Prevalence and Potential Risk Factors of Human Cystic Echinococcosis in Selected Districts of South Omo Zone, Ethiopia

By:

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A Dissertation submitted to:
The Department of Microbial, Cellular and Molecular Biology
in Fulfillment of the Requirements for the Degree of Doctor
of Philosophy in Biomedical Sciences

Addis Ababa University

Addis Ababa, Ethiopia

January, 2019

ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

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A Thesis Presented to the School of Graduate Studies of the Addis Ababa University in Partial Fulfillment of the Requirements for the PhD Degree in Biology (Biomedical Sciences)

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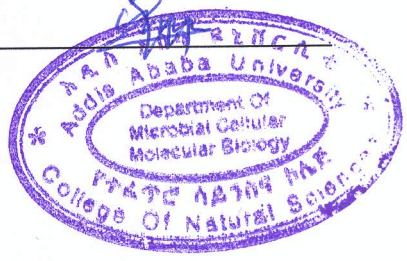

DEDICATION

This Thesis is dedicated to my dearest adviser, the late Professor Getachew Tilahun (1951-2018).

DECLARATION

I, the undersigned, declare that the thesis hereby submitted for the Degree of Doctor of Philosophy in Biology (Biomedical Sciences) to the Department of Microbial, Cellular and Molecular Biology, Addis Ababa University is my own work and has not been previously submitted at another university. The materials obtained from other sources have been duly acknowledged in the thesis.

Signed on May 2018, Department of Microbial, Cellular and Molecular Biology, College of Natural and Computational Sciences, Addis Ababa University:

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Prof. Getachew Tilahun

ACKNOWLEDGEMENTS

My sincere gratitude will go to my dearest advisers, Prof. Beyene Petros and Prof. Getachew Tilahun (late), AAU, for their guidance, critical comments and encouragement during the entire period of this study.

I would like to thank Prof. Peter Kern (University of Ulm, Germany), Prof. Thomas Romig (University of Hohenheim, Germany) and Dr. Eberhard Zeyhle (Meru University, Kenya) for their unreserved support and encouragement.

I'm grateful for Dr. Teklu Wogayehu, Community Service Director of AMU, Ato Alemayehu Bawude, Chief Administrator of South Omo Zone and Ato Mintesnot Melka, Head, Health Department of South Omo, for their kind collaboration.

I'm indebted for the department of MCMB, AAU, especially the malaria and other diseases thematic research for providing financial support.

I'm very grateful to Arba Minch University for sponsoring my graduate study.

I would like to appreciate the South Omo Chief Administration Office together with the Community Service Directorate of AMU for their financial support for the treatment of the study participants.

I'm grateful for the German Research Foundation, DFG for its financial support for the study through the Cystic Echinococcosis in Sub-Saharan Africa Research initiative, CESSARi.

I would like to thank AHRI, AMREF Ethiopia and HELMEX plc for their sincere collaboration during the ultrasound and slaughter house survey.

I would like to appreciate the contribution of University of Pavia, Italy, University of Hohenheim, Germany, for providing trainings and laboratory facility for molecular work.

I am very thankful to my friends at AAU, AMU and AHRI, my relatives, my wife and kids for their encouragement during my study.

Last but certainly not least, the study participants of Hamer, Nyangatom and Dasenech are thanked very much for their willingness to participate in this study.

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AAU	Addis Ababa University
AE	alveolar echinococcosis
AHRI	Armaur Hansen Research Institution
AMU	Arba Minch University
CE	cystic echinococcosis
CESSARi	cystic echinocococcosis in Sub-Saharan Afica Research initiative
Cox1	Cytochrome c oxidase subunit I
CT	Computed tomography
DFG	German Research Foundation (Deutsche Forschungsgemeinschaft)
FAO	Food and Agricultural Organization
MCMB	Microbial, Cellular and Molecular Biology
MRI	Magnetic Resonance Imaging
Nad1	NADH (Nicotinamide Adenine Dinucleotide) dehydrogenase subunit 1
PCR	Polymerase Chain Reaction
PE	polycystic echinococcosis
RFLP	Restricted Fragment Length Polymorphism
SNNPR	Southern Nations, Nationalities and Peoples Region
WHO	World Health Organization
WHO-IWGE	World Health Organization – informal working group for echinococcosis

ABSTRACT

Cystic echinococcosis (CE) is a cestode parasitic zoonosis caused by the larval stage of various species of the genus *Echinococcus* and/or the strains of *Echinococcus granulosus*. This study was aimed to determine the current status and public health importance of cystic echinococcosis (CE) in the pastoralist areas of South Omo Zone, SNNPR, Ethiopia. Prior to the commencement of the study institutional and national ethical approvals were obtained. Ultrasound survey was conducted on 2838 consenting study participants in Hamer, Nyangatom and Dassenech Districts of South Omo Zone. 311 were randomly selected for interview to assess their awareness and attitude about the disease and the practices that may be related to the transmission of the disease. Among the study participants, 56 (2.0%) were found infected with CE on US. Human CE was more prevalent (3.3%) in Hamer District. Liver was the most affected organ and CE1 stages showed high frequency (23 cases). Increased CE1 prevalence was observed as the age increased. The questionnaire survey indicated that most respondents had never heard of CE; had large number livestock and dogs; practiced home slaughtering; did not dispose infected offal properly; and had a wrong perception about the infection. The level of awareness and the attitude of the community about CE was very low and wrong. Sharing of water sources with animals was a potential risk factor, which was found to have significant association with CE infection. 1338 slaughtered cattle were inspected at Jinka and Arba Minch Abattoirs to estimate the distribution of CE among livestock of the pastoralist in the study area. Slaughter house survey was conducted on 4312 small ruminants at HELMEX Export Abattoir plc, meat processing center for export in Bishoftu town, Oromia Region. Cysts were excised from liver and lungs of the animals and examined. Size, number and vital status of the cysts were determined. 75 (61 from Arba Minch and 14 from Jinka) out of 1338 cattle were found infected and lung infection was more common. 23 (7 from Babile and 16 from Borena) out of 4312 small ruminants were also found positive for CE during abattoir inspection. Molecular characterization of cysts from cattle and small ruminants showed that the causative agent was predominantly G1 strain of *E.granulosus*. However, the cyst excised from the lower leg muscle of one human study participant was characterized and found to be a novel strain and given a provisional name, G_{Omo}. G_{Omo} is a new strain discovered in the study area and definite elucidation of its life cycle awaits further research. Thus, the study showed that CE is common in humans and animals in the pastoralist community of the study districts, Hamer, Nyangatom and Dassenech. Further epidemiological and molecular studies are recommended for a further in depth elucidation of the intensity of infection in the human population and to establish the life cycle of the G_{Omo} strain.

Key words: Cystic echinococcosis, *Echinococcus granulosus* ss, G_{Omo}, South Omo, Ethiopia,

1 INTRODUCTION

1.1 Historical background of Echinococcosis

Echinococcosis in humans and animals had been recognized by ancient scholars - Hippocrates (460 – 377 BC), Galen (129 – 200 BC) and Aretaeus (around 50 AD) (Grove, 1990). These scholars considered the hydatid cysts as degenerated glands or accumulations of serum or mucus between laminar cell layers. The parasitic nature of hydatid cysts was shown during the early modern age by other scholars such as Francisco Redi (1626 – 1697), Philip Jacob Hartmann (1648 – 1707), Edward Tyson (1650 – 1708) and Peter Simon Pallas (1741 – 1811). In 1688, Johann Jacob Wepfer (1620 – 1695) made a link between the adult tapeworms in the intestine of definitive hosts and its cystic stage developing in internal organs of intermediate hosts (Grove, 1990).

The term *'Echinococcus'* was used in 1801 by Carl Asmund Rudolphi (1771 – 1832), referring to the small, round, 'spiny' protoscolices found in the cysts (Romig *et al.,* 2015). The relationship between cysts in the intermediate host and adult worms in the definitive host had been proven through independent feeding and infection experiments conducted by von Siebold (1804 – 1885), Küchenmeister (1821 – 1890) and Rudolf Leuckart (1822 – 1898). Development of metacestodes in intermediate hosts from intestinal tapeworms of carnivores was demonstrated based on infection experiments on sheep by Kuchenmeister and by Leuckart on piglets (Romig *et al.*, 2015; Eckert and Thompson, 2017).

Felix Deve (1872 – 1951) in France and Harold Robert Dew (1891 – 1962) in Australia independently showed that the oncospheres actively penetrated the intestinal wall, entered small portal veins and were subsequently carried in the bloodstream to the liver. Both Dew and Deve, in the beginning of 20th century, had observed larvae in portal veins of the liver a

few hours after experimental infection of pigs. It was concluded that most larvae remain in the liver, but a few of them may pass the liver 'filter' and are transported to the lung or other organs. This view was consistent with the distribution of cysts in the human body, predominantly localized in the liver and less frequently in other organs (Eckert and Thompson, 2017).

The two pathological forms were recognized in the mid-19th century, which later on were characterized as cystic echinococcosis (CE) and alveolar echinococcosis (AE). However, it was unclear whether these two forms were caused by a single or by two different *Echinococcus* species. In 1901/1902, Adolf Posselt (1867 – 1936) was the first to provide clear experimental evidence that human AE is caused by *E. multilocularis* (then called *Taenia echinococcus alveolaris*) (Romig *et al.*, 2015). He isolated from a human patient alveolar parasite tissue containing numerous protoscolices and infected a parasite-free dog. After 49 days, he found in the intestine numerous small tapeworms with typical morphological features of the adult stages of *E. multilocularis*. After half a century, Johannes Vogel (1900 – 1980) re-examined Posselt's feeding experiment and recognized 'all characteristics' of *T. echinococcus alveolaris* (later renamed as *E. multilocularis*) (Eckert and Thompson, 2017).

Hydatigena granulosa was the first valid name for causative agent of echinococcosis, which was given by Batch in 1786 based on a fertile *Echinococcus* cyst of sheep from Germany (Romig *et al.*, 2015). Pursuant to this, several other *Echinococcus* species were named – *E. granulosus* by Batsch in 1786 and *E. multiloculais* by Leuckart 1863, *E. oligarthra* by Diesing in 1863, *E. felidis* by Ortlepp in 1937, *E. ortleppi* by Lopez-Neyra and Soler Planas in 1943, *E. equinus* by William and Sweatman in 1963, *E. vogeli* by Rausch and Bernstein in 1972, *E. shiquicus* by Xiao and colleagues in 2005, were described (Xiao *et al.*, 2005; Romig *at al.*, 2015).

1.2 Aetiology of Echinococcosis

The causative agents of echinococcosis belong to the genus *Echinococcus*, which is composed of eight species and one genotypic cluster, *Echinococcus canadensis* (Figure 1). These species usually utilize predator-prey systems for the maintenance of their life cycles. Definitive hosts are exclusively members of the order Carnivora, mainly of the dog family (Canidae), to a lesser degree cats (Felidae) and hyenas (Hyaenidae). Intermediate hosts cover a much wider range, marsupials, rodents and ruminants. All species of *Echinococcus*, except *Echinococcus shiquicus* and *E. felidis*, are geographically spread over vast areas, and some agents of cystic echinococcosis (CE) can be considered as cosmopolitan (Romig *et al.*, 2017).

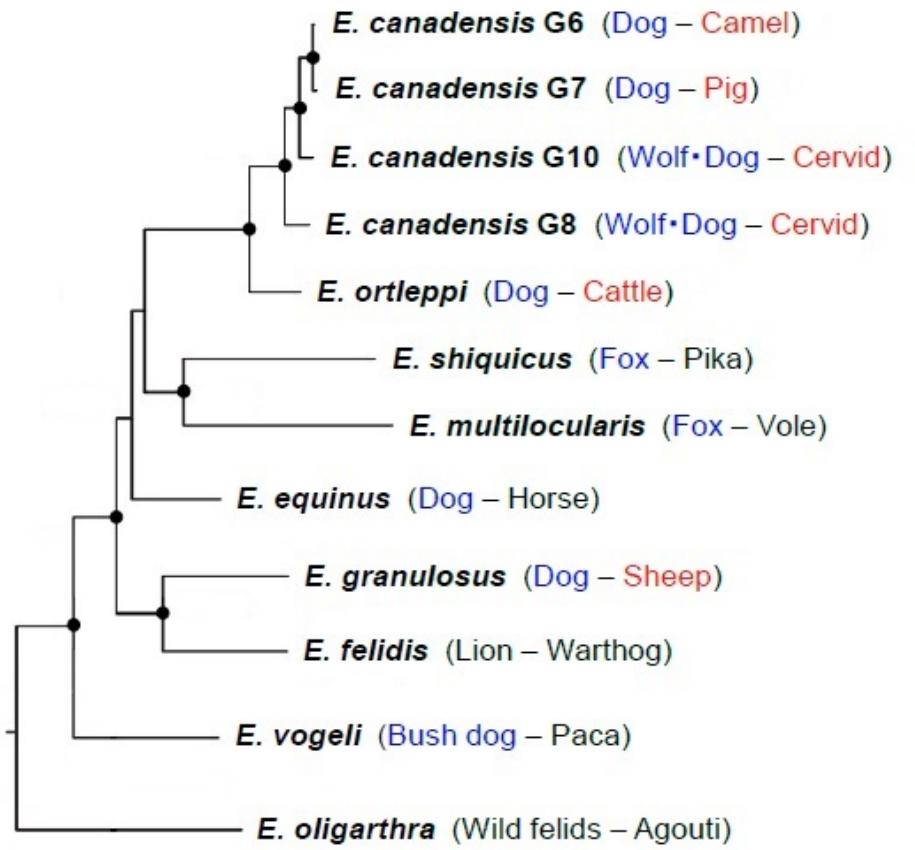


Figure 1. Phylogenetic tree of currently recognized *Echinococcus* species and species cluster and the possible hosts that maintain the life cycle. (Modified from Nakao *et al.*, 2013b; Ito *et al.*, 2017).

1.2.1 Echinococcus oligarthra

This species is known to be confined to the new world, i.e. to the Americas (D'Alessandro and Rausch, 2008). The life cycle involves different species of wild cats and large rodents. However, host ranges are geographically widely scattered and do not allow conclusions on the local transmission patterns. *E. oligarthra* causes unicystic echinococcosis. The disease has been reported in two loci, orbit of the eye and myocardium, which make this tapeworm to be of little public health significance as compared to that of *E. vogeli* (D'Alessandro and Rausch, 2008).

1.2.2 Echinococcus vogeli

The geographic range of *Echinococcus vogeli* is assumed to be largely identical to that of *Echinococcus oligarthra.* The only verified natural definitive host of this species is the bush dog, which stretches from Panama through northern South America and the Amazon basin (east of the Andes) into Paraguay and northeastern Argentina. The parasite is transmitted between bush dogs and large rodent species. While all records of *E. vogeli* in animals are confined to the bush dog's distribution range, there are some reports on suspected human cases. The two species of rodents that serve as intermediate hosts are pacas and agoutis (Mayor *et al.,* 2015). *E. vogeli* causes polycystic echinococcosis to humans (D'Alessandro and Rausch, 2008). Polycystic echinococcosis is severe progressive disease that may cause damage to liver, lung and heart. Domestic dogs may be involved in the life cycle as a spillover from the wildlife cycle due to human behaviour. Pacas are traditionally hunted by people for their meat, and their viscera are fed to dogs giving them infection (Mayor *et al.,* 2015).

1.2.3 Echinococcus multilocularis

Despite the large geographical distribution range that includes most of the temperate and cold zones of the northern hemisphere, no subspecific taxonomic units are presently recognized within *E. multilocularis* (Nakao *et al.,* 2013b; Knapp *et al.,* 2015). *E. multilocularis* is adapted to circulate between wild (foxes, raccoon dogs, wolves, golden jackals, coyotes, dholes and wild cats) and domestic (dogs and cats) carnivores as definitive hosts and small mammals (voles, muskrats and mice) as intermediate hosts. Translocations of wildlife species and of domestic animals create new host populations and/or introduce *E. multilocularis* into new areas (Davidson *et al.,* 2012). Some canid species are very adaptable and show a great resilience to human activities and interventions. The red fox, as the major definitive host in the largest part of the parasite's range, has recently established populations living in close contact to humans even in urban areas (Deplazes *et al.,* 2004). This adds additional complexity to the life cycle, and different transmission patterns may exist on small spatial scales in landscapes fragmented by human activities (Liccioli *et al.,* 2015).

1.2.4 Echinococcus shiquicus

E. shiquicus seems to be geographically restricted to the Qinghai-Tibet plateau region of China. It has only recently been described as a sister species of *E. multilocularis* based on differences in adult and larval morphology and gene sequence data (Xiao *et al.,* 2005; 2006b). Its only known wild definitive host is the Tibetan fox but has never been found in red foxes (*V. vulpes*), which can be sympatric with Tibetan foxes locally. The adult stage of *E. shiquicus* has also been detected by PCR in domestic dogs, but the potential role of dogs as definitive hosts for this parasite remains to be elucidated (Boufana *et al.,* 2013). Only the plateau pika is known as intermediate host so far, while various other species of small

mammals in this region were only found infected with *E. multilocularis*. From the limited data available, it seems that *E. shiquicus* is restricted to the predator-prey system between Tibetan foxes and plateau pikas, and its geographical range may be defined by the overlapping distribution of these species, which are endemic to the Tibetan highlands. In contrast, *E. multilocularis*, which is also present in this region, utilizes a clearly wider range of hosts in the same region (Jiang *et al.*, 2012b). *E. shiquicus* is specific to its intermediate host. No human case has been reported so far (Xiao *et al.*, 2006a).

1.2.5 Echinococcus equinus

E. equinus has formerly been included in *E. granulosus* as the 'horse strain'. Its transmission pattern (horses and domestic dogs), morphological and epidemiological data make it distinct from the 'sheep strain' (Kumaratilake *et al.*, 1986). However, it is now known that other agents of CE (e.g., *E. granulosus* **ss**, *E. ortleppi*) can also develop metacestodes in species of the Equidae (Boufana *et al.*, 2014; Obwaller *et al.*, 2004), so any records without molecular data are questionable. The sylvatic transmission of *E. equinus* is maintained by lions and black-backed jackals as definitive hosts, and plains zebras as intermediate host (Wassermann *et al.*, 2015). The parasite clearly exploits the predator-prey system between lions and zebras, while jackals are likely to acquire infection through scavenging.

1.2.6 *Echinococcus granulosus* senso stricto (ss**)**

Following a revision of the various species that had been described earlier as agents of CE, the name *E. granulosus* was widely used in the second half of the 20th century to cover all taxa of the genus *Echinococcus* that produced cystic metacestodes (Romig *et al.*, 2015). To accommodate the high diversity regarding host range, morphology, developmental biology and geography, a number of 'strains' were erected (Thompson and McManus, 2001). Some

are now regarded as separate species, while the ‘sheep’, ‘Tasmanian sheep’ and ‘buffalo’ strains (G1-3), now constitute the species *E. granulosus* ss (Nakao *et al.*, 2013a; Romig *et al.*, 2015). Transmitted predominantly in domestic a life cycle involving dogs and livestock, *E. granulosus* **ss** has the widest geographical distribution of all *Echinococcus* species, and, with 88% of 1661 human CE cases characterized to species level worldwide, it has by far the largest impact on public health (Alvarez Rojas *et al.*, 2014). The high number of human cases certainly reflects the wide distribution and high frequency in dogs and livestock, but an apparently low specificity at the intermediate host level may also contribute to an enhanced infectivity or pathogenicity for humans compared to other *Echinococcus* spp. causing CE.

Epidemiological data suggest that this species is particularly well adapted to sheep as intermediate hosts, which is reflected in high prevalence (Cardona and Carmena, 2013; Deplazes *et al.*, 2017) and high cyst fertility rates. In addition, almost all other livestock species (goats, cattle, yak, camels, alpacas, pigs, donkeys) are known to develop fertile cysts of *E. granulosus* ss and thereby contribute to transmission, but are usually considered to be less important for the life cycle due to lower prevalence, cyst fertility or availability to dogs. Cattle are frequently found infected. Cyst fertility is low according to most studies (<20%), but can reach 75% (Latif *et al.*, 2010).

Transmission in domestic settings involves dogs as definitive hosts and livestock as intermediate hosts. Infection of dogs occurs by feeding of contaminated offal after home slaughter, improper management of abattoirs and slaughterhouses, where roaming dogs have access to condemned offal, or by stray or semistray dogs scavenging on livestock carcasses left on the pasture. The apparently low host specificity for development of the *E. granulosus* ss metacestode is reflected in the long list of accidental intermediate hosts that do not play a

part in the life cycles, ranging from cyst development in rodents (Yang *et al.,* 2009) to abdominal CE in domestic cats (Deplazes, 2015).

1.2.7 Echinococcus felidis

Echinococcus felidis was long known as the 'lion strain' of *E. granulosus* and could only recently be confirmed as an independent species (Huttner *et al.,* 2008). Originally, it was described 80 years ago in the lion (*Panthera leo*) from the Northern Transvaal (present Limpopo province), South Africa (Ortlepp, 1937 cited in Huttner *et al.,* 2008). Molecularly diagnosed eggs in faeces of lions confirmed the actual presence of this parasite in Uganda (Queen Elizabeth National Park) (Huttner *et al.,* 2009), Kenya, Zambia and Namibia (Huttner *et al.,* 2009; Kagendo *et al.,* 2014). Eggs were also detected in faeces of spotted hyenas, but not in any other large carnivore (Huttner *et al.,* 2008, 2009; Kagendo *et al.,* 2014). The life cycle was speculative due to the lack of diagnostic morphological criteria for the metacestode stage. Lions prey on a large number of African herbivore species, and many of these are known to develop *Echinococcus* cysts (Huttner and Romig, 2009; Deplazes *et al.,* 2017). However, during recent molecular surveys a wide spectrum of *Echinococcus* species was found in African herbivores, including *E. equinus, E. granulosus* **ss**, *E. ortleppi and E. canadensis* G6/7 (Obwaller *et al.,* 2004; Huttner *et al.,* 2008, 2009; Kagendo *et al.,* 2014), so that the intermediate host range of *E. felidis* cannot be based on previous lists of wild mammal species as host of CE. There are six genetically verified records of *E. felidis* metacestodes from warthogs (*Phacochoerus* species) from Uganda and Namibia (Huttner *et al.,* 2009), and recently one metacestodes from hippopotamus (*H. amphibius*) from South Africa (Halajiana *et al.,* 2017). Despite being widespread in eastern Africa, cysts of this species were never recorded from humans, nor from any species of livestock in this region, even where pastoralists graze their animals near or in conservation areas (Huttner *et al.,*

2009). There is no reasonable doubt that this species is a primary wildlife parasite, probably restricted to sub-Saharan Africa.

1.2.8 Echinococcus ortleppi

The species was described from adult worms of dogs that had been fed with cysts from South African cattle. It was initially characterized as the 'cattle strain' of *E. granulosus* and finally recognized as a separate species (Nakao *et al.*, 2013a; Romig *et al.*, 2015). In contrast to *E. granulosus* ss, it is well adapted to cattle as intermediate hosts, producing cysts with a high fertility rate predominantly in the lungs (Grenouillet *et al.*, 2014; Mbaya *et al.*, 2014; Urach Monteiro *et al.*, 2016). It shows distinct morphological features of the adult worms, and the development time in dogs before the onset of egg production is shorter than in other *Echinococcus* species causing CE (Thompson *et al.*, 1984). *E. ortleppi* occurs worldwide in domestic life cycles between cattle and dogs. Compared to *E. granulosus* ss, this species is usually far less frequent even in cattle-raising regions. This apparent paradox has been tentatively explained by the fact that even in traditional pastoralist societies (e.g., in sub-Saharan Africa), cattle are a valuable asset which is mostly sold alive to be transported to distant slaughterhouses. In contrast to cysts developing in sheep and goats (that are often slaughtered at home), cysts in cattle are therefore less frequently available for local dogs, which creates a barrier for cattle infection with *E. ortleppi* (Addy *et al.*, 2012). This is in contrast to infection with *E. granulosus* ss that is acquired by cattle as spillover from the concurrently running sheep-dog cycle. Even where standards of cattle-slaughterhouses permit access to cysts for stray dogs, such slaughterhouses are often in urban environments far away from the cattle-raising areas (Wachira *et al.*, 1993). Sharp increases of *E. ortleppi* infections in cattle in Rio Grande do Sul (Brazil) (de la Rue *et al.*, 2006; Balbinotti *et al.*, 2012; Urach Monteiro *et al.*, 2016) was explained by the improved availability of electricity that enables

rural farmers to run deep freezers. In western Zambia, the frequent infection of cattle was explained by the common presence of unsupervised cattle slaughter slabs in villages (Banda and Sikasunge, personal communication). Although *E. ortleppi* is the most important species of *Echinococcus* that produces fertile cysts in cattle, there is no strict intermediate host specificity. Goats and pigs are known to develop fertile cysts also (Mbaya *et al.,* 2014; Pednekar *et al.,* 2009), and metacestodes have been reported from a number of other mammals: an unreported zebra species and oryx antelopes (Oryx gazella) were found naturally infected in Namibia (Obwaller *et al.,* 2004). Human infections are known from all over the world, but seem to be extremely rare (Alvarez Rojas *et al.,* 2014). It is unclear if this is caused by low exposure due to the sporadic occurrence in animal hosts or by partial resistance to infection.

1.2.9 *Echinococcus canadensis* cluster

This species complex currently comprises the genotypes G6/7 (camel and pig strain), G8 ('American' cervid strain) and G10 ('Fennoscandian' cervid strain) (Nakao *et al.,* 2013a; Romig *et al.,* 2015). The three genotypic groups seem genetically sufficiently distant from each other to warrant recognition as species, and G6/7 seems to be epidemiologically distinct from G8 and G10, which are mainly present in wildlife cycles and are restricted to the north of Eurasia and America. The erection of three species has been proposed, with the name *Echinococcus intermedius* for G6/7; *Echinococcus borealis* for G8; and *E. canadensis* for G10 (Lymbery *et al.,* 2015). The genotypic cluster G6/7 includes the 'camel strain' and the 'pig strain' that have been described morphologically and based on their transmission patterns in dog-camel and dog-domestic pig cycles (Thompson *et al.,* 1995). Based on short sequences of the mitochondrial *cox1* and *nad1* genes, they were characterized as genotypes G6 and G7 (Bowles *et al.,* 1992; Bowles and McManus, 1993b). Consecutive studies using

longer sequences including entire mitochondrial genome data concluded that both genotypes differ only in few base pair exchanges and might be better regarded as geographical microvariants of the same taxon (Romig *et al.,* 2015). G6/7 is transmitted in domestic life cycles with dogs as definitive hosts and a rather wide range of livestock species as potential intermediate hosts. Domestic transmission of G6/7 can also persist where neither pigs nor camels are kept as livestock. Examples are parts of southern Europe and eastern Africa, where goats were identified as important hosts, while sheep are rarely infected (Addy *et al.,* 2012). The role of cattle in the domestic life cycle of G6/7 needs further study since few data are available on cattle infection with this parasite (Romig *et al.,* 2017).

There are numerous reports about the involvement of wildlife in the transmission of G6/7. A wide range of wild mammals, lions and oryx antelopes, in Africa are shown to be suitable hosts for the parasites and might be important for transmission. In Europe, wolves and wild boars seem to play important role in the sylvatic transmission of G6/7 (Guerra *et al.,* 2013; Umhang *et al.,* 2014). Some authors argued that infection of wild boar could be a spillover from the domestic cycle, although involvement of red foxes (by scavenging on boar carcasses) might also contribute (Romig *et al.,* 2017).

The geographic distribution and host records of G8 and G10 correlate well with the ‘northern form’ of *E. granulosus* that was considered to have a primary wildlife transmission between wolves and wild cervids in the northern hemisphere (Rausch, 2003). Sylvatic transmission depends on the wolf as definitive host and occurs throughout the circumpolar north (Davidson *et al.,* 2016; Oksanen and Lavikainen, 2015). Intermediate host seems to be predominantly moose, which is frequently infected and harbours cysts. A domestic cycle occurs between herding dogs and domesticated reindeer. This was described for northern Fennoscandia, where the parasite (assumed to conform to G10) had been abundant in herded

reindeer well into the second half of the 20th century. This transmission system was accompanied by high incidence of human CE (Oksanen and Lavikainen, 2015). Recently, this life cycle seems to occur in a modified form, where the increasing wolf population in eastern Finland is assumed to take the role of definitive hosts from dogs and provide an infection source for semidomesticated reindeer (Lavikainen *et al.*, 2006; Romig *et al.*, 2017).

1.3 Global Distribution and Prevalence of Cystic Echinococcosis

Cystic echinococcosis is widely distributed throughout the world (Figure 2). It is not distributed uniformly, even in the similar areas. Dispite its patchy distribution, the infection is cosmopolitan.

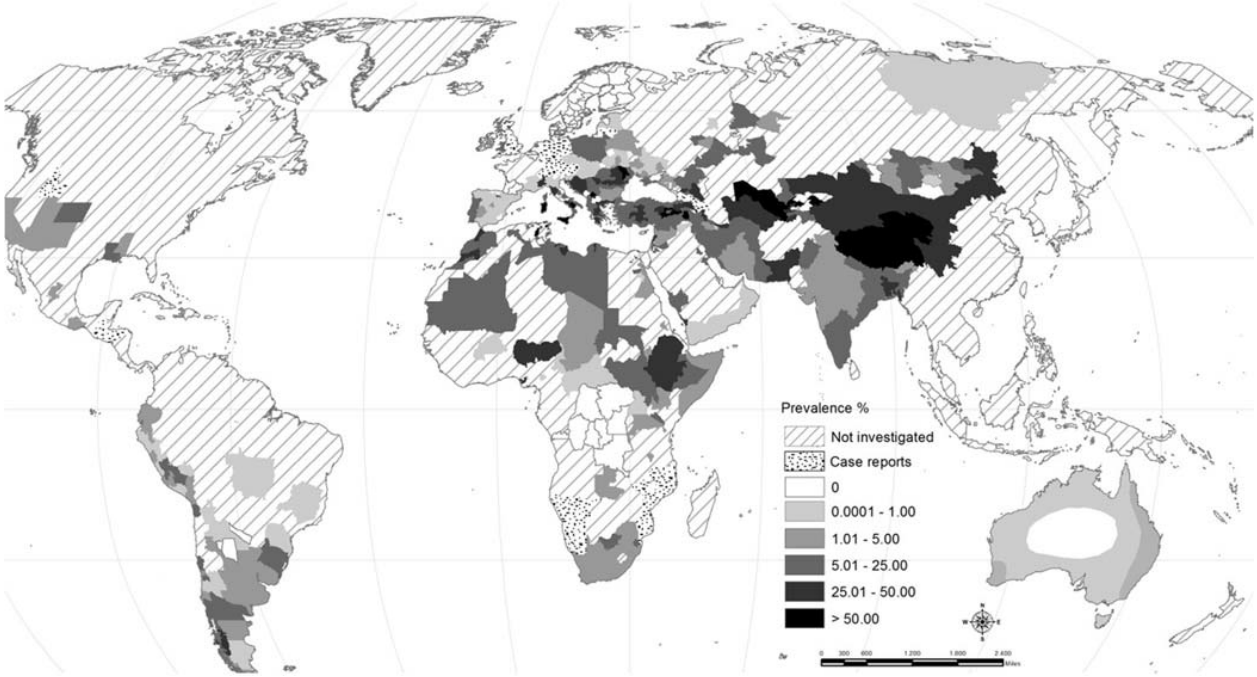


Figure 2. Global Distribution of Cystic Echinococcosis (Deplazes *et al.*, 2017).

In Europe, it is present in each country with the exceptions of Ireland, Iceland and Denmark. It is most intensely endemic in the Mediterranean areas and parts of Eastern Europe such as Bulgaria (Torgerson and Budke, 2003). The disease seems to be less prevalent in the UK, Central Europe, the Baltic States and the other Scandinavian countries (Dakkak, 2010; Romig

et al., 2006). As in other endemic areas of the world, transmission of CE in Europe relies primarily on dogs serving as definitive hosts and domestic ungulates including sheep, goats, cattle, buffaloes, equines and pigs as intermediate hosts. A sylvatic cycle maintained among wild carnivores (wolves, jackals and foxes) acting as definitive hosts and wild boars as intermediate hosts has been documented in Spain (Carmena *et al.,* 2008), Italy (Busi *et al.,* 2007; Guberti *et al.,* 2004), and Bulgaria (Breyer *et al.,* 2004). A substantial amount of data on genotyping is currently available from most of the European countries affected by CE, allowing a rather complete picture of the molecular diversity and geographical distribution of *Echinococcus* infections in production animals. At least four *Echinococcus* species have been documented to be circulating in Europe, including *E. granulosus* **ss**, *E. equinus, E. ortleppi,* and *E. canadensis*. As in the case of Asia, *E. granulosus* **ss** is the species most frequently identified among livestock isolates. Notably, *E. granulosus* **ss** G1 is the only variant found in all the isolates analyzed from intermediate and definitive wild hosts (Busi *et al.,* 2007; Sobrino *et al.,* 2006; Breyer *et al.,* 2004). Taking together, these findings emphasize the dominant role of the dog-sheep cycle in the transmission of the parasite in Europe and also provide evidence that wild animals may act as disease reservoirs for livestock and human infections. *E. equinus* has been documented infecting horses in Italy and Spain, although this specific genotype was previously known to be also present in Belgium, Ireland, Switzerland and UK (Eckert and Thompson, 1988).

In Asia the parasite is intensely endemic in large parts of China and is an important re-emerging zoonosis in the former Soviet Republics in Central Asia (Torgerson *et al.,* 2002). The parasite is also found throughout the Indian Subcontinent and the Middle East. CE in Asia is mainly transmitted through dogs acting as definitive hosts and a variety of intermediate hosts species including sheep, goats, cattle, buffaloes, camels, pigs and yaks.

Wild carnivores including jackals, wolves and probably red foxes have also been found to be infected with *Echinococcus* adult worms, demonstrating the co-existence of a sylvatic cycle (Abdybekova and Torgerson, 2012; Wang *et al.*, 2008; Sadjjadi, 2006; Dalimi *et al.*, 2002). Three species of the *E. granulosus* complex have been described in Asian livestock in recent years. These include *E. granulosus* **ss**, *E. ortleppi*, and *E. canadensis*. A recently described *Echinococcus* species (*E. shiquicus*) is also known to be transmitted in the wild, with red foxes and pikas serving as definitive and intermediate hosts, respectively (Xiao *et al.*, 2005). *E. granulosus* **ss** is by far the *Echinococcus* genotype most frequently found circulating among livestock species in Asia.

Although it seems mainly to be sylvatic, the parasite is common in North America, especially in Canada and Alaska (Torgerson and Budke, 2003). In the United States, CE does not appear to represent a major animal health problem, although it is known that several genotypes of *E. granulosus* have been introduced in imported, infected livestock hosts and have become now establish in local livestock populations (Moro and Schantz, 2006). Sporadic autochthonous transmission is currently recognized in California, Utah, Arizona, and New Mexico (Moro and Schantz, 2009). In the Northernmost regions of the Americas (Canada and Alaska) the parasite is also maintained in a sylvatic cycle involving wolves and moose, caribous and other cervids. Domestic livestock species seem to remain uninfected and do not play a role in the transmission of the parasite in these regions (Moro and Schantz, 2006). CE affects more severely South American countries characterized by extensive grazing livestock farming, including Argentina, Brazil, Chile, Peru, and Uruguay (Moro and Schantz, 2006). In these countries the infection is transmitted through the classical domestic cycle involving dogs as definitive hosts and sheep, goats, cattle, equines, pigs, and alpacas as livestock intermediate hosts. The disease seems to be rare or non-existent in countries of Northwestern South

America and Central America, with few reports of infection in animal or human hosts from these regions. Three *Echinococcus* species have been found infecting host livestock species, including *E. granulosus* **ss**, *E. ortleppi* and *E. canadensis*. *E. equinus* has not been formally documented so far, although the previous finding of horses infected with hydatid cysts in Chile (Acosta-Jamett *et al.,* 2010) support the presence of this taxon in the continent. The *E. canadensis* G8 variant ('cervid' or Northern sylvatic genotype) is known to be maintained among wolves, moose, and reindeers in Alaska and Canada (Moro and Schantz, 2006; Rausch, 2003).

CE is an animal and human health concern in Australia since the end of the eighteenth century, when the disease was accidentally introduced by the country probably in infected sheep soon after European settlement (Jenkins, 2005). CE is common in sheep-farming areas of New South Wales, the Australian Capital Territory, Victoria, southwest Western Australia and eastern Queensland. It has also been found in cattle populations in the Kimberley region of Western Australia, in northern Queensland and near Darwin in the Northern Territory (Jenkins and Macpherson, 2003). The conventional domestic life cycle of the parasite involving dogs and livestock species (mainly sheep and cattle) is known to occur in mainland Australia, although it is well recognized now that the main source of infection for domestic livestock, domestic dogs and humans today is via wildlife reservoirs (Jenkins, 2006). This is a striking feature as the overlapping and potential flow among domestic and sylvatic cycles are uncertain or not yet demonstrated in other endemic regions of the world. A number of wildlife species have been reported as suitable hosts for the transmission of *E. granulosus*. These include dingoes and foxes acting as definitive hosts and a range of macropodid species (kangaroos, wallabies, and wombats) and feral pigs as intermediate hosts (Lidetul and Hutchinson, 2007; Banks *et al.,* 2006; Jenkins, 2006). Regarding molecular genotyping data,

E. granulosus **ss** G1 is the only strain currently found in Australia (Jenkins *et al.,* 2005). This fact, together with the remarkable adaptability that this genotype has developed to infect whatever hosts become available, may explain the wide range of animal species susceptible to harbour echinococcal infections, and also the particular connection among domestic and sylvatic cycles that is characteristic of this region.

In Africa, *E. granulosus* is widespread. It is highly endemic in all North African countries including Algeria, Egypt, Libya, Morocco, and Tunisia (Dakkak, 2010), and also in sub-Saharan Africa including Ethiopia, Kenya, Mauritania, Sudan, and Tanzania (Romig *et al.,* 2011; Magambo *et al.,* 2006). It causes a considerable public health problem, especially among the pastoralists of eastern Africa (the Karamajong, Maasai, Nyangatom, Toposa and Turkana peoples) (Casulli *et al.,* 2010). The disease has also been previously recorded from most of the countries of Western, Central and Southern Africa (Eckert *et al.,* 2001; Schantz *et al.,* 1995). CE is primarily maintained in a domestic life cycle involving dogs as definitive host and various livestock species (sheep, goats, cattle, equines, camels and pigs) as intermediate hosts. Consequently, CE particularly affects pastoralists and semi – pastoralists living in arid or semi-arid areas. A wildlife cycle is also known to occur through jackals, hyenas, African wild dogs/cats, foxes and lions acting as definitive hosts and a large number of ungulates as intermediate hosts. The direct contribution of wild mammals in the transmission of CE to domestic animals is a possibility that should not be underestimated as both domestic and sylvatic cycles may coexist and/or overlap (Huttner and Romig, 2009; Eckert and Deplazes, 2004). Molecular evidence shows that at least five species of the *E. granulosus* complex are found in Africa. These include *E. granulosus* **ss** (G1–G3 genotypes), *E. ortleppi* (G5 genotype, cattle strain) and *E. canadensis* (G6/7 genotypes). *E. equinus* (G4

genotype, horse strain) has been previously documented in the continent (Macpherson and Wachira, 1997), and *E. felidis* is recently characterized (Huttner *et al.,* 2008).

In Ethiopia, cystic echinococcosis has been recorded in all corners of the country. In spite of the fact that CE is common in the country, human CE is not well studied and documented. Only few data are available for human CE. Clinical examination and serology showed that the disease is highly endemic in South-western Ethiopia. Earlier studies showed that CE was very common among the Hammer, Dassanech and Nyangatom tribes in South Omo Zone (Fuller and Fuller, 1981). Other clinical data from North-western Ethiopia reveals that the disease occurs at an average rate of 2.3 per 100,000/year (Kebede *et al.,* 2010). Most of the studies of CE in Ethiopia were done on cattle, sheep, and goats. Slaughter house surveys in different localities showed that CE is widespread in Ethiopia and causes serious economic and veterinary health problems (Sissay *et al*., 2008; Fikire *et al*., 2012; Terefe *et al*., 2012; Dawit *et al*., 2013). Survey of the disease in cattle and stray dogs in Eastern Ethiopia showed that cystic echinococcosisis highly prevalent in those animals (Mersie, 1993). Another study from North-Eastern Ethiopia further revealed that cystic echinococcosisis to be a serious public and veterinary health problem (Gebremeskel and Kalayou, 2009). High prevalence rate of CE in cattle and camel in Ethiopia could be attributed to age factor and husbandry methods. These animals are generally slaughtered at an older age than sheep and goats, and consequently are exposed to infection over a longer period of time (Abebe and Jobre, 2011). High prevalence could be due to home slaughtering practice of small ruminants without inspection, feeding of infected offal to dogs (Fikire *et al*., 2012). Poor public awareness and wrong attitude about the disease are other factors that may contribute to a higher prevalence rate (Abiyot *et al.,* 2011; Woldegeorgis *et al.,* 2009).

1.4 Life Cycle and Biology of *E. granulosus*

Echinococcus granulosus is a small tapeworm (usually 2 – 7mm in length) and morphologically recognizable by a flattish body containing 2 – 6 segments, with a scolex that bears two rows of hooks and four suckers. The adult worm lives firmly attached to the mucosa of the small intestine in the definitive hosts, usually dogs and other canines (coyotes, dingoes, red foxes), where the adult-stage reaches sexual maturity within 4 to 5 weeks. This is followed by the shedding of gravid proglottids (each containing several hundred eggs) and/or of released eggs in the feces of definitive hosts (McManus *et al.,* 2003). These eggs remain alive and keep their capability to produce infection for a long time after defecation (Thevenet *et al.,* 2005). If eggs are ingested by the intermediate hosts, mainly ungulates such as sheep, pigs, goats, horses, every egg releases an embryo (onchosphere) which possesses six hooklets.

Onchospheres can penetrate the intestinal lamina propria and travel via blood or lymph, thus are trapped in the liver, lungs, and other sites where cystic development begins. This process involves transformation of the onchospheral stage to the metacestode larva. *Echinococcus* larvae can inhabit a broad range of mammals, such as marsupials, hares, rabbits, rodents, carnivores and primates. Humans can accidentally become “aberrant” intermediate hosts, after ingestion of some species of *Echinococcus* eggs excreted by infected carnivores (Ammann and Eckert, 1996; Eckert and Thompson, 1997).

About 5 days after ingestion of eggs, the metacestode evolves into a large unilocular cyst, referred to as hydatid cyst, which is usually surrounded by a host-derived collagen capsule (adventitial layer or the pericyst), but can also be enclosed by host inflammatory cells (McManus *et al.,* 2003; McManus, 2013). The cyst wall comprises of an inner cellular layer

with 20–25 micron thickness (germinal epithelium or the endocyst) and an outer protective acellular layer (laminated membrane or the ectocyst) (McManus *et al.,* 2003). Microvilli-like extensions (microtriches) are extended from the germinal layer towards the apical membrane of its syncytial tegument and protrude into the matrix of the laminated layer. These microvilli increase the resorbing surface of the cyst (Morseth, 1967; Lascano *et al.,* 1975).

The brood capsules (daughter cysts) are small vesicular structures that are originated from the germinal layer toward the cyst cavity (Lewall, 1998; McManus *et al.,* 2003). These vesicles are primarily attached to the germinal layer by pedicles and may resemble a cluster shape in microscopic images. A large number of spherical larval heads (protoscolices) that typically possess a set of hooklets and visible suckers are asexually generated from the germinal epithelium and/or in the daughter cysts and later every one of them can evolve to an adult worm. The sucker discs are sometimes pulled inside (invaginated) or are extended out (evaginated) (Ammann and Eckert, 1996; Lewall, 1998). When the surrounding membrane of brood capsules rupture, the protoscolices and other larval particles such as hooks and proboscis are released inside the cyst lumen and because of their microscopic appearance are usually known as hydatid sand.

Protoscolices are only produced in fertile cysts (contain germinal layer) of 5 to 20 mm in diameter. Due to structural defect or absence of the germinal epithelium, some cysts do not produce protoscolices and remain sterile (Morseth, 1967; Lascano *et al.,* 1975; Lewall, 1998).

The outer laminated layer is a hyalinated carbohydrate-rich structure which is synthesized by the parasite and is secreted into periphery of the cyst (Morseth, 1967; Ammann and Eckert, 1996; Diaz *et al.,* 2011). As there is no junction between cells toward the cyst cavity, the

intercellular fluid of the germinal layer is apparently linked with the cyst vesicular fluid (Diaz *et al.,* 2011).

The size of cysts is highly variable and usually ranges between 1 and 15 cm, but much larger cysts (>20 cm in diameter) may also occur. The exact time required for the development of protoscolices within cysts in the human host is not known, but is thought to be more than 10 months post- infection. The cyst cavity is filled with a clean, clear, aseptic liquid (hydatid fluid) containing secretions from either the parasite or the host (Ammann and Eckert, 1996; Lewall, 1998). It may consist of some elements identical to that of the host's serum (Na, K, Cl, CO2, a density between 1.008 and 1.015, alkaline pH) and some proteins with antigenic properties such as Ag5 and AgB (Kagan and Agosin, 1968; Mamuti *et al.,* 2006; Czermak *et al.,* 2008). If fertile metacestodes in the cyst-containing organs of the infected intermediate host are ingested by a suitable definitive host, the life cycle of the parasite is completed, in that the protoscolices evaginate, attach to the intestinal mucosa and develop into adult stage and begin to produce parasite eggs in 32 to 80 days (Morseth, 1967; McManus *et al.,* 2003).

1.5 Pathogenesis of Cystic Echinococcosis

Definitive hosts do not usually show any disease symptoms even if they have huge parasite burden. On the contrary, the larval stage of *E. granulosus* induces significant pathology in the intermediate host. However, the pathogenicity of hydatid cyst differs from host to host and depends on many factors such as age, sex, genetic traits, physiological condition and the species of the host. Also, the severity of clinical symptoms is closely correlated with the size, number and localization of evolved cysts (Torgerson and Budke, 2003).

Almost in all intermediate hosts, hydatid cyst is principally located in the liver with a frequency of about 70%, although it can be found in other organs such as the lungs (20%),

kidneys, spleen, brain, heart and bones with less frequency (Zhang *et al.*, 2012). About 20–40% of human patients have multiple cysts or multiple organ involvement. After an undefined incubation period which may last months or years, the exerted pressure on adjacent tissue by a grown cyst may cause symptoms and can be followed by other pathologic events. As hydatid cysts grow slowly, the host often tolerates it remarkably well and therefore hydatid patients may come to clinical attention only when the normal function of the infected organs is interfered by the mechanical pressure of the cyst. Other clinical signs such as allergic reactions, eosinophilia or accidental cyst rupture which triggers acute hypersensitivity responses can also indicate the existence of the infection. Cysts or a cystic mass may also be discovered by chance during body scanning or surgery for other clinical complications (Nunnari *et al.*, 2012).

Natural history of the hydatid cyst can be divided into two phases (Stojkovic and Junghanss, 2013). During the first phase, continuous growth and the enlargement of the cyst can cause increased compression on the surrounding parenchyma and may result in upper abdominal pain and other non-specific signs. While hydatid cyst is growing, the cyst wall may lose its resistance against the pressure of the hydatid fluid, thus cyst rupture occurs. Also, this condition can happen due to a trauma or even surgical intervention. In general, symptoms such as acute allergic reactions, obstructive jaundice and emesis can be detectable as consequences of the cyst rupture. During subsequent phase, the hydatid fluid will be replaced by the components which result in stiffness of the cyst cavity and is followed by the calcification of the cyst wall. In this phase, cyst growth usually halts and the ectocyst is detached from the fibrous capsule. Although partial calcification of the cyst does not always indicate the death of the parasite, densely calcified cysts may be assumed to be inactive.

1.5.1 Cystic echinococcosis of the liver

Once the onchosphere passes through the intestinal wall, it is carried by the portal venous or lymphatic system to the liver. That is why the liver is the most frequently involved organ. Most cysts tend to be harbored in the right lobe. Hepatic cysts can cause complications in about 40% of cases. These can be commonly observed as secondary infections, whereby the cyst ruptures to the biliary tree and to the peritoneal or the pleural cavities (Kammerer and Schantz, 1993; Derici *et al.,* 2006).

Secondary infection (i.e. bacterial, fungal) of the hydatid lesion is the most common complication and can be somewhat symptomatic. The evolvement of an infected hydatid cyst is usually dormant, sub-acute and is clinically identified by pain in the right hypochondrium, hepatic abscess, and fever (Moro and Schantz, 2006; Shaw *et al.,* 2006).

Biliary rupture may occur through a small fissure or bile duct fistula (Kammerer and Schantz, 1993; Derici *et al.,* 2006; Yilmaz *et al.,* 2012). A wide perforation allows the access of hydatid membranes to the main biliary ducts, which can cause symptoms simulating choledocholithiasis (Kammerer and Schantz, 1993; Bricault, 2012). Intrabiliary rupture of a hepatic cyst can be indicated as an occult drainage of hydatid fluid into the biliary tree and is observed in 10-37% of patients mainly in centrally localized cysts (Kammerer and Schantz, 1993; Ramia *et al.,* 2012; Touma *et al.,* 2013). The increased pressure of the hydatid fluid can also be a prompting factor of the rupture usually in the right hepatic ducts, although the left hepatic ducts are sometimes involved (Kammerer and Schantz, 1993; Ramia *et al.,* 2012; Touma *et al.,* 2013). More severe complication can be detected due to an overt passage of intra-cystic material to the biliary tract in 3-17% patients (Stamatakos *et al.,* 2007). Perforation into the gallbladder can be detected in 5-6% of cases. The hydatid cyst rupture to

the biliary ducts and the dissemination of the hydatid material in the biliary tree lead to the occurrence of other biliary complications such as biliary obstruction, ascending cholangitis and hydatid-induced biliary lithiasis (Ramia *et al.,* 2012; Bricault, 2012).

Although hepatic cyst rupture in the gastrointestinal tract is very rare (Kammerer and Schantz, 1993), involvement of the diaphragm and thoracic cavity can be detected in 0.6-16% of patients (Gomez *et al.,* 1995). Perforation of the hydatid cyst into the peritoneal area and the thorax may cause serious consequences such as anaphylactic shock due to the discharge of highly antigenic compounds from cyst contents (Shaw *et al.,* 2006; Busic *et al.,* 2012; Dhua *et al.,* 2012). On the other hand, released protoscolices may settling in other visceral organs can potentially evolve into a hydatid cyst. This condition is known as secondary cystic echinococcosis and may occur either spontaneously or after trauma. Formation of secondary cysts has been also observed as a complication after inattentive surgery (Shaw *et al.,* 2006; Busic *et al.,* 2012; Dhua *et al.,* 2012). Involvement of the pulmonary parenchyma or peritoneum is usually the most frequent trait of secondary cystic echinococcosis. Nonetheless, primary infection of the peritoneum has been also reported (Smego and Sebamego, 2005; Wani *et al.,* 2005).

1.5.2 Cystic echinococcosis of other organs

In human hosts, the lungs are the second most frequent sites of infection in adults, while the involvement of the lungs is the most common feature of cystic echinococcosis in children (Beggs, 1985). In organs such as the lungs and the brain, hydatid cysts may grow faster and achieve larger size more likely due to the softness of the tissues which is easy to compress. Calcification in pulmonary cysts is very rare (0.7% of cases) (Jerray *et al.,* 1992), although it may be seen in pericardial, pleural, and mediastinal cysts (Lewall, 1998). Sudden coughing

attacks, hemoptysis, and chest pain are the most common clinical symptoms (Dogan *et al.,* 1989; Jerray *et al.,* 1992). Expectoration of the fluid or other materials of the cyst and its rupture into the pleural cavity may also occur. Bacterial infection of the cyst is the most serious complication commonly seen after rupture (Dogan *et al.,* 1989; Jerray *et al.,* 1992).

The prevalence of renal infection is 3% and the involvement of the kidneys usually remains asymptomatic for many years, although symptoms such as flank mass, pain and dysuria can be commonly seen (Von Sinner *et al.,* 1993; Odev *et al.,* 1996). Renal hydatidosis can be characterized more frequently by solitary cysts located in the cortex, and they may reach 10 cm before any clinical symptoms are noted (Odev *et al.,* 1996). Uncomplicated cysts may produce a lump of rounded mass in the outline of the kidney that extends the infundibula and calices. In up to 18% of cases, the cyst may rupture into the collecting system, a situation which may induce acute renal colic and hydatiduria. Several rounded masses may be seen in the excretory system due to daughter cysts (Odev *et al.,* 1996).

The splenic involvement in human hydatid disease has been reported in 8% to 9% of cases (Franquet *et al.,* 1990). The metacestode can be harbored in the spleen mainly after systemic or inter-peritoneal dissemination of protoscolices due to the rupture of hepatic cysts. Consequently, the spleen is usually considered the third most frequent site of infection in humans. Clinical symptoms such as abdominal pain, splenomegaly and fever are often observed in patients with splenic hydatid infection (Franquet *et al.,* 1990; Akhan and Koroglu, 2007).

The osseous involvement in hydatid disease is most commonly seen in the spine and pelvis, followed by the femur, tibia, humerus, skull, and ribs with a frequency ranging between 0.5-4% (Beggs, 1985; Torricelli *et al.,* 1990; Islekel *et al.,*1998). The absence of the cellular

infiltrate and fibrosis (pericyst) around the cysts in the skeletal system allows them to grow vastly in an irregular manner and they may produce subsidiary branches that penetrate through less resistant compartments of the tissue, especially in the bone canals (Torricelli *et al.,* 1990). The growing metacestode in the bone can also possess a vesicular shape which fills the space between trabecular structures and destroys the osseous tissue. If the tissue damage extends to the cortex, the subsequent diffusion of the parasite materials into the surrounding tissues may occur (Torricelli *et al.,* 1990; Banerjee *et al.,* 2012).

Hydatid disease affects the central nervous system in 1% of cases (Odev *et al.,* 1996) and is usually diagnosed during childhood. The hemispheres are the most common locations of the cerebral cysts, particularly around the middle cerebral artery, although the metacestode may be harbored anywhere in the brain (Kovoor *et al.,* 2007).

The infection may also involve almost any anatomic site due to hematogenous dissemination. These locations include the heart, pericardium, orbit, retrocrural space, mediastinum, subcutaneous space, muscle, and adrenal glands (Beggs, 1985; Bashour *et al.,* 1996; Kiresi *et al.,* 2003; Benhaddou *et al.,* 2010; Kayaalp *et al.,* 2011).

1.6 Public Health and Economic Importance of CE

Cystic echinococcosis has a considerable impact in both human and animal health, with important economic consequences arising from the cost of medical treatment and morbidity and losses in animal productivity (Torgerson, 2003). For example, in North African countries, the cost to human health treatment and animal losses was estimated at US$ 60 million per year (Budke *et al.,* 2006; Moro and Schantz, 2006). In Jordan alone, an equivalent to 21 million US dollars was reported (Torgerson *et al.,* 2001; Conteh *et al.,* 2010). The annual

economic losses, due to cystic echinococcosis, on a global basis have been estimated to be over 2 billion US dollars (Budke *et al.*, 2006).

While in animals clinical symptomatology may be relatively unusual, there are reports of decrease in feed conversion ratios, lowering of milk production in lactating animals, decreases in reproduction rates and decreases in the value of wool or hides from infected animals. These effects have been analysed economically (Torgerson *et al.*, 2000; 2001), and it is possible that in some societies the economic effects of infection in domestic stock may be the most important effect costing the livestock industries millions of dollars in endemic areas. In Uruguay, for instance, the annual losses were estimated at 6.2 million US dollars from the organs condemnation and the loss of livestock productions (Torgerson *et al.*, 2000). In Queensland Australia, hydatid disease was estimated to cost the meat industry, conservatively about 2.7 million US dollars annually through lost offal sale (McManus and Thompson, 2003). For instance, in Yugoslavia, a 10% reduction in milk yield and 5% in carcass weight due to cystic echinococcosis has been described (Torgerson, 2003). In severe infection, the parasite may causes retarded performance and growth and reduced quality and yield of meat and milk (Getaw *et al.*, 2010).

1.7 Diagnosis of CE

The clinical diagnosis of cystc echinococcosis, in previous times, was dependent on symptoms which could be detected by inspection and palpation (e.g., distended abdomen). Although the clinical manifestations, caused by cysts of *E. granulosus* in various organ systems, were quite well known to authors in the second half of the 19th century, differential diagnosis (tumours, liver abscess etc.) was difficult in many cases. Diagnostic puncture was widely used in the 19th and the beginning of the 20th century. This method was regarded as

useful and mostly harmless, but the risks like dissemination of protoscoleces, allergic reactions and bacterial infections, were recognized. Regardless of these risks, the fine-needle puncture is used occasionally (e.g., cysts or unclear lesions in seronegative persons). Over the years, attempts were made to introduce and apply improved diagnostic methods.

1.7.1 Immunodiagnosis

Immunodiagnosis of cystic echinococcosis in humans dates from the beginning of the 20th century. For the detection of circulating anti-*Echinococcus* antibodies a complement fixation test (Ghedini-Weinberg test) and precipitation test (Casoni test) were developed. These tests were complemented or gradually replaced by better methods, such as indirect haemagglutination test, bentonite and latex agglutination tests, immunoprecipitation and immunoelectrophresis since the middle of 20th century. Some of these tests were quite sensitive in detecting CE, especially of the liver, but a general problem was the low degree of specificity. Later on, the repertoire of antibody tests was extended by further procedures, including the indirect fluorescent antibody test, the enzyme-linked immunosorbent assay (ELISA) and some secondary tests. The use of purified or recombinant antigens in modern testing procedures significantly improved the reliability of diagnostic results (WHO, 2001).

E. granulosus cysts induce a strong antibody response in most patients, triggering different isotypes (IgG, IgM, IgA and IgE), although the sensitivity and specificity of the response depend on several factors. The first antibodies appear few weeks after infection against oncosphere antigens. Subsequently, antibodies are developed against the laminar layer and later against the cyst fluid (HF) and protoscoleces, if present.

The main serological methods used for CE diagnosis and follow up are based on the detection of specific IgG antibodies. The most widely used antigen for the detection of specific IgG

antibodies is the HF. This antigen mixture is currently used in several techniques such as the ELISA, the indirect haemagglutination test (IHA) and the immunoblotting (IB). The immunoprecipitation agar technique, which was used for the detection of arc5, is nowadays rarely used due to several disadvantages. Both the ELISA and the IHA are usually the first line tests for CE patients, while the IB is used as confirmatory test. In this context, a number of drawbacks have been detected, including low sensitivity and specificity and a poor prognostic value for followup due to the long-lasting persistence of antibodies against HF (Barnes *et al.*, 2012).

A number of recent studies that used the IgG-ELISA against HF antigens for CE diagnosis reported variable sensitivity (Sarkari and Rezaei, 2015; Manzano-Roman *et al.*, 2015). False negative results depend on several factors, including early cyst stages (Tamarozzi *et al.*, 2013), and cyst location (Kilimcioglu *et al.*, 2013). Parasite genotype is an additional source of potential false negative results in serological tests with HF (Jiang *et al.*, 2012a &b). Another problem is the percentage of false positive results. IgG-ELISA based on the use of HF as antigen gives rise to variable false positive results in healthy donors from different geographical areas (Mohammadzadeh *et al.*, 2012; Zhang *et al.*, 2012; Tamarozzi *et al.*, 2013). Cross-reactivity of antibodies against HF is found in patients with other parasitic and non-parasitic diseases (Manzano-Roman *et al.*, 2015; Tawfeek *et al.*, 2011; Chirag *et al.*, 2015; Dekumyoy *et al.*, 2005). The antibody response against the HF is variable in different patients and in the same patient at different times post infection. This variability depends on several clinical variables, including cyst stage, number of cysts, cyst size and location, treatment followed by each patient and parasite genotype, among others (Manzano-Roman *et al.*, 2015; Lissandrin *et al.*, 2016).

The total somatic extract of protoscoleces has been assayed by several authors. The results showed wide range of sensitivity (Chen *et al.,* 2015; Manzano-Roman *et al.,* 2015; Sarkai and Rezaei, 2015). Moreover, specificity has been worse for the extract of protoscoleces, compared to HF. Analysis of other extracts, i.e adult worms, protoscoleces tegument and cyst wall, showed that sensitivity was ranging from 81.3% to 96.7% with false negative results (Schweiger *et al.,* 2012; Mohamed *et al.,* 2014). A common problem for all these extracts is their cross-reactivity and their heterogeneity, very similar to that found for HF.

A number of purified native antigens have been tested for the detection of antibodies in CE patients, mainly representing AgB and Ag5. Unfortunately, similar drawbacks for HF are encountered when purified antigens from this source are used, including false positive results probably due to the presence of cross-reacting carbohydrate moieties in purified native antigens, false negative results attributed to clinical variables already pointed out for HF, and variability in sensitivity and specificity using the same purified antigen due to lack of standardization of purification methods, among others, both in ELISA and immunoblot (Manzano-Roman *et al.,* 2015).

1.7.2 Diagnostic Imaging

Various imaging modalities, including ultrasonography (US), computed tomography (CT), magnetic resonance imaging (MRI) and conventional radiography, are important for the diagnosis of CE (Polat *et al.,* 2003). These techniques are used for classification, staging, identification of possible complications and monitoring the response to treatment.

Ultrasonography

Classifications of the various appearances of CE cysts are based on US features obtained from liver scans. When US first became widely available, Gharbi *et al.* (1981) classified liver cysts into five groups: type I (pure fluid collection), type II (fluid collection with a split wall), type III (fluid collection with septa), type IV (cysts with heterogeneous echogenicity) and type V (cysts with thick walls). In 1995 the WHO-IWGE evaluated this classification schemes for advantages and weaknesses with reference to simplicity, pathophysiological relevance and utility for the follow-up of treated patients. Final agreement was achieved in 2001, with details of the consented classification were issued in 2003 (Fig 3). (WHO-IWGE, 2003).

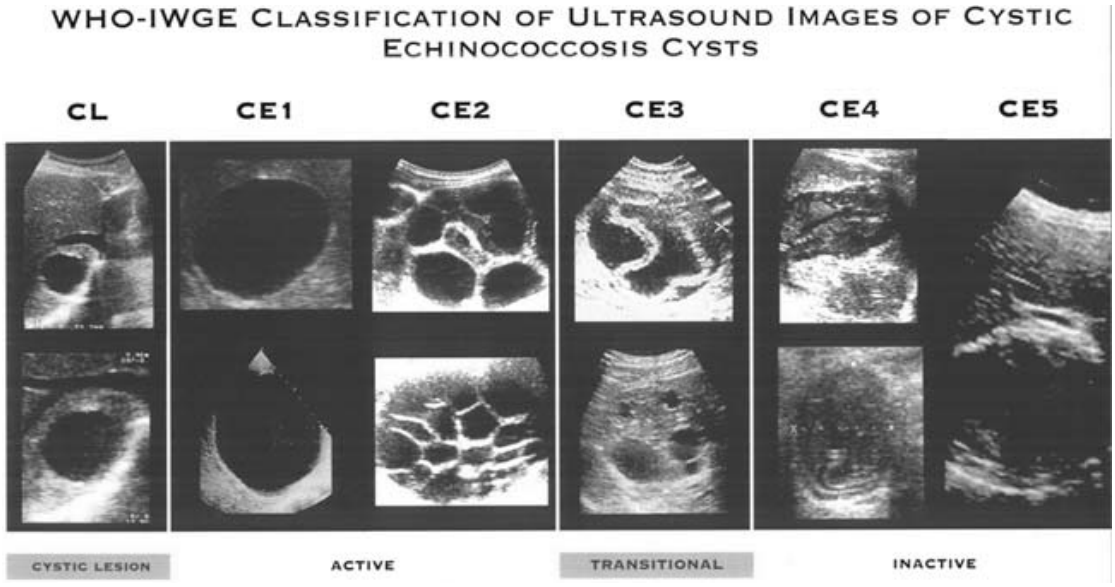


Figure 3. WHO-Informal Working Group on Echinococcosis standardized classification (Brunneti *et al.*, 2010)

Two main differences between the WHO-IWGE and 'Gharbi's' classification were the addition of 'cystic lesion' (CL) stage and the reversing of type II and III into CE3 and CE2, respectively. Thus the WHO-IWGE classification grouped cysts into three clinical categories: active cysts that are developing and usually fertile (CE1, CE2); transitional cysts that are

degenerating but usually still containing viable protoscoleces (CE3) and inactive cysts that have degenerated or are calcified and unlikely to be fertile (CE4 and CE5). For each category, cyst diameter was also considered, with cysts classified as small (<5.0 cm), medium (5 to <10 cm) or large (>10 cm) (WHO-IWGE, 2003). US is considered the gold standard imaging method. The following stages are based on the WHO-IWGE classification (Brunneti *et al.*, 2010).

Active stage CE1: CE may manifest as a well-defined anechoic cyst. The cyst wall is usually observed as the double echogenic lines separated by a hypoechogenic layer termed the 'double contour sign'. No internal structures are observed in simple cysts. However, by repositioning the patient multiple echogenic foci, due to the presence of hydatid sand, may be detected within the lesion. The echogenic foci quickly fall to the lowest portion of the cavity without forming visible strata. This finding has been referred to as the 'snowstorm' or 'snowflakes sign'.

Active stage CE2: These multiseptated cysts manifest as well-defined fluid collections in a 'honeycomb pattern', with multiple septa representing the walls of the parasitic vesicles. Separated, entire vesicles appear as 'cysts within a cyst' and are commonly referred to as 'daughter cysts'.

Transitional stage CE3: These cysts have multiple internal septa, daughter cysts, multiple echogenic foci and floating membranes inside the cyst cavity. A decrease in intracystic pressure, cyst degeneration, trauma, host response or response to therapy may lead to detachment of the parasite from the host-derived adventitial layer, which may show the 'water-lily sign' (***CE3a***) or in some cases, a 'wheel spoke' pattern (***CE3b***). The matrix represents echinococcal fluid containing membranes of broken vesicles, brood capsules,

protoscoleces and ‘hydatid sand’. Membranes may appear within the matrix as serpentine linear structures, a finding that is highly specific for diagnosis of CE.

Inactive stage CE4: In this stage the matrix fills the cyst completely, creating a mixed echogenic pattern that mimics a solid mass. This appearance is called as the ‘ball of wool sign’. Because differentiation of this cyst type from other hepatic masses or abscesses is often difficult, it is important to look for membranes within the lesion that may help in making a correct diagnosis. While most CE4 cysts are inactive, the parasite may still be alive (Wang *et al.,* 2006). Contrast-enhanced CT or MRI may be necessary to correctly identify this stage.

Inactive stage CE5: In this stage, calcification of cyst wall occurs. Calcification of the internal matrix may also be seen. These cysts have a hyperechoic contour, with a cone-shaped acoustic shadow. When the cyst wall is heavily calcified, only the anterior portion of the wall is visualized and appears as a thick arch with a posterior concavity which is an important sign for the diagnosis of inactive stage CE5.

Computed Tomography (CT)

While there is no cyst classification scale specific for CT, US classification methods may be used (Stojkovic *et al.,* 2012). CT is actually the method of choice to study extra-hepatic dissemination of cysts because it allows for imaging of the entire abdomen, pelvis and thorax (Oto *et al.,* 1999). It is also commonly used in obese patients and those who have had previous abdominal surgery or who suffer from excessive intestinal gas. Common locations for extra-hepatic dissemination of cysts include the other abdominal organs, peritoneum, the diaphragm, the thoracic cavity, the abdominal wall, the portal system and other vessels (Chawla *et al.,* 2003). Administration of intravenous (IV) contrast medium may be warranted to obtain a vascular map or if infection or communication with the biliary tree is suspected.

Abscesses typically appear as a highly attenuated rim surrounding the lesion. Patchy areas of contrast-enhanced liver parenchyma, in the vicinity of the lesion, can represent inflammatory changes. Indirect signs of infection and/or communication with the biliary tree, including finding evidence of gas, air or gas inside the cyst.

Calcification of the cyst wall can be detected by CT with completely calcified inactive CE5 cysts appearing as round hyperattenuating masses. However, CT has limited ability to evaluate internal septa, floating membranes and daughter cysts. On CT, a CE cyst typically appears as a round lesion with water attenuation density, surrounded by a calcified ring-like or highly attenuated wall, representing the host-derived adventitial layer. Detachment of the parasite membranes from the adventitia is seen as linear areas of increased attenuation within the cyst. If daughter cysts are visualized, they typically contain fluid with a lower attenuation than the fluid in the main cyst. Positron-emission-tomography using 18F-fluoro-desoxyglucose as a tracer (FDG-PET), with or without CT, is not currently recommended since there is no FDG uptake except in CE cysts with bacterial infection (Niccoli Asabella *et al.*, 2013).

Magnetic Resonance Imaging (MRI)

MRI allows the visualization of cysts in multiple planes (Marrone *et al.*, 2012). It is also the best imaging modality to detect biliary tree involvement MRI. If US cannot be performed due to cyst location or patient-specific reasons, T2-weighted MRI is often preferable to CT except when it comes to evaluating calcifications (Stojkovic *et al.*, 2012). MRI is the best diagnostic imaging modality to evaluate floating membranes and membrane detachment.

Diffusion-weighted MRI (DW-MRI) is helpful in differentiating purely liquid cysts (active CE1) from benign liver cysts (CL). With this method, CE cysts appear hyperintense, whereas,

benign cysts do not. Magnetic resonance cholangiopancreatography (MRCP) enhances visualization of communication between the CE cyst and biliary tree and dilatation of the biliary system secondary to compression of the cyst (Hosch *et al.,* 2007). Proton magnetic resonance spectroscopy (1H-MRS) provides additional information based on the metabolic composition of the CE cysts (Barker, 2005; Limanond *et al.,* 2004). This method may aid in determining the viability of the germinal layer and protoscoleces (Hosch *et al.,* 2008).

X-Ray

On chest X-ray, intact cysts typically appear as homogeneous round or oval-shaped structures with smooth borders surrounded by normal lung tissue. Large cysts can shift the mediastinum, induce a pleural reaction or cause atelectasis of the adjacent parenchyma. Cyst growth produces erosions in the bronchioles and, as a result, air is introduced between the adventitia and laminar layer, producing the ‘crescent’ or ‘meniscus’ sign. Air penetrating the interior of the cyst may outline the inner surface of the laminar layer, producing parallel arches of air that are referred to as ‘Cumbo’s sign’. This phenomenon has also been described as having an onion peel appearance (Morar and Feldman, 2003). If a ruptured cyst communicates with the tracheobronchial tree, evacuation of the cyst contents results in an air/fluid interface. After partial expectoration of the cyst fluid and protoscoleces the cyst empties and the collapsed membranes can be seen inside the cyst producing the ‘serpent sign’. When the crumpled endocyst floats freely in the cyst fluid, it is known as the ‘water-lily sign’. In a minority of cases, when the fluid is completely evacuated by expectoration, the remaining solid components fall to the lower part of the cavity, with the resulting mass called ‘Monod’s sign’.

1.7.3 Molecular Diagnosis

With the advent of molecular approaches for the detection of parasites, various techniques were developed during the last three decades to identify *Echinococcus* species and strains from animal and human hosts. PCR-based methodologies have found a broad applicability for detection, population studies and epidemiological investigations. These techniques were used on different analytes such as eggs, worms, protoscolices or germinal layer from metacestodes, and from heterogeneous matrices such as soil, vegetables, host intestinal mucosa and faeces (Siles-Lucas *et al.,* 2017).

The target nucleotide sequences used for the identification of *Echinococcus* spp. are located within the mitochondrial genes or nuclear ribosomal DNA (rDNA). Mitochondrial DNA (mtDNA) has been widely used for the identification of closely related species or strains. Among the 12 protein-coding genes mtDNA: cytochrome c oxidase complex (cox1), cytochrome b (cob) and nicotinamide dehydrogenase (nad1) genes are widely used targets for *Echinococcus.* Nuclear rDNA markers used for the identification and characterization of genus *Echinococcus* include the internal transcribed spacer (ITS), external transcribed spacer (ETS) and the 28S rRNA gene (Bowles *et al.,* 1992; Bowles and McManus, 1993a & b; Nakao *et al.,* 2000).

At least three steps are required in the molecular diagnostic procedure: sample preparation, DNA extraction and specific amplification of *Echinococcus* DNA followed by visualization and measurement of the PCR products. Parasite specimens can be collected from the environment (tissue or eggs contained in faeces or dispersed in the soil). These samples can be preserved in ethanol (>70% v/v), frozen at least at -20^{o}C or in paraffin-embedded tissues (Siles-Lucas *et al.,* 2017).

Several laboratory techniques can be used to isolate and concentrate eggs, worms and parasitic material from animal definitive hosts. Regarding human hosts, depending on the clinical management of CE, several options are available to isolate metacestode material. The samples are usually available after surgical treatment, percutaneous interventions by puncture-aspiration-injection- re-aspiration (PAIR) or catheter drainage (Brunetti and Filice, 2001; Brunetti *et al.*, 2010).

Various DNA extraction procedures exist depending on the matrix (intestines, faeces, soil, vegetables) and parasitic analyte (eggs, cysts, worms) investigated. For DNA extraction, the principal method consists of the classic phenol-chloroform DNA extraction with alkaline lyses step and organo-solvent extraction (Monnier *et al.,* 1996); as well as the use of commercial DNA isolation kits (Ni *et al.*, 2014); and DNA fishing/magnetic capture method (Isaksson *et al.*, 2014). DNA extraction can be also performed directly from faeces (Knapp *et al.*, 2014). This method generally cannot handle more than a maximum of 0.5 g, but will extract all taeniid DNA and also DNA from other organisms present in the sample.

DNA extraction can be performed using worms, protoscolices or eggs. The specimen is handled under a stereo microscope, washed three times with distilled water and lysed in 10 ml of 0.02N NaOH at 95^{o}C for 10 min (Huttner *et al*., 2008; 2009). The lysate can then be directly used as template for PCR. DNA can be easily extracted from ethanol (>75% v/v) preserved metacestode tissue retrieved from human and animal hosts. Protoscoleces are the tissue of choice when performing such DNA extractions. Germinal layers can be also used for these purposes.

Polymerase chain reaction (PCR) is the method of choice for parasite identification, molecular epidemiological studies and confirmatory purposes, although several traditional

molecular approaches have been used in the past such as PCR/RAPD (Random Amplification of Polymorphic DNA) and PCR/RFLP (Restriction fragment length polymorphism) (Bowles and McManus, 1993a; Xiao *et al.*, 2006a). Conventional and robust approaches have been used for genus/species detection such as conventional-PCR, nested-PCR to test faecal samples or multiplex-PCR for a more differential detection of *Echinococcus* spp. along this path, sensitive approaches involving PCRs and sequencing have been developed to detect variability within species and genotypes (Bowles *et al.*, 1992; Bowles and McManus, 1993b; Nakao *et al.*, 2000).

Sequencing is used for detecting genetic variation and species identification within genus *Echinococcus*. More recently, affordable and easy to use approaches such as LAMP (loopmediated isothermal amplification method), were developed and tested (Ni *et al.*, 2014 Wassermann *et al.*, 2014). LAMP is an affordable tool to use in low resource settings endemic for alveolar and cystic echinococcosis because DNA can be amplified using a simple water bath avoiding the need for complex instruments. However, the system is prone to the introduction of false positives. Real-time PCR (qPCR) (Dinkel *et al.*, 2011; Knapp *et al.*, 2014) offers several advantages over conventional PCR for the detection of parasitic infections, including increased sensitivity and specificity, reduction in reaction time and a quantitative estimate of the amount of DNA in the sample. In addition, Real-time PCR with high-resolution melting (qPCR, HRM) has become a sensitive genotyping method, based on the characteristics of thermal denaturation of the amplicons (Rostami *et al.*, 2013; Santos *et al.*, 2013; Safa *et al.*, 2015).

1.8 Treatment of CE

For many years, surgery has been considered the only treatment available for cystic echinococcosis because of the potential radical removal of the parasite. Nowadays, besides surgery, clinical management of cystic echinococcosis relies on several therapeutic approaches ranging from chemotherapy with benzoimidazole carbamates (mebendazole and albendazole) to percutaneous nonconventional treatment, like puncture – aspiration – injection and reaspiration (PAIR), or Radio-Frequency Thermoablation, or "wait and see" approach (Pawlowski, 1997). There is no single "golden rule", but it is very important to individualize the treatment of choice based on the patient and the nature of hydatid cyst (Pawlowski, 1997; Junghanss *et al.,* 2008; Teggi, 2004; Garcia *et al.,* 2007). For non-complicated small hepatic cysts, it is possible to take into account the "watch and wait" approach because inactive cysts that do not compromise organ function or cause discomfort, seem to remain unchanged or stabilize even further. When the cysts are complicated, surgery represents the first choice of treatment (Pawlowski, 1997; Junghanss *et al.,* 2008). Surgery is indicated for large hepatic cysts with multiple daughter cysts; for single hepatic cysts, situated superficially, which may rupture spontaneously, or because of trauma; for cysts that are infected; for cysts communicating with biliary tree and/or exerting pressure on adjacent vital organs. Because curative surgery is not always possible, there is a 2–15% risk of relapse in hyperendemic areas and moderate ranges of morbidity in particular when the surgery is repeated. Extra caution must be there during surgery, since the cyst can break spontaneously or the surgical damage of the cyst can lead to spillage and widespread dissemination (Pawlowski, 1997; Buttenschoen and Buttenschoen, 2003; Saimot, 2001).

Chemotherapy with benzoimidazole carbamates (mebendazole or albendazole), once reserved for inoperable cases of cystic echinococcosis, is widely used (Teggi, 2004; Saimot, 2001;

Franchi *et al.,* 1999; Falagas and Bliziotis, 2007; Teggi *et al.,* 1993). Sometimes cysts of the same patient with the same morphology and localized in the same organ may differently respond to therapy probably because they have a different intrinsic sensitivity to drugs. Chemotherapy may be less effective for thin-walled daughter cysts within a mother cyst (Pawłowski *et al.,* 2001). Some treated patients exhibit relapses, but these are usually sensitive to retreatment in high proportion of cases (up to 90%) (Teggi *et al.,* 1995). The rate of relapses after chemotherapy is relatively high. Chemotherapy is contraindicated for large cysts with a risk of rupture (notably superficially situated, infected cysts) or for inactive or calcified cysts. Patients with severe chronic hepatic diseases and with bone marrow depression should not be treated (Pawłowski *et al.,* 2001). Early pregnancy is also a contraindication.

Combined therapy of praziquantel and albendazole may reduce the risk of disease recurrence and intraperitoneal seeding of infection that develops via cyst rupture and spillage occurring spontaneously or during surgery or percutaneous procedures (Bygott and Chiodini, 2009). However, there is insufficient published evidence to support a clear recommendation for the use of praziquantel in prolonged chemotherapy for established hydatid disease for which surgery is not indicated or in severe disseminated disease (Bygott and Chiodini, 2009). The percutaneous treatment by puncture of the cyst, aspiration of cyst fluid, injection of a scolicidal agent, and reaspiration of the cyst content percutaneous (PAIR) under sonographic guidance played an important role in the treatment of cystic echinococcosis. The aim of this treatment is to destroy the germinal layers with scolicidal agents or to evacuate the germinal and laminated layers, i.e. the entire endocyst (Filice and Brunetti, 1997). Its efficacy has been confirmed both by short- and long-term follow-up. Percutaneous drainage of echinococcal cysts is effective and relatively safe. The major risks of percutaneous techniques are

anaphylactic shock, secondary echinococcosis caused by spillage of cystic fluid, and chemical angitis caused by contact of the scolicidal agent with the biliary tree (Pawłowski *et al.,* 2001). Radiofrequency thermal ablation uses the same needle electrodes used for local treatments of hepatocellular carcinomas (Brunetti and Filice, 2001). The experience with radiofrequency thermal ablation is still very limited; however, it does not seem to be very effective at long-term follow-up (Junghanss *et al.,* 2008).

1.9 Control and Prevention of CE

Control of CE can be implemented through either horizontal or vertical approaches. The former emphasizes long-term primary health care (education, sanitation, upgrading of meat inspection, etc.) with the aim to reduce disease transmission. The vertical approach is targeted to the parasite; and it is based on specific control measures (dog population control, dog-dosing, etc.) and must include a base-line survey and surveillance of intermediate hosts to monitor progress (Gemmell *et al.,* 2001).

Control strategy can be implemented in 4 phases, namely preparatory or planning, attack, consolidation and the maintenance of eradication phase (Eckert and Deplazes, 2004; Gemmell *et al.,* 2001). Preparatory or planning phase may include appointment of an appropriate authority supported by legislation, collection of baseline data, selection of appropriate control strategies, selection and training of staff and provision of sufficient fund for the program. During the attack phase, control measures are applied non-discriminately to the entire host population at risk. Examples of this are mass dog-dosing campaigns and the introduction of restrictive regulations on dog feeding practices. In the consolidation phase, risky areas or farms are identified through surveillance and control measures are targeted at these only. Surveying involves the gathering of data about past infections and present status

of the disease in the local community. In addition to this, it is also done by mass detection of serum antibodies against *E. granulosus* infection in the community and detection of the egg in the stool of the definitive hosts in the surroundings (Eckert and Deplazes, 2004). Meat inspection and legislation to quarantine infected premises are also included in this phase. The maintenance of eradication phase can be entered once the parasite has possibly been eliminated.

It is very important to have some piece of information about the prevalence and incidence of the disease before any control measure is implemented. It prevents unnecessary expenditure of time and energy and makes the control strategies more targeted and effective. However, it is very difficult to define the level of *E. granulosus* infection in a given community. This is because of several factors. First, in some countries, echinococcosis is a zoonotic infection and may be monitored by more than one governmental body. Usually, these bodies may not coordinate such public concerns. So, it is unlikely to come up with reliable data about the burden of infection on the community. The second problem is that the infection is asymptomatic in its clinical manifestation, especially during the early stage of infection (Eckert and Deplazes, 2004). It is not possible to determine the disease burden by using the data obtained from local health centers because of difficulties associated with diagnosis (Craig *et al*, 2003).

Cystic echinococcosis can be controlled through preventive measures that break the cycle between the definitive and the intermediate host. These measures include dosing dogs, inspecting meat and educating the public on the risk to humans and on avoiding feeding offal to dogs, as well as introducing legislation (McManus *et al.,* 2003; Kachani *et al.,* 2003). However, none of these measures will work in isolation. The disease can be controlled successfully through health education and appropriate legislation only when people

understand the life cycle of the parasite (Gemmell *et al.,* 2001). It is of the utmost importance that the government be involved, for example, through the Ministries of Health and Agriculture.

Research on mathematical models of cystic echinococcosis control indicates that vaccination of sheep would be an effective control strategy, provided that over 90% vaccine coverage of the sheep population was achieved (Craig *et al.,* 2007). However, the most effective intervention that was revealed by the modeling was a combination of vaccinating sheep and dog anthelmintic treatment. If about 75% vaccine coverage of the sheep population is achieved, anthelmintic treatment of dogs could be reduced to 6-month intervals while still achieving a high level of control of disease transmission, thereby greatly reducing the cost of a control programme and probably also increasing compliance from dog owners (Torgerson, 2006).

Echinococcus vaccines would ideally prevent oncosphere development to hydatid cysts in sheep, and thus stop the development of adult gravid tapeworms in dogs (Zhang *et al.,* 2001; Zhang *et al.,* 2003). With an efficient vaccine the attack phase can be shortened considerably. Great efforts have been made in Australia and New Zealand to develop vaccines which can protect sheep or cattle against infections with metacestode stages of taeniid cestodes. A defined recombinant vaccine for ovine cystic echinococcosis (EG95) was developed by the groups led by Lightowlers and Heath in 1996 in Australia and New Zealand (Heath and Lawrence, 1996; Lightowlers *et al.,* 1996). The native molecule is 24.5 kDa and cloned as a 16.5 cDNA fusion peptide of 155 amino acids with a fibronectin-like motif under the control of seven closely related genes (Zhang *et al.,* 2003; Woollard *et al.,* 1998; Heath and Lawrence, 1996; Lightowlers *et al.,* 1996). Large controlled studies with sheep have shown that vaccination with a recombinant oncospheral *E. granulosus* antigen (EG95) induces high

degrees of protection, reducing the cyst numbers in vaccinated animals by approximately 90 to 100%. Field trials in Australia, New Zealand, Argentina, Italy, and China demonstrated more than 95% protection for at least 12 months in sheep following two injections in Quil A adjuvant (Eckert and Deplazes, 2004, Lightowlers *et al.*, 1996; Woollard *et al.*, 1998; Heath *et al.*, 2003). Research into improved delivery is focused on the use of heterologous vectors (eg, *Corynebacterium* spp, Salmonella, and adenovirus), and also delivery as a DNA vaccine, although the latter produced equivocal results. Perhaps the most attractive option for this kind of zoonoses vaccine in view of the low disease/pathology status of infected sheep is the incorporation of EG95 with an existing commercial livestock vaccine such as tetanus and leptospirosis (Lightowlers and Gauci, 2001).

2 RATIONALE OF THE STUDY

CE is a neglected disease with negative impact on human health and the economy of endemic areas, which mostly consists of developing countries (Budke *et al.,* 2006). It is a typical affliction of rural pastoralist societies which have little access to health facilities, and whose economic losses are rarely considered and are difficult to quantify (Huttner and Romig, 2009). Control of CE has been difficult to sustain in these areas because of financial and logistic constraints (Magambo *et al.,* 2006). Individuals will be forced to spend a lot of time and money for diagnosis and treatment of CE, which is unaffordable by pastoralist communities. Even those who managed to have surgical treatment remain morbid with a significant decrease in their quality of life.

CE causes direct economic loss by affecting livestock through condemnation of infected organs like liver and lungs. Indirectly, it adversely affects the economy by causing reduction in live weight-gain, fecundity, and milk yield and wool production of the livestock. The ban on meat and meat product export due to hydatid infected livestock will significantly increase economic losses in foreign currency earnings and by affecting employment opportunities in the animal products processing industries.

Although few prevalence studies have been reported from the present study area about CE, the studies did not determine the burden of the disease in humans, animals and neither did they assess the major risk factors to human infection. Moreover, the parasite species involved and its strains have not been characterized. Therefore, the study seeks to provide relevant information that can be used in planning the control and prevention of CE in South Omo Zone.

3 HYPOTHESIS

CE is endemic in South Omo Zone with possible detection of additional new strains of the parasite in human population.

4 OBJECTIVES

4.1 Major Objective

The major objective of this study is to assess the prevalence and identify the risk factors of human cystic echinococcosis in South Omo Zone, Ethiopia.

4.2 Specific Objectives

- To determine the prevalence of CE in humans and animals in the study area.
- To identify the major risk factors associated with human CE in the study area.
- To determine the strains or genotypes of *E. granulosus* in the study area using molecular techniques.

5 MATERIALS AND METHODS

5.1 Study area and study population

The study was conducted in Hamer, Nyangatom and Dasenech districts of South Omo Zone, SNNPR, Ethiopia. The total area of South Omo Zone is 24,249 km^2. The Zone has eight Districts and the Jinka town administration (the Zone Capital), which is located about 775kms from Addis Ababa. Sixteen ethnic groups are indigenous to the Zone, while a good number of individuals from other ethinicities from other parts of the country also live there. The study area is characterized by diverse altitudes, from 380masl to over 2000masl, and hot, dry *Acacia* grassland receiving rain from under 400 mm up to 800 mm per year. The mean maximum daily temperature in the extreme southwest averages nearly 34°C annually. In March, the daily average maximum temperature exceeds 36°C.

According to the population projections for 2016/17 based on the population and housing census conducted in 2007 (Central Statistical Agency, 2008), it is estimated that the Zone has a total population of 767,915. The population of the three districts, Hamer, Nyangatom and Dasenech is 79,419; 23,250 and 70,133, respectively. Among these, 7.53% are urban dwellers and 4.45% are pastoralists (Central Statistical Agency, 2008). The major ethnic groups in the study areas were Hamer, Kara and Arebore (Hamer Districts), Nyangatom and Murule (Nyangatom District), Dasenech (Dasenech District). Almost all of these ethnic groups are pastoralists and semi-pastoralists. Cattle, goats, sheep, donkeys are reared. Dogs are common and serve as guards in all groups of the population.

Since the only abattoir in South Omo was found in Jinka, additional animal slaughter house survey was conducted at Arba Minch Abattoir to get more data. For human ultrasound survey, Turmi, Achemusa and Borea towns and villages were selected from Hamer district.

Aipa, Kangaten and Kibish sites were selected from Nyangatom district. From Dasench district, Omorate, Delegnemore and Torongole localities were selected.

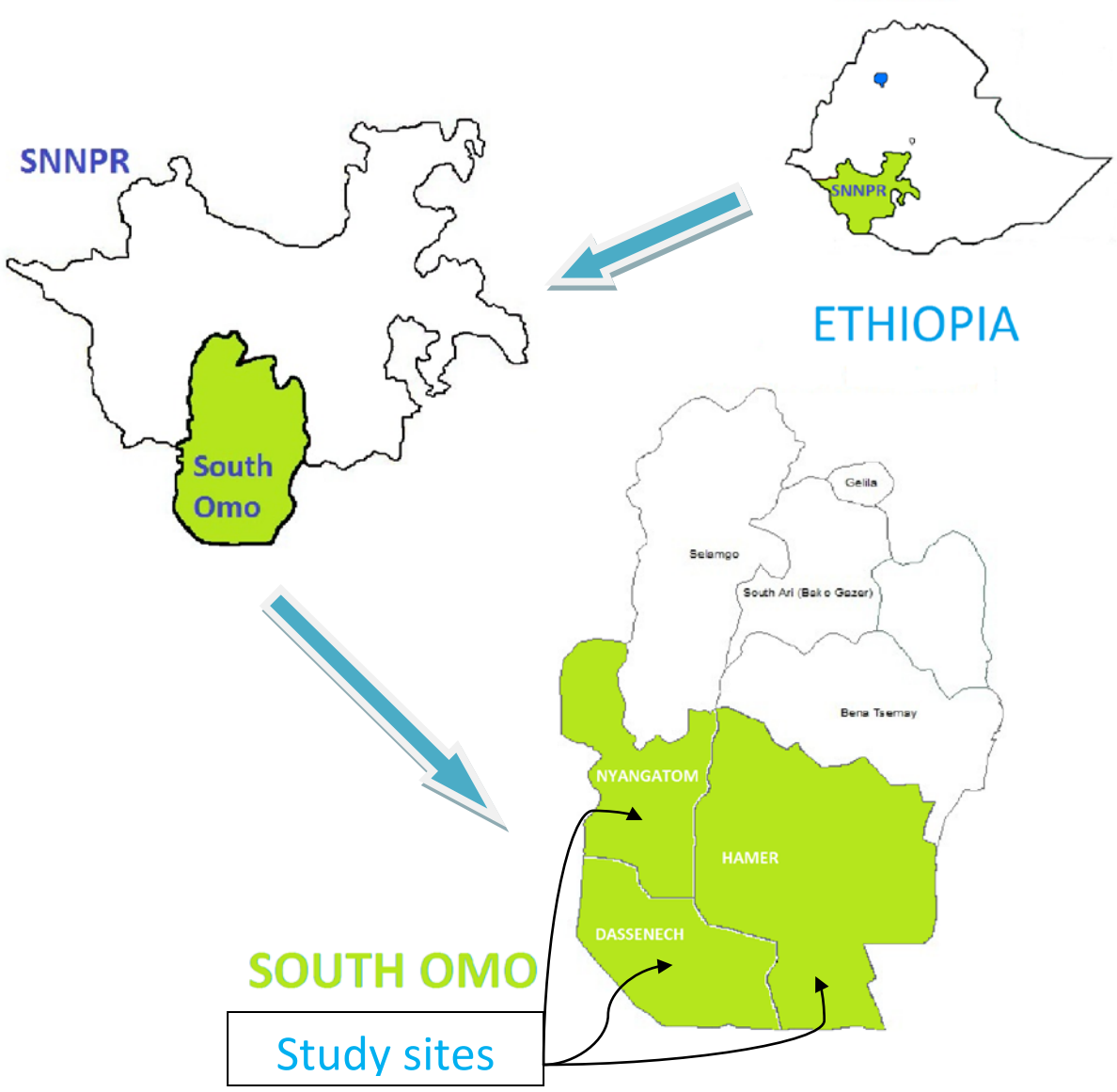


Figure 4. Map of South Omo Zone (Courtesy of South Omo Zone Health Department).

5.2 Sample size determination

A total of 665 individuals of all sex and age groups in the three districts of the South Omo Zone will be included in the study. The sample size is determined by taking the previous

result of seroprevalence of the infection, which is 31.7%, in the Nyangatom and Dassenech District (Fuller and Fuller, 1981).

$N = gZ^2p(1 – p)/d^2$, where N= required sample size, Z= confidence level at 95% (1.96 standard value), p= estimated prevalence in the study area (31.7% 0r 0.317) and d= marginal error at 5% (0.05 standard value), g=design effect. i.e 2.

$N = 2\ (1.96)^2 \times 0.317\ (1 – 0.317)/\ (0.05)^2 = 665.39892608$

$= \underline{665}$

For bovine CE, by using similar calculation for sample size determination, a total of 501 cattle were included for slaughter house inspection at Jinka and Arba Minch abattoirs by taking the previous result of abattoir inspection at Arba Minch Municipal Abattoir by Tilahun and Terefe (2013).

5.3 Study design

The study design was cross sectional prevalence study for the ultrasound examination and followed by cohort for ultrasound positive cases. The prevalence of human infection among the sex and age groups as well as groups based on their period of residence in the study area were assessed. Major exposure risk factor of each study subject was examined through questionnaire and observation of human activities related to potential CE risk. Contribution of the exposure to cystic echinococcosis was analyzed.

5.4 Ultrasound survey

The ultrasound survey was done in different villages and towns of the three districts by using portable ultrasound and videographic printer. Each study participant was scanned after necessary demographic data properly registered. A portable ultrasound (SonoSite Titan Ultrasound System, Germany) with a 3.5-mHz real time sector probe was used throughout the survey. Sonograms of imaged cysts were recorded on a monochrome video graphic

printer (Sony Videoprinter 897MD, Japan) with details of the individual recorded. Primary health care centers and schools were selected in each of the 11 villages of the three districts to conduct the ultrasound scanning activities. Individuals were scanned standing to facilitate rapid screening from the front and back. The liver, pancreas, spleen, and kidneys were carefully examined and any pathologic cystic images were recorded. Cystic echinococcosis was differentiated from other cystic lesions if one or more of the characteristic diagnostic criteria of CE were present, namely, a laminated membrane and/or daughter cysts. All CE cysts were classified according to their size, morphology, and echotomographic appearance. Ultrasound gel was used to create uniform optical medium between the surface of the participants' abdomen and the probe. The images were carefully observed and categorize as CE1-CE5 based on the WHO CE classification (Brunetti *et al.*, 2010). Each positive case was requested to have a picture and the print copies were given to each positive participant and the study participants were provided treatment and consultation by the respective district health service providers. Patients' ultrasound images were interpreted in accordance with the WHO-IWGE classification (Brunetti *et al.*, 2010). Based on this classification, liver cysts were categorized as cystic echinococcosis in stages 1-5 (CE1-CE5). CE stages 1 and 2 are considered as active disease stages in the WHO-IWGE guidelines (Brunetti *et al.*, 2010).

5.5 Questionnaire Survey

Questionnaires were carefully designed and administered to the study participants during ultrasound survey to assess the knowledge and attitude towards cystic echinococcosis and to determine the major risk factors associated with its transmission in animals and humans. The questionnaire was orally administered by using local translator. Every 6^{th} person was randomly selected and requested for questionnaire. All questionnaires were identified by date, participant ID and GPS coordinate. It covered questions on; (i) dog ownership and

number of dogs owned (ii) livestock owned per household (iii) offal disposal, (v) public health awareness of the disease, (vi) use of anti-parasitic treatment of dogs in the household (vii) co-mingling between dogs and other livestock. Information on stray dogs having access to domestic animal offal was also collected.

5.6 Slaughter house survey

The survey was conducted on cattle between January and February 2013 at the Jinka abattoir and on small ruminants (goats and sheep) between November 2014 and March 2015 at Debre Zeit HELMEX plc, and between April 2016 and May 2016 at Arba Minch Abatoir. Cattle that were slaughtered at Jinka abattoir originated from four districts (Hamer, Bena-Tsmai, Ari and Salamago) of South Omo districts. Before the commencement of the study, appropriate orientation was given to meat inspectors at the slaughterhouses about the objective of the study and on sampling of hydatid cysts for this particular study.

Small ruminants were purchased by HELMEX plc from different districts of Borena, Babile, South Omo and Arsi Zones. Cattle that were slaughtered at Arba Minch Abattoir originated from different localities of Gamo Gofa and South Omo Zones. All the cattle and small ruminants slaughtered during the time of survey were inspected both at ante-mortem and post mortem inspection and samples with lesions were collected. Each animal was traced back to its area of origin. During ante-mortem examination, all cattle were inspected for general health condition and place of origin was determined at the same time. At this stage, age of the particular animal was estimated by asking the owner or purchaser. During post-mortem examination, visceral organs including lungs, liver, heart, spleen and kidneys were inspected visually, by palpation and systematic incision on each carcass according to procedures recommended by FAO (1994). All organs were examined but special attention was paid to

the liver, lungs and kidneys. Some hydatid cysts that were found during inspection were removed whole and collected in polythene bags. Separate polythene bag were used for each hydatid cyst, animal and organ.

These bags were labeled with the animal identification, stored in ice boxes, transported to the nearest laboratory and examined.

5.6.1 Hydatid Cyst Charactrization

Hydatid cysts were collected from different organs and taken to the Veterinary Laboratory in Jinka Town and Parasitology and Pathology Laboratory, College of Veterinary and Agricultural Sciences, Addis Ababa University. Microbiology and Parasitology Laboratory of College of Natural Sciences, Arba Minch University, was also used to process samples collected from Arba Minch abattoir. These cysts were grossly examined for degeneration and calcification. In the laboratory, each cyst wall was carefully incised with a scalpel blade and the contents poured into a sterile glass petri dish. Based on the presence or absence of brood capsules containing protoscoleces, the cysts were classified as fertile or infertile. The infertile cysts were further classified as sterile if they were filled with fluid without protoscoleces or calcified if they were calcified based on the procedure described by Macpherson (1985).

Viability of the protoscoleces was examined under the microscope. A drop of the sediment from the hydatid cysts was placed on a glass slide and covered by a 22 X 22 mm cover slip. Amoeboid-like peristaltic movements (flame cell activity) of viable cysts were checked under the microscope at X40 (Smyth and Barrett, 1980). Doubtful specimens were further examined after being stained with 0.1% aqueous eosin solution mixed with equal volume of hydatid fluid containing protoscoleces and allowed to stand for fifteen minutes on a microscopic glass slide. The protoscoleces were classified as dead when they took up the stain and viable when

they did not (Macpherson, 1985). Thus viability of protoscoleces was determined by exclusion of eosin dye and flame cell motility (Daryani *et al.,* 2007; Dalimi *et al.*, 2002).

5.7 Cyst Collection from Surgically Operated Patients

Hydatid cysts were taken from patients surgically operated at Turmi Health Center, Hamer District, and Jinka Genaral Hospital. It was done after physically examined by the physician and scanned with ultrasound. All CE confirmed patients were under albendazole treatment for at least four days before surgery. Surgery was conducted based on laparatomy and cystoctomy. The cysts were counted and the sizes of some representative cysts were measured. Two cyst samples were taken from each of them was transferred into a 15ml vial filled with 70% EtOH and stored at room temperature for further analyses. Each patient was informed about the objectives of the study to obtain his consent for further participattion. After surgery, each patient was received Albendazole treatment and he was re-examined after six months to make sure that there is uneventful recovery without signs of relapse.

5.8 Molecular Diagnosis

DNA extraction

Single protoscolix, was visualised from a petri-dish using the lower power magnification (X10), and then picked with a 1 µl pipette and transferred to 10 µl 0.02N NaOH. DNA was extracted from protoscolices or tissue pieces by lysing in 0.02N NaOH at 95°C for ten minutes as previously described by Nakao *et al*. (2003). The lysate was used directly as template in a nested PCR targeting the NADH dehydrogenase subunit 1 (nad-1) gene as described by Hüttner *et al*. (2009). When the above process failed to yield adequate DNA, genomic DNA was extracted as described in Dinkel and colleagues (2004). About 0.5g cyst wall (germinal layer) was cut into small pieces and digested in the presence of 2 mg/ml

proteinase K in 500 µl 10 mM Tris–HCl (pH 7.5), 10 mM EDTA, 50 mM NaCl, 2 % sodium dodecyl sulphate and 20 mM dithiothreitol. DNA was extracted using phenol–chloroform–isoamyl alcohol (25:24:1) with subsequent ethanol precipitation. After drying, the DNA was dissolved in 100-µl nuclease-free water.

5.8.1 Nested-PCR

A nested PCR assay was run to amplify the NADH dehydrogenase subunit 1 (nad-1) gene (1075 BP) using the following primer pair: TGT TTT TGA GAT CAG TTC GGT GTG/rev.CAT AAT CAA ACG GAG TAC GAT TAG, for the primary reaction and internal primer pair: CAG TTC GGT GTG CTT TTG GGT CTG/rev.GAG TAC GAT TAG TCT CAC ACA GCA, for the nested reaction (Huttner *et al.,* 2008). In both reactions, a 50-µl reaction mixture was made up of DNase/RNase-free water, 25µl reaction mixture containing PCR Buffer (10 mM Tris-HCl, pH 8.3; 50 mM KCl), 2mM MgCl2, 200 µM of dNTP, 0.25 µM of each primer, 0.625 U Taq polymerase (Thermo Scientific, Germany), and 1 µl of protoscolex/tissue lysate (or 1 µl of PCR product). The cycling conditions included 35 cycles of denaturation (94ºC for 30 s), annealing (55ºC for 30 s) and extension (72ºCfor one min). These amplification steps were sandwiched by an initial denaturation at 94ºC for 5 min and a final elongation at 72ºC for five min. The PCR products were visualised on 1.5% agarose gel and stained with ethidium bromide (Biotium, Inc.).

Restriction fragment length polymorphism (RFLP) of nad-1

The nad-1 amplicons were digested as described previously (Huttner *et al.,* 2009) with the restriction enzyme Hph I (Fermentas GmbH, Germany). A total reaction mixture of 30.5µl constituted of 10µl PCR reaction mixture, 2µl buffer B (supplied with enzyme), 18µl DNase/RNase-free water and 0.5µl HphI. Reaction mixture was incubated at 37 °C for 3h,

followed by deactivation of the enzyme at 65 °C for 20 min. Banding patterns were detected on 3% (w/v) agarose stained with Ethidium Bromide solution (Biotium, Inc.). Genotypes of samples were determined by comparing their banding patterns to defined patterns of *E. granulosus G1*, *E. ortleppi* and *E. canadensis G6*.

The nad-1 PCR product of samples with different or unclear banding patterns were analyzed by partial DNA sequencing (Seqlab GmbH, Göttingen). DNA sequences were compared with existing sequences in the GenBank databases using the BLAST (www.blast.ncbi.nlm.nih.gov/Blast.cgi).

5.8.2 Gene Sequencing

DNA was obtained from two different daughter cysts with different or unclear banding patterns during nested PCR and RFLP analysis. For this, approximately 0.5 cm2 of the germinal layer from each cyst was cut into small pieces and transferred separately into 1.5 ml tubes containing 500 µl of 0.02 M NaOH solution (Nakao *et al.,* 2003). The tubes were placed into boiling water for 20 min, centrifuged and the supernatants transferred to new tubes. The cyst lysates were used directly as templates in the PCR. Primers for several fragments of approximately 1200 bp in length, covering the complete mt genome, were designed based on the published mt genome of E. granulosus (GenBank accession number AF297617) (Le *et al.,* 2002). Amplicons were sent to GATC Biotech AG (Konstanz, Germany) for sequencing. The partial sequences were joined to a total mt genome sequence. In addition to the mt genome, the two nuclear genes, elongation factor 1 alpha (ef1a) and ezrin-radixin-moesin (ERM)-like protein (elp), were partially amplified as described previously and sequenced (Huttner *et al.,* 2008).

Molecular Phylogenetic Analyses

Complete mt genomes were available from 12 *Echinococcus* spp. genotypes and *Taenia solium* as the out-group (Table 1). The sequences of the 12 mt protein coding genes were extracted (total 10,912 bp) and used for further analysis. Multiple alignments were created with ClustalX 2.0.12 (Larkin *et al.*, 2007) and saved in PHYLIP format. The online program PhyML 3.0 calculated the Maximum Likelihood (ML) phylogeny with the best fitting substitution model GTR + G6 + I (Guindon *et al.*, 2010). The robustness was tested with 1000 replicates for bootstrapping. The phylogenetic trees were drawn with FigTree v1.4.2 and rooted with *T. solium* as the out-group. In order to show the relationship of the isolates with the defined genotypes G1, G2 and G3 of *E. granulosus* (Bowles *et al.*, 1992; Casulli *et al.*, 2012), a second phylogenetic analysis was undertaken using a 351 bp long fragment of the cox1 of all *Echinococcus* spp. and *T. solium*. The ML phylogeny was calculated by PhyML 3.0 using the HKY85 + G6 + I substitution model.

Table 1. Mitochondrial genomes and nucleotide sequences used in this study.

Analysis and species used	**Accession Nos.**	**References**
ML analysis; complete mt genome		
G_{Omo}	**KX037021**	**This study**
Echinococcus granulosus s.s. (G_{1-3})	AB786664	Nakao *et al.* (2013c)
Echinococcus felidis	NC021144	Nakao *et al.* (2013a)
Echinococcus equinus	NC020374	Nakao *et al.* (2013c)
Echinococcus ortleppi	NC011122	Nakao *et al.* (2007)
Echinococcus canadensis G_6	NC011121	Nakao *et al.* (2007)
Echinococcus canadensis G_7	AB235847	Nakao *et al.* (2007)
Echinococcus canadensis G_8	AB235848	Nakao *et al.* (2007)
Echinococcus Canadensis G_{10}	AB745463	Nakao *et al.* (2013c)
Echinococcus vogeli	AB208546	Nakao *et al.* (2007)
Echinococcus oligarthra	NC009461	Nakao *et al.* (2007)
Taenia solium	NC004022	Nakao *et al.* (2003)

5.9 Data Analysis

Data obtained from hydatid cyst sample collection, ultrasound survey, questionnaire and molecular diagnosis were analyzed by using SPSS version 20.0 statistical software package. Differences in prevalence of the infection in different districts were checked by chi-square test. A significance level of 0.05 was used for all tests.

5.10 Ethical Considerations

The ethical aspect of this study was reviewed and clearance was obtained from the Ethical Committee of College of Natural Sciences, Addis Ababa University, and from National Research and Ethics Review Committee