. granulosus* isolates were obtained and analyzed. Sequence analysis confirmed the presence of *E. canadensis* (G6) and *E. ortleppi*. Table 4.9 shows isolates characteristics and their molecular characterization. 40 camel isolates had a homology with the camel strain (G6) while 2 cattle isolates had sequences identical to the cattle strain (G5).
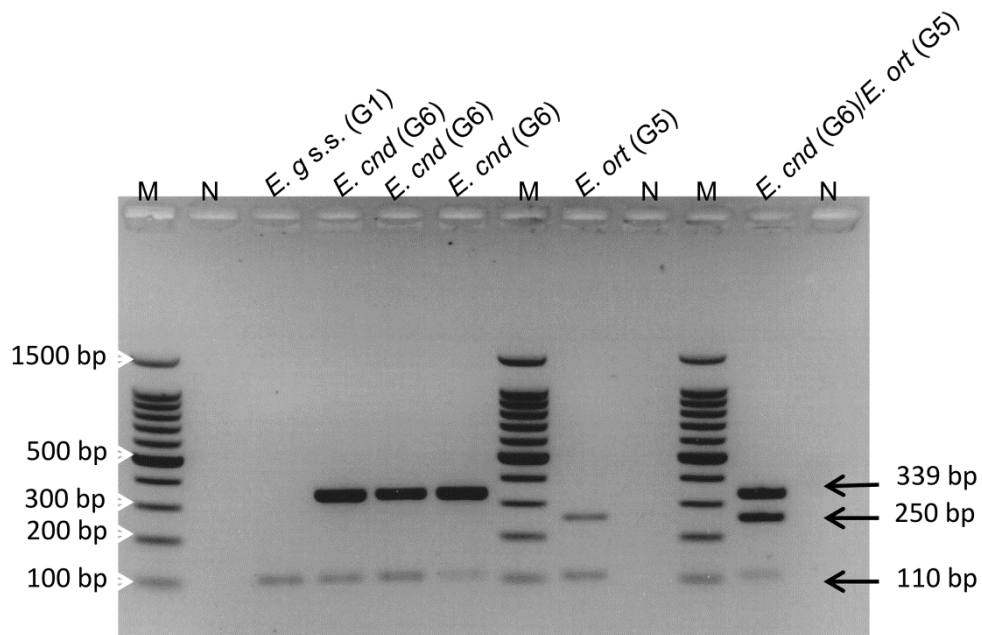
Figure 4.4 Genotype profile of sub-mPCR amplification of hydatid cyst isolates. Lane M:100-bp DNA ladder, N: negative control, E.g s.s. (G1) : *E.granulosus senso.stricto* **(G1) as positive control with only 100 bp banding pattern, E. cnd (G6):** *E. canadensis* **(camel strain) with banding pattern (100-339), E. ort (G5):** *E. ortleppi* **(cattle strain) withbanding pattern (100-250), E. cnd (G6)/ E. ort (G5): DNA mixture of** *E. canadensis* **(G6) and** *E. ortleppi* **(G5).**
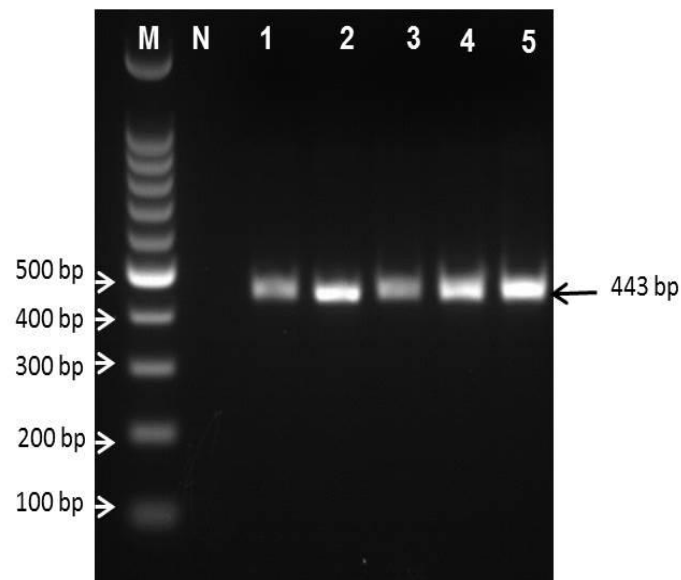
Figure 4.5 Amplification product of the mitochondrial *cox1* **gene with EgCO1/2 primers**

Table 4.9 Total number of isolates analyzed and engrouped by host species and host origin

Sample No.	Host	Origin	Molecular characterization		Species
			mPCR	COX1	
1	Camel	Tamboul	G6/G7	G6	*E. canadensis* G6
2	Camel	Tamboul	-	G6	*E. canadensis* G6
3	Camel	Tamboul	G6/G7	G6	*E. canadensis* G6
4	Camel	Tamboul	G6/G7	G6	*E. canadensis* G6
5	Camel	Tamboul	G6/G7	G6	*E. canadensis* G6
6	Camel	Tamboul	G6/G7	G6	*E. canadensis* G6
7	Camel	Tamboul	G6/G7	-	*E. canadensis* G6
8	Camel	Tamboul	-	-	-
9	Camel	Tamboul	-	G6	*E. canadensis* G6
10	Camel	Tamboul	G6/G7	G6	*E. canadensis* G6
11	Camel	Tamboul	G6/G7	-	*E. canadensis* G6
12	Camel	Tamboul	G6/G7	G6	*E. canadensis* G6
13	Camel	Tamboul	G6/G7	G6	*E. canadensis* G6
14	Camel	Tamboul	G6/G7	G6	*E. canadensis* G6
15	Camel	Tamboul	G5	G5	*E. ortleppi*
16	Camel	Tamboul	G6/G7	-	*E. canadensis* G6
17	Camel	Tamboul	G6/G7	G6	*E. canadensis* G6
18	Camel	Tamboul	G6/G7	-	*E. canadensis* G6
19	Camel	Tamboul	G6/G7	-	*E. canadensis* G6
20	Camel	Elobeid	G6/G7	G6	*E. canadensis* G6
21	Camel	Elobeid	G6/G7	G6	*E. canadensis* G6
22	Cattle	Elobeid	G6/G7	-	*E. canadensis* G6
23	Cattle	Elobeid	G6/G7	-	*E. canadensis* G6
24	Cattle	Elobeid	-	-	-
25	Cattle	Elobeid	G5	G5	*E. ortleppi*
26	Camel	Elobeid	G6/G7	G6	*E. canadensis* G6
27	Cattle	Khartoum	G6/G7	-	*E. canadensis* G6
28	Cattle	Khartoum	-	G6	*E. canadensis* G6
29	Cattle	Khartoum	G6/G7	G6	*E. canadensis* G6
30	Camel	Tamboul	G6/G7	G6	*E. canadensis* G6
31	Camel	Tamboul	-	G6	*E. canadensis* G6
32	Camel	Tamboul	G6/G7	G6	*E. canadensis* G6
33	Camel	Tamboul	G6/G7	-	*E. canadensis* G6
34	Camel	Tamboul	G6/G7	-	*E. canadensis* G6
35	Camel	Tamboul	G6/G7	G6	*E. canadensis* G6
36	Camel	Tamboul	G6/G7	G6	*E. canadensis* G6
37	Camel	Tamboul	G6/G7	G6	*E. canadensis* G6
38	Camel	Tamboul	G6/G7	-	*E. canadensis* G6
39	Camel	Tamboul	G6/G7	G6	*E. canadensis* G6
40	Camel	Tamboul	G6/G7	-	*E. canadensis* G6

41	Camel	Tamboul	G6/G7	-	*E. canadensis* G6
42	Camel	Tamboul	G6/G7	-	*E. canadensis* G6
43	Camel	Tamboul	-	-	-
44	Camel	Tamboul	G6/G7	G6	*E. canadensis* G6
45	Camel	Tamboul	G6/G7	-	*E. canadensis* G6
46	Camel	Tamboul	G6/G7	-	*E. canadensis* G6
47	Camel	Tamboul	G6/G7	-	*E. canadensis* G6
48	Camel	Tamboul	-	G6	*E. canadensis* G6
49	Camel	Tamboul	G6/G7	G6	*E. canadensis* G6
50	Camel	Tamboul	G6/G7	G6	*E. canadensis* G6
51	Camel	Tamboul	G6/G7	G6	*E. canadensis* G6
52	Camel	Tamboul	G6/G7	G6	*E. canadensis* G6
53	Camel	Tamboul	G6/G7	G6	*E. canadensis* G6
54	Camel	Tamboul	G6/G7	G6	*E. canadensis* G6
55	Camel	Tamboul	G6/G7	G6	*E. canadensis* G6
56	Camel	Tamboul	G6/G7	G6	*E. canadensis* G6
57	Camel	Tamboul	G6/G7	G6	*E. canadensis* G6
58	Camel	Tamboul	G6/G7	-	*E. canadensis* G6
59	Camel	Tamboul	G6/G7	G6	*E. canadensis* G6
60	Camel	Tamboul	G6/G7	G6	*E. canadensis* G6
61	Camel	Tamboul	G6/G7	G6	*E. canadensis* G6
62	Camel	Tamboul	G6/G7	G6	*E. canadensis* G6
63	Camel	Tamboul	G6/G7	-	*E. canadensis* G6
64	Camel	Tamboul	G6/G7	-	*E. canadensis* G6
65	Camel	Tamboul	G6/G7	-	*E. canadensis* G6
66	Camel	Tamboul	G6/G7	G6	*E. canadensis* G6
67	Camel	Tamboul	G6/G7	-	*E. canadensis* G6
68	Camel	Tamboul	G6/G7	-	*E. canadensis* G6
69	Camel	Tamboul	G6/G7	-	*E. canadensis* G6

4.2 Echinococcosis in dogs

4.2.1 Isolation of taeniid eggs

From July 2011 to January 2012, 143 dogs were examined for canine echinococcosis in the same areas where the status of hydatidosis in slaughtered animals was determined. To identify taeniid eggs, 143 fecal samples of stray as well as domestic dogs (80 and 63, respectively) were investigated by direct microscopic examination after submitted to the combined flotation and sedimentation method. Eggs were detected in 33 of 143 (23.1%, 95% CI: 16.4-30.9%) dog fecal samples (Table 4.10).

Table 4.10 Frequency of taeniid and *E. granulosus* egg-positive fecal samples in owned and stray dogs

Total dogs examined	No of taeniid eggs-positive (%)	No of PCR positive (%)
63 household dogs	11 (17.5 %)	8 (12.7 %)
80 stray dogs	22 (27.5 %)	5 (6.25 %)
143	33* (23.1 %)	13 (9.1 %)

*10/33 taeniid-egg positive samples were not included in the mPCR

4.2.2 Molecular analysis

Taeniid-egg positive samples were further processed by multiplex PCR (mPCR). The result of mPCR by amplification of 117 bp fragment of *rrn S* revealed that 13dogs were infected with *E. granulosus* (Table 4.10) and 3 from those 13 had co-infection with *E. granulosus* and *Taenia* spp. as demonstrated by amplification of both 117bp and 267bp fragments of *rrnS* (Figure 4.6).

Overall, *E. granulosus*-specific PCR patterns were obtained in 13 out of 143 dogs examined in different sites in the Sudan, corresponding to a total prevalence of 9.1% (Table 4.11).

Table 4.11 *Echinococcus granulosus* prevalence in dogs in different studied areas

| Region	No of examined dogs	No of infected dogs	Infection % (95% CI)
Khartoum	75	8	10.7 (4.7-19.9)
Tamboul	20	3	15 (3.2-37.9)
Senga	21	0	0 (0-16.1)
Elobeid	27	2	7.4 (0.9-24.3)
Total	143	13	9.1 (4.9-15)
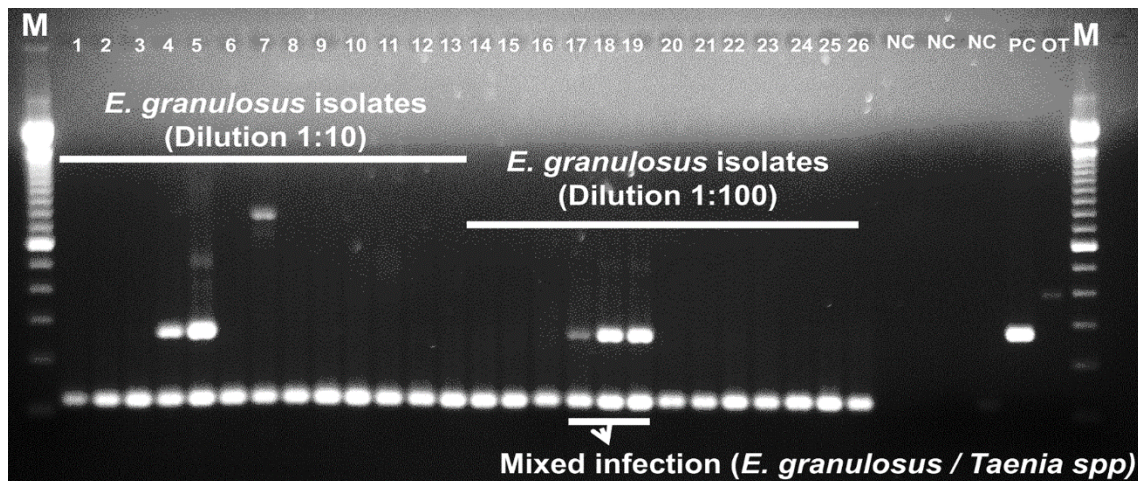
Figure 4.6 Multiplex PCR for fecal samples. Lane M: 100-bp DNA ladder, lane 1,2,3,4,5,6,7,8,9,10,11,12,13: *E. granulosus* **isolates (1:10), lane 14,15,16,17,18,19,20,21,22,23,24,25,26:** *E. granulosus* **isolates (1:100), lane 27,28,29: negative controls, lane 30: positive control for** *E. granulosus***, lane 31: positive control for** *Taenia* **spp. Lane 17,18,19: mixed infection of** *E. granulosus* **and** *Taenia* **spp.**

4.2.3 Risk factors analysis

Questionnaires (n= 63) were assessed for risk factors associated with the acquisition of canine echinococcosis. Univariate analysis using chi-square test demonstrated that none of the possible risk factors was found to be significant for *E. granulosus* infection except for the application of anthelmintic treatment (Table 4.12). De-worming a dog was a significant risk factor for canine echinococcosis infection.

The higher prevalence of canine echinococcosis was detected among dogs aged between 1-2 years old and less than that (16.7%, 16%, respectively) compared with those aged more than 2 years old (5.6%). In addition, the prevalence of *E. granulosus* was found to be higher in males (14.3%) than females (10.7%). Higher prevalence was also detected in both guarding and pet dogs with 22.2% and 19%, respectively, compared with those used for hunting (6.7%). The *E. granulosus* infection was less in dogs that were restrained all the time (9.5%) compared with those which were allowed to roam freely (17.1%). Also higher prevalence of *E. granulosus* was observed in dogs that were kept outside houses (21.4%) than those that were kept inside houses (9.5%). Dogs that fed on raw meat showed a high prevalence of canine echinococcosis (21.7%) compared with dogs that were fed prepared meals without raw meat (8.1%). Dogs that had no access to viscera were more often infected (15.4%) than those

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that had the same access. 14.6% of the infected dogs had close contact to humans particularly with playing. *E. granulosus* infection was recorded in dogs regardless if the dog owners practiced home slaughter or not. A high prevalence of the disease was found in dogs whose owners burned the viscera of slaughtered animals (25%) compared to owners who used to throw viscera out in the roads (8.7%). A high prevalence was also found in dogs that were not allowed to be present near the area of slaughter (14.3%). Hundred percent of dog owners had no knowledge about canine echinococcosis and 93.7% had no knowledge about its possible risk for humans (Table 4.13).

Table 4.12 Univariate analysis of possible risk factors associated with the occurrence of canine echinococcosis

Risk factor	No. examined	No. infected	Prevalence (%)	95% CI	x^2	P-value
Age					**1.285**	**0.525**
0-1	25	4	16	4.5-36.1		
1-2	18	3	16.7	3.6-41.4		
>2	18	1	5.6	1-27.3		
Unknown[a]	2	0	0	0-84.2		
Sex					**0.179**	**0.672**
Male	35	5	14.3	4.8-30.3		
Female	28	3	10.7	2.3-28.2		
Breed					**0.166**	**0.683**
Local	2	0	0	0-84.2		
Foreign	39	3	7.7	1.6-20.9		
Unknown[a]	22	5	22.7	7.8-45.4		
Dog use					**2.921**	**0.571**
Hunting	30	2	6.7	8-22.1		
Guarding	9	2	22.2	2.8-60		
Pet	21	4	19	5.4-41.9		
Herding	1	0	0	0-97.5		
Other	2	0	0	0-84.2		
De-worming					**5.292**	**0.021**
Yes	24	6	25	9.8-46.7		
No	39	2	5.1	6-17.3		
Dog restraining					**1.833**	**0.400**
Tied all the time	21	2	9.5	1.2-30.4		
Free roaming	35	6	17.1	6.6-33.6		
N/A[b]	7	0	0	0-41		
Place where dog sleep					**1.360**	**0.507**
Inside house	42	4	9.5	2.7-22.6		
Outside house	14	3	21.4	4.7-50.8		
N/A[b]	7	1	14.3	4-57.9		
Dog food					**2.835**	**0.242**

Canned	3	0	0	0-70.8		
Raw	23	5	21.7	7.5-43.7		
Other[c]	37	3	8.1	1.7-21.9		
Dog has access to viscera					**1.938**	**0.379**
Yes	7	0	0	0-41		
No	52	8	15.4	6.9-28.1		
N/A[b]	4	0	0	0-60.2		
Close contact to human					**1.172**	**0.556**
Playing	48	7	14.6	6.1-27.8		
Home access	7	0	0	0-41		
Other	8	1	12.5	3-52.7		
Home slaughter					**0.261**	**0.609**
Yes	58	7	12.1	5-23.3		
No	5	1	20	5-71.6		
Disposal of viscera					**4.911**	**0.297**
Feeding to dog	6	0	0	0-45.9		
Burying	8	0	0	0-36.9		
Burning	4	1	25	6-80.6		
Other[d]	23	2	8.7	1.1-28		
N/A[b]	22	5	22.7	7.8-45.4		
Dog near place of slaughter					**1.575**	**0.455**
Yes	9	0	0	0-33.6		
No	42	6	14.3	5.4-28.5		
N/A[b]	12	2	16.7	2.1-48.4		

[a] Unknown category in both age and breed was not included in the analysis

[b] No answer

[c] Prepared meal without raw meat

[d] Throwing viscera out in the roads

Table 4.13 Frequencies of responses of dog owners on knowledge of echinococcosis and owner occupation

Risk factor	Number (Total = 63)	Percentage (%)
Knowledge of echinococcosis in dogs		
Yes	0	0
No	63	100
Knowledge of infection risk for humans		
Yes	4	6.3
No	59	93.7
Owner occupation		
Pastoralist	3	4.8
Other	60	95.2
Antiparasitic drug (Total = 24)		
Caniverm	13	54.2
Drontal	7	29.2
Mebendazole	2	8.3
Unknown	2	8.3
Drug interval (Total = 24)		
Every 3 months	14	58.3
3-6 months	5	20.8
Every year	3	12.5
No answer	2	8.3

5 Discussion

5.1 Livestock hydatidosis

Cystic echinococcosis is an important animal and human health concern in Africa. CE is predominantly maintained in a domestic life cycle through dogs as definitive hosts and a range of domestic intermediate hosts species such as sheep, goats, cattle and camels (Cardona and Carmena, 2013). A measurement of the prevalence of CE infections in intermediate hosts is the most reliable indicator of the level of local environmental contamination with *Echinococcus* eggs from carnivores (Schwabe, 1968). One of the objectives of our study was to estimate the prevalence of hydatidosis in slaughtered animals in certain regions in the Sudan. The current survey, conducted in numerous abattoirs over 7 months, indicated that CE is endemic in the country with statistically significant differences between host species. Camels had the highest CE prevalence (25.34%), followed by cattle (1.5%), sheep (0.4%) and goats (0.34%). Previous reports from the Sudan confirmed the endemicity of the disease in different livestock species with variation of prevalence estimates within different parts of the country (Elmahdi et al., 2004; Omer et al., 2010; Ibrahim et al., 2011). The comparatively lower prevalences of livestock hydatidosis recorded in this study compared to the previous ones are probably attributable to differences in the age of slaughtered animals examined. In other words, all these studies are abattoir-based surveys and the use of such surveys can introduce bias when certain groups of animals are sent to the slaughterhouse. As the Sudanese consumer prefers the meat of young animals, it is very common to find high proportions of young animals in some slaughterhouses but other slaughterhouses are specialized in exporting livestock meat, where high percentages of older animals may be found. Therefore, in presenting data for such diseases like hydatidosis, it is important to use the age stratification rather than crude prevalence estimates.

According to the current study, there was a significant difference in the proportion of animals with CE between studied areas. The highest levels of camels with CE were documented in Elobeid (western Sudan) and Tamboul (central Sudan). The highest percentage of cattle hydatidosis was also recorded in Elobeid with lower prevalences in Madani and Khartoum. These differences in the proportion of animals with CE infection between regions could be related to a number of factors including local circumstances of livestock husbandry, types of animal maintained, livestock capacity, environmental conditions, movements of animals, the *E. granulosus* genotype and the extent of environmental contamination with the parasite eggs.

For example, Elobeid (the capital of North Kurdufan State) has a large livestock industry and Tamboul is one of the most important camels grazing areas in the Sudan and these two areas are mainly dependent on pastoralism as the animal husbandry system. This can also explain why there is no association found between camel CE and the origin of examined camels. Similarly, higher prevalences of CE were also described among different intermediate hosts in western Sudan compared to the other locations in the country (Omer et al., 2010).

In the Sudan, according to the previous research, the camel CE prevalence has been reported to be 44.6%, 59.9% and 29.7% in central Sudan, together central and western Sudan and in Blue Nile State, respectively (Elmahdi et al., 2004; Omer et al., 2010; Ibrahim et al., 2011). Our finding of a 25.34% prevalence of hydatid cysts in camels was higher than reports from neighboring countries such as Ethiopia (Debela et al., 2014), Egypt (Omar et al., 2013), Libya (Kassem and Gdoura, 2006) and Tunisia (Lahmar et al., 2013). The significantly higher prevalence of camel CE recorded in camels over 3 years of age was almost 2.6 times more than that observed in camels aged less than 3 years. An increase in the infection rate of camels with age could be elucidated to the fact that camels are always slaughtered in the Sudan at older ages and therefore high probability of exposure over a long period of time to the contaminated environment with *E. granulosus* eggs. Our results are in agreement with other studies in the Sudan and other countries (Azlaf and Dakkak, 2006; Ibrahim et al., 2011; Debela et al., 2014).

Female camels were more likely to have CE infection than males ($p = 0.018$). This is possibly due to that female camels live longer than males for the purpose of the reproduction. Lahmar et al. (2013) stated that females are more heavily infected and live longer than males, therefore they play an important role in CE transmission. Debela et al. (2014) indicated that females have a higher possibility of acquiring the infection because of the management practices that keep females around homesteads at the backyard for milk production, and are consequently more likely to acquire the infection from the contaminated environment with shedding of gravid segment of *E. granulosus* by infected dogs.

The distribution pattern of CE in the internal organs of the examined camels revealed that the lungs were the most infected organs. 221out of 256 cysts were found in the lungs whilst only 4 were found in the liver. The same pattern of cyst infection was observed in 85.4%, 85% and 49.9% in camels in Libya, Algeria and Iran, respectively (Ibrahim and Craig, 1998; Bardonnet et al., 2003; Ahmadi, 2005). This agrees with reports from Sudanese camels (Omer et al.,

2010; Ibrahim et al., 2011) but in contrast to the findings of Lahmar et al. (2013) in Tunisian dromedaries, where the liver was more infected than lungs. According to Debela et al. (2014), this might be due to the presence of large capillary beds in the lung than any other organ. Our finding that 47.3% of hydatid cysts were fertile, 19.5% sterile and 20.7% calcified suggests that most hydatid cysts from camels are capable to infect dogs and other definitive hosts of the parasite. 24%, 74% and 85.4% of the hydatid cysts recovered from camels in the previous research in the country were found to be fertile (Elmahdi et al., 2004; Omer et al., 2010; Ibrahim et al., 2011). The significance of camels in the transmission of *E. granulosus* in the Sudan is emphasized by the high cyst fertility rate of 47.3% (41% in lungs) and a viability of 100%.

In the current study, the prevalence of cattle *Echinococcal* cysts was lower than previously reported throughout the country which were 3% in central regions (Elmahdi et al., 2004), 6.1% in central and western Sudan (Omer et al., 2010) and 2.7% (Ibrahim et al., 2011) in Blue Nile State. The prevalence of cattle cystic echinococcosis in other African countries has been reported to be 19.4% in Kenya (Njoroge et al., 2002), 13.9% in Algeria (Bardonnet et al., 2003), 22.9% in Morocco (Azlaf and Dakkak, 2006) and 22.1% in Ethiopia (Kebede et al., 2009). While no association was found between cattle CE infection rate and the age of cattle examined, but it was obviously that all infected cattle were adult. Moreover, the absence of echinococcal infection in the present work in younger cattle highlighted that the animal age is an important risk factor in the occurrence of hydatidosis. Similar to camel CE, the prevalence of hydatidosis was also found to be significantly higher in female cattle than the male ($p =$ 0.002). In Saudi Arabia, 12.44% of female cattle had a CE infection compared to 7.45% in males (Ibrahim, 2010). This is could be elucidated to the long life of females or related to pregnancy and lactation that affect the immunity and thus females more exposed to such infections like Hydatidosis.

Our data showed that cattle were more likely to have pulmonary (72.7%) than hepatic cysts (27.3%) and that all fertile cysts were in the lungs of infected cattle. The same organ distribution of cysts was recorded in 27.3% of cattle in Turkey (Umur, 2003), 13% in Ethiopia and 22.5% in Tanzania (Nonga and Karimuribo, 2009). In contrast, the liver was the predominant infected site in cattle in Saudi Arabia and Tunisia (Ibrahim, 2010; Lahmar et al., 2013). The similarities between the pattern of camel and cattle infection observed in our study were possibly due to the *E. granulosus* genotype circulating in the area, but such an assumption needs further epidemiological studies for confirmation. Reports from the Sudan

documented the high fertility rate of hydatid cysts in cattle (Elmahdi et al., 2004; Omer et al., 2010; Ibrahim et al., 2011). However, the overall prevalence of cattle cystic echinococcosis was considerably lower in the present survey, but the high cyst fertility ratio of 70.6% underlined the importance of cattle in the transmission of *E. granulosus* and confirmed that they could still act as a vital reservoir of the parasite.

According to our results, 0.4% of the sheep were found infected with hydatid cyst, which is less than formerly published (Elmahdi et al., 2004; Omer et al., 2010; Ibrahim et al., 2011). In addition, the same researchers recorded fertile cysts prevalence in sheep in a range between 0-1% in central Sudan, 0% in southern Sudan, 19% in western Sudan and 39% in Blue Nile State. These low prevalences of sheep hydatidosis and cyst fertility suggest that this species may play a negligible role in the epidemiology of echinococcosis in the Sudan. In contrast, sheep are considered as the most important intermediate host in the maintenance of the domestic life cycle of *E. granulosus* in other regions of Africa, including Morocco, Tunisia, Algeria and Egypt (Azlaf and Dakkak, 2006; Lahmar et al., 2013; Omar et al., 2013; Mokhtaria et al., 2013), where sheep had not only high prevalence estimates but also in some of these countries significantly higher ratios of fertile cysts than other animal species. The prevalence of goat hydatidosis described in the present study was lower than those stated in different regions in the Sudan ranging between 1.9% in western Sudan and 7.1% in Southern Sudan with a cyst fertility ratio of 33% and 0%, respectively (Omer et al., 2010). Torgerson et al. (1998) hypothesized that lower CE prevalences in goats were due to their grazing behavior as browsers and therefore consuming smaller numbers of viable eggs. The lower prevalence recorded in both sheep and goats may be partly due to the higher proportions of lambs and kids in the examined sample. We recovered only three hydatid cysts recovered from young sheep and goats, but we observed that they were all sterile. This is consistent with previous studies suggesting that sheep and Nubian goats in the country have natural resistance to infection with *E. granulosus*, a suggestion that needs investigations (Ahmed et al., 2013).

Molecular characterization of hydatid cysts from natural intermediate hosts in an endemic area is crucial to determine the life cycle, host specificity and *E. granulosus* taxa in order to achieve an effective control measures for the parasite transmission. In the current study, 69 *E. granulosus* isolates collected from the Sudan have been investigated by sub-mPCR and PCR-sequencing of the *cox1* mitochondrial marker. The molecular characterization demonstrated the presence of *E. canadensis* (G6) and *E. ortleppi* (G5). *E. canadensis* (G6) was detected in camels and cattle samples from Tamboul, Elobeid and Khartoum

slaughterhouses presenting 64 of 66 typed isolates. The presence of the camel strain has been previously confirmed in several intermediate hosts including humans as the dominant genotype prevailing in the country. Fourty-five out of 46 isolates from sheep, cattle and camel origin were identified as *E. canadensis* (G6) (Dinkel et al., 2004). In an abattoir survey, Omer et al. (2010) genetically typed a total of 490 hydatid cysts from camels, cattle, sheep and goats and additionally 5 human isolates. 99.8% of cysts examined belonged to *E. canadensis* (G6). Furthermore, PCR and sequencing of 120 cysts confirmed that all cysts belonged to *E. canadensis* (G6) (Ibrahim et al., 2011) and Ahmed et al. (2013) identified 98% of the camel isolates from Tamboul area as *E. canadensis* (G6). The predominance of the camel strain in our survey and in previous studies underlines the importance of the camel/dog cycle in the transmission of *E. granulosus* in the Sudan and the putative role of camels as reservoir for human CE infection. According to the previously cited studies, the presence of *E. canadensis* (camel strain) has been reported in Algeria (Bardonnet et al., 2003), Tunisia (M'rad et al., 2005), Egypt (Abdel Aaty et al., 2012), Kenya (Casulli et al., 2010) and Iran (Sharbatkhori et al., 2010).

On the other hand, we showed that *E. ortleppi* (G5) was present in one camel from Tamboul and one cow from Elobeid. Two out of seven isolates from examined cattle were identified as *E. ortleppi*, which indicates that this species may be one of the genotypes predominant in the area. Molecular analysis performed by some authors had already earlier indicated the presence of this species in the Sudan. Dinkel et al. (2004) demonstrated its existence for the first time in two cattle from the country. In addition, Omer et al. (2010) proved the presence of this species in a southern Sudanese bovine and in a cow from eastern Sudan. However recently, *E. ortleppi* (G5), which has limited occurrence in the country, has been reported for the first time in a camel in Tamboul area (Ahmed et al., 2013). Our results confirmed the presence of this species in the same area and also confirmed that this species is not only adapted to cattle, buffaloes, goats and sheep as previously described (McManus, 2013), but also able to infect camels as Ahmed et al. (2013) recorded. At the present study, the documented high fertility ratio in cattle (70.6%) with the majority of cysts in the lungs could be elucidated to the presence of *E. ortleppi* (G5). Thompson and McManus (2002) stated that *E. ortleppi* is characterized by the development of pulmonary metacestodes and the production of predominantly fertile cysts. Our findings proved that the cattle/dog cycle occurs in Tamboul and Elobeid in camels and cattle and therefor, potential implications of CE in humans. The possible causes for the increased occurrence of *E. ortleppi* (G5) in the Sudan necessitate further studies.

5.2 Canine echinococcosis

Determination of *Echinococcus* spp. prevalence in the definitive host population is the most accurate index of the degree of transmission of *E .granulosus* in a local region (Jenkins et al., 2000), a fact that is essential to formulate effective control polices. Although the Sudan is an endemic area of *E. granulosus*, limited investigations have been performed on the epidemiology of canine echinococcosis and to the best of our knowledge the present study was the first one to generate current data on the occurrence of the infection in Sudanese dogs combining with molecular analysis. Results obtained from this cross sectional survey could be used to design appropriate control strategies to prevent human cystic echinococcosis.

We found a high percentage of taeniid egg-positive samples in stray dogs (27.5%) compared with the household dogs (17.5%). 12.7% and 6.25% of the taeniid egg-positive samples from household or stray dogs, respectively, were identified as *E. granulosus* with an overall prevalence of 9.1% (13/143, 95% CI 4.9-15). These dogs, primarily domestic ones, may represent a potential risk for human infection and the development of CE in the household surroundings and also a major source of *E. granulosus* infections for livestock species in the country. In previous research, Gemmell (1990) estimated that a single infected dog sheds an average of 8470 *Echinococcus* eggs per day leading to considerable environmental contamination, hence a greater risk for humans and production animals in a given region. In a comparison to the prevalence of infection in dogs in Egypt (5-16%), Ethiopia (7-62%), Kenya (26-33%), Libya (20-26%) and Tunisia (3-21%) (Carmena and Cardona, 2013), it is assumed that the prevalence of the dog echinococcosis recorded in the present study was in the range of some neighboring countries. The proportion of *E. granulosus*-positive dogs from Tamboul area were higher (15%), followed by the area of Khartoum (10.7%) and Elobeid (7.4%). This regional difference could be explained by the estimates of the regional prevalences of cystic echinococcosis in intermediate hosts species recorded in this study in the same areas, which were 17.5%, 0.9% and 5.1%, respectively. The high proportion of canine echinococcosis in Tamboul (east central Sudan) indicated that this area is an endemic region. This is consistent with the previous findings of Saad and Magzoub (1986), who recorded an estimated prevalence of 51% from a total of 49 dogs in a necropsy survey in the same region. Both Tamboul and Elobeid are rural municipals, where sometimes animal slaughtering does not meet hygienic standards and meat inspection may be inefficient. For example, in Tamboul area, livestock slaughtering is commonly done in open space combined with a presence of

large numbers of stray dogs. During slaughtering, offal unsuitable for human consumption is deliberately given to dogs. Under these circumstances, the perpetuation of the *E. granulosus* cycle through easy access of dogs to infected viscera is likely. The high prevalence of canine echinococcosis in Khartoum was due to the fact that all household dogs examined originated from this area. Khartoum State, the capital of the Sudan, is an urban area with well-established veterinary clinics, where we collected the fecal samples from household dogs. Due to the limited campaigns against stray dogs, a number of them are still free roaming in the area gathering around slaughterhouses, contaminating the environment with several infections such as echinococcosis.

Risk factors related with canine echinococcosis have not been evaluated in the Sudan so far. In the present study, most of the potential risk factors hypothesized to be associated with the acquisition of *E. granulosus* in dogs were not statistically significantly associated with the infection status when tested by univariate analysis. This is probably due to the small number of positive copro-PCR dogs. We found that younger dogs (0-2 years) were three times more frequently infected with *E. granulosus* than those older in age (> 2 years). This finding is in agreement with results obtained in other studies in dog populations in Peru (Moro et al., 2005), Libya (Buishi et al., 2005a), Uganda (Inangolet et al., 2010) and Chile (Acosta-Jamett et al., 2010). These surveys showed a pattern of age-related infection, indicating that young dogs were at high risk of infection with higher worm abundance. Torgerson et al. (2003) suggested that acquired immunity against *E. granulosus* in older dogs may possibly explain the pattern of age-related infection.

Although the prevalence was higher in male dogs (14.3%) than in females (10.7%), no statistically significant association was found between dog sex and the likelihood of a dog being infected with *E. granulosus*. Similar results have been reported in necropsied dogs from high endemic areas in Libya (Buishi et al., 2005a). In contrast, Budke et al. (2005) indicated that male dogs were a significant risk factor for echinococcosis and that is possibly due to the fact that male dogs are more likely to hunt and maintain territory compared to female dogs.

According to our observations, hunting dogs were less frequently infected than pet and guarding dogs. This result could be explained by the controlled diet presented by the owners to their hunting dogs that is supposed to preserve them from gaining too much weight for the purpose of hunting. Because of the greater access to offal from animals carcasses in the field, a number of field studies revealed a high prevalence and parasite abundance in farm (herding or shepherd) and hunting dogs (Buishi et al., 2005a; Moro et al., 2005) compared to pet and

guard dogs that received partially or fully controlled diet. The high proportion of *E. granulosus* infection in our guard and household pets may be due to other factors such as allowing these dogs to roam without restrictions or access to livestock offal. Our results indicate that controlled dogs (tied all the time) and dogs that were kept inside houses had a lower prevalence of echinococcosis than those allowed to roam freely and kept outside houses. These findings are in the line with previous investigations (Buishi et al., 2005a; Acosta-Jamett et al., 2010). Controlling and keeping dogs indoors protects them from scavenging trashes and waste materials. It is obvious that dogs that were frequently fed raw meat were almost three times more often infected than those that served meals without raw meat. Therefore this finding confirms two main facts: (i) human behavior plays an important role in the maintenance of zoonosis such as echinococcosis and (ii) the lack of knowledge about *E. granulosus* and its transmission among dog owners. In a highly endemic area of *E. granulosus* in Peru, 23% of the villagers reported to feed their dogs with hydatid infected offal (Moro et al., 2005).

One of the interesting findings of this survey suggests that households that reported de-worming their dogs were highly infected with echinococcosis (25%) compared to those reported no application of anthelmintic treatment (5.1%). We found that this result was inconsistent with the results of the questionnaire, which revealed that 14 and 8 of the dog owners treated their dogs every 3 months or >3 months (3-6 months, every year), respectively. Cabrera et al. (2002b) evaluated different intervals (6, 12 and 16 weeks) of treatment with Praziquentel against *E. granulosus* for dogs and studied its transmission to sentinel sheep in Uruguay. Treatment of dogs every 6 weeks had stopped the transmission, while treatment intervals of 12 and 16 weeks failed to accomplish this. It has been previously documented that the prepatent period of *E. granulosus* is approximately 6 weeks and therefore the recommended treatment interval should not be longer than this period (Torgerson and Budke, 2003). In the present study some findings appeared to be contradictory regarding the access of dogs to viscera, home slaughter and the presence of the dog near the slaughtering area. Even if the dog owner prevented his dog from having access to slaughtering areas and subsequently gave the dog no access to viscera and if he practices home slaughtering or not, this dog would contract the infection if allowed to roam freely at least sometimes.

It is very common in the Sudan, especially during religious festivals, and in places where the slaughtering of animals takes frequently place, to find the viscera of the slaughtered livestock thrown into the street where dogs easily feed on them, thus providing favourable conditions

for maintaining the life cycle of echinococcosis. The results of the questionnaire strongly support these findings as 23/63 (36.5%) of dog owners reported that they disposed the viscera by throwing them away outside houses. These data demonstrate that home slaughter is a significant factor in the occurrence of echinococcosis and reflected the poor awareness of the people that practices lead to the perpetuation of the life cycle of parasite. The observed prevalence of echinococcosis (12.7%) in household dogs was high and is sufficient for a high contamination of the household vicinities by *E. granulosus* eggs. Moreover, 14.6% of the infected dogs had close contact to their owners, therefore the potential risk for human infection is increased.

Recommendations

1. The current findings and the previous research in different part of the country confirmed the continuous present of echinococcosis /hydatidosis in the Sudan that act as a serious public health threat in the country. We recommend further spatial epidemiological studies in both definitive and intermediate hosts in order to determine the high risk areas and transmission foci.

2. Camels play a key role in the epidemiology of hydatidosis in the country through the dominant *E. canadensis* G6 and camel/dog cycle should be targeted in any control programs.

3. The possible causes for the increase occurrence of *E. ortleppi* in the Sudan need further epidemiological studies and also the sympatrically occurrence of *E. canadensis* and *E. ortleppi* and the possibility of the cross breeding.

4. Risk factors that analyzed in the present study will provide reliable information that lead to better understanding of CE transmission before implementing any control measures, basically that related to dog populations.

5. The existence of *E. canadensis* and *E. ortleppi* in the livestock species suggests the active transmission of *E. granulosus* in the selected regions and since both species are able to infect humans, control measures should be taken to minimize the infection risk for humans. Control measures should emphasize the interruption of the parasite transmission. They need to be based on legislation specifically for dog ownership and dog registration, control of the dog population and improvement of veterinary services, mainly offal condemnation, and the proper disposal of viscera and offal in slaughterhouses (burning, proper rendering; but not burial). Due to the lack of knowledge about echinococcosis transmission, intervention

measures should focus on educating the human population, in particular dog owners and communities considered at a high risk of infection with *E. granulosus*. These interventions should raise awareness about echinococcosis and its public health impact on humans and animals as well, change behavioral patterns that facilitate transmission of the parasite and highlight the importance of prophylactic treatment programs.

6 Summary

An Epidemiologic Study of Canine Echinococcosis and Livestock Hydatidosis in the Sudan

Echinococcus granulosus infections are a major public health problem in livestock-raising regions worldwide. Cystic echinococcosis is endemic in some areas of the Sudan, nevertheless, its presence in other parts of the country is basically unknown.

In this project, a retrospective cross-sectional study was conducted to update the CE prevalence and to investigate epidemiological factors associated with the infection in sheep, goats, cattle and camels slaughtered at urban and rural abattoirs of five regions in the Sudan (Khartoum, Wad Madani, Tamboul, Elobeid and Senga). A total of 1,991 domestic ruminants (535 sheep, 291 goats, 735 cattle, 430 camels) were examined for the presence of *E. granulosus* hydatid cysts in the period from July 2011 to January 2012. The prevalence of cystic echinococcosis (CE) was 25.3% (109/430, 95% CI 21.30-29.74%) in camels, 1.4% (11/735, 95% CI 0.74-2.67%) in cattle, 0.3% in sheep (2/535, 95% CI 0.04-1.34%) and 0.3% (1/291, 95% CI 0.00-1.90%) in goats. 92.7% , 2.7% and 4.6% of the camels were found infected only in the lungs, only in the liver or in both, liver and lungs, respectively. Overall, 256 hydatid cysts were collected from a total of 109 infected camels. 121 (47.3%) were fertile, 50 (19.5%) sterile and 53(20.7%) calcified and the highest percentage of fertile cysts was recorded in the lungs. Hydatid cyst prevalence was significantly higher in older and female camels. Of 17 cysts recovered from infected cows, 12 (70.6%) were fertile, 2 (11.8%) sterile and 1(5.9%) calcified and cyst prevalence was significantly higher in female cattle.

Molecular analysis of both sub-multiplex PCR (Sub-mPCR) and sequencing of the mitochondrial marker *coxI* performed on cyst material from 62 camels and 7 cattle demonstrated that 92.7% of the study animals were infected with *E. canadensis* (G6) and 2.9% with *E. ortleppi* (G5), while 4.3% of the isolates could not be genotyped.

Another emphasis of this thesis was the assessment of the prevalence of canine echinococcosis and potential risk factors associated with the acquisition of the disease in owned dogs. Between July 2011 and January 2012, a cross-sectional survey was conducted in the same areas where the

status of hydatidosis in slaughtered animals was determined. Fecal samples from 143 dogs (63 domestic and 80 stray dogs) were examined for *E. granulosus* infection by direct microscopic examination after combined flotation and sedimentation method followed by multiplex PCR of mitochondrial genes for positive taeniid eggs samples. The combined flotation and sedimentation technique revealed taeniid eggs in the fecal samples of 33 dogs (23.1%, 95% CI: 16.4-30.9%). Multiplex PCR revealed that 13 out of 143 dogs examined in different sites in the Sudan were infected with *E. granulosus* and 3 from those 13 had co-infection with *E. granulosus* and *Taenia* spp.. 8 household (12.7 %) and 5 (6.25 %) stray dogs were infected with *E. granulosus* corresponding to a total prevalence of 9.1% (95% CI: 4.9-15%).

Since no studies have been conducted so far in the Sudan to evaluate the risk factors related with canine echinococcosis, the present survey focused on investigating these potential factors in owned dogs based on responses to a questionnaire administrated to dog owners. Significant risk factor for a copro-positive owned dog was associated only with de-worming. High proportions of *E. granulosus* were recorded in younger, male, guarding and pet dogs. Higher prevalences of canine echinococcosis were also detected in free roaming dogs and dogs that were kept outside houses and fed on raw meat.

7 Zusammenfassung

Eine epidemiologische Studie über Hundeechinokokkose und Hydatidose beim Vieh im Sudan

Echinococcus granulosus-Infektionen sind ein großes Problem der öffentlichen Gesundheit in Viehzuchtregionen weltweit. Die zystische Echinokokkose ist endemisch in einigen Gebieten des Sudan, während die mögliche Verbreitung in anderen Teilen des Landes im Grunde unbekannt ist.

Im Rahmen dieser Arbeit wurde eine retrospektive Querschnittstudie durchgeführt, um aktuelle Daten zur CE-Prävalenz zu erhalten und Faktoren, die mit der Infektion bei Schafen, Ziegen, Rinder und Kamele assoziiert sind, in städtischen und ländlichen Schlachthöfen in fünf Regionen im Sudan (Khartum, Wad Madani, Tamboul, Elobeid und Senga) zu ermitteln. Insgesamt wurden 1991 Hauswiederkäuer (535 Schafe, 291 Ziegen, 735 Rinder, 430 Kamele) auf das Vorhandensein von *E. granulosus*-Zysten in der Zeit von Juli 2011 bis Januar 2012 untersucht. Die Prävalenz der zystischen Echinokokkose betrug 25,3 % (109/430, 95% CI 21,30-29,74%) bei Kamelen, 1,4% (11/735, 95% CI 0,74 - 2,67%) bei Rindern, 0,3% bei Schafen (2/535, 95% CI 0,04 - 1,34 %) und 0,3% (1/291, 95% CI 0,00 - 1,90%) bei Ziegen. Bei 92,7%der Kamele fanden sich Zysten nur in der Lunge, bei 2,7% nur in der Leber und bei und bei 4,6% in beiden Organen, Leber und Lunge. Insgesamt wurden 256 Zysten von 109 infizierten Kamelen gesammelt. Von den Zysten waren 121 (47,3%) fertil, 50 (19,5%) steril und 53 (20,7%) verkalkt. Der höchste Prozentsatz an fertilen Zysten fand sich in den Lungen der Kamele. Die Prävalenz war bei älteren und bei weiblichen Kamelen deutlich erhöht. Von 17 Zysten, die aus infizierten Kühen gewonnen worden waren, erwiesen sich 12 (70,6%) als fruchtbar, 2 (11,8%) waren steril und eine (5,9%) war verkalkt. Die Prävalenz war bei weiblichen Rindern deutlich höher als bei männlichen Tieren.

Eine molekulare Typisierung durch Sub-Multiplex PCR (Sub-mPCR) und Sequenzierung des mitochondrialen Markers Cox *I* an Zystenmaterial aus 62 Kamelen und 7 Rinder zeigte, dass 92,7% der Tiere mit *E. canadensis* (G6) und 2,9% mit *E. ortleppi* (G5) infiziert waren. 4,3% der Isolate ließen sich nicht genotypisieren.

Ein weiterer Schwerpunkt der Arbeit war die Schätzung der Prävalenz der Echinokokkose bei Hunden und die Ermittlung potenzieller Risikofaktoren für die Infektion mit dem Parasiten durch gehaltene und streunende Hunde. Zwischen Juli 2011 und Januar 2012 wurde eine Querschnitterhebung in den gleichen Bereichen durchgeführt, in denen der Status der Hydatidose bei den geschlachteten Tieren erhoben worden war. Kotproben von 143 Hunden (63 gehaltene und 80 streunende Hunde) wurden auf eine Infektion mit *E. granulosus* durch direkte mikroskopische Untersuchung nach kombinierter Flotation und Sedimentation untersucht, gefolgt von einer Multiplex-PCR mit Zielsequenzen in mitochondrialen Genen bei Proben, die Eier vom Taenien-Typ enthielten. Mit der kombinierten Flotation und Sedimentationstechnik fanden sich Eier vom Taenien-Typ in Kotproben von 33 Hunden (23,1%, 95% CI: 16,4-30,9%). Die Multiplex-PCR ergab, dass 13 von 143 Hunden, die an verschiedenen Standorten im Sudan beprobt worden waren, mit *E. granulosus* infiziert waren und 3 von denen 13 eine Ko-Infektion von *E. granulosus* und *Taenia* spp. aufwiesen. 8 (12,7%) gehaltene und 5 (6,25%) streunende Hunde waren mit *E. granulosus* infiziert. Das entsprach einer Gesamtprävalenz von 9,1% (95% CI: 4.9-15%).

Da im Sudan bisher keine Studien vorlagen, in denen Risikofaktoren für die Infektion von Hunden mit Echinokokken ermittelt wurden, konzentrierte sich die vorliegende Studie auf die Identifizierung potenzieller Risikofaktoren bei gehaltenen Hunden, die mit Hilfe eines Fragebogens ermittelt wurden, mit dem Hundebesitzer befragt wurden. Als statistisch signifikanter Risikofaktor für einen in der koproskopischen Untersuchung positiven gehaltenen Hund wurde nur die Entwurmung ermittelt. Hohe Anteile von *E. granulosus*-Infektionen wurden bei jüngeren, männlichen Hunden, Wachhunden und als Haustieren gehaltenen Hunden gefunden. Darüber hinaus fanden sich höhere Prävalenzen bei streunenden Hunden und solchen, die außerhalb von Häusern gehalten wurden und mit rohem Fleisch gefüttert worden waren.

8 Appendices

Appendix 8.1   Investigation of Livestock Hydatidosis

Location ID……………………………………………………………………………

Animal ID…………………………………………………………………………………

Date...…………………………………………………………………………………..

1. (General information)

Owner……………………………………………………………………………..…

Origin of the herd/flock……………………………………………………………….

Species……………………………………………...…………………………………

Breed…………………………………………………………….………………….…..

2. (General Information about the animal)

Age: kid/ calf / lamb ☐   adults ☐

Sex: male ☐   female ☐   neutered ☐

Infected organ(s)………………………………………………………………….…

Number of cysts……………………………………………………………………..…

Fertility of cysts: Yes ☐   No☐

Appendix 8.2 Investigation of Canine Echinococcosis

Location ID…………………………………………………………………………………..
Animal ID…………………………………………………………………………………
Date…………………………………………………………………………………..

1. (Questions about the dog owner)

Dog Owner…………………………….…

Occupation……………………………..

Address…………………………………………………………………………………

2. (Questions about the dog)

Name…………………………………………………………………….…………

Age……………………………………………………………………………………

Sex: male☐   female ☐   neutered☐

Breed…………………………………………………………………………………

Dog use: shepherd dog   pet dog☐   guard dog☐   hunting dog☐   other ☐

(specify)……………………………………………………………………………

Dog deworming strategy: Yes☐   No☐

If yes: How

often…………………………………………………………………………………

Name of drug………………………………………………………..   Unknown ☐

Dog restraining: restrained all the time☐ allow to roam some or all the time☐   N/A☐

Place where dog keep & sleep: inside the house ☐   outside the house☐   N/A ☐

Dog feeding: canned food☐☐   raw meat☐   other☐

(specify)……………………………………………………………………………..

Access to viscera: Yes☐   No ☐   N/A ☐☐

Close contact to human: play with dog☐   has the dog access to home☐   dog sleeps in the

same room as owner/family☐ ☐   other☐

3. (Questions about Livestock)

How often the owner slaughtered per year inside the house…………………………..……

Disposal of viscera: dog feeding☐   burying ☐   burning☐   N/A☐

Presence of dog near the area of slaughtering: Yes☐   No☐   N/A☐

Knowledge of Hydatidosis:

In dogs: Yes☐   No☐
Infection risk for humans: Yes☐   No☐

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Finally, I would like to dedicate this thesis to my beloved country **the Sudan**.

Naglaa Abass

Selbstständigkeitserklärung

Hiermit bestätige ich, dass ich die vorliegende Dissertation mit dem Titel „An Epidemiologic Study of Canine Echinococcosis and Livestock Hydatidosis in the Sudan" selbstständig angefertigt habe. Ich versichere, dass ich ausschließlich die angegebenen Quellen und Hilfen in Anspruch genommen habe. Keine entgeltliche Hilfe von Vermittlungs- bzw. Beratungsdiensten (Promotionsberater oder anderer Personen) wurde in Anspruch genommen. Niemand hat von mir mittelbar oder unmittelbar entgeltliche Leistungen für Arbeiten erhalten, die im Zusammenhang mit dem Inhalt der vorgelegten Dissertation stehen.

Ich habe die Dissertation am Institut für Epidemiologie des Friedrich-Loeffler-Institutes fertiggestellt. Die vorliegende Arbeit wurde bisher nicht für eine Prüfung oder Promotion oder für einen ähnlichen Zweck zur Beurteilung eingereicht.

Berlin, den 20.11.2014

Naglaa Abass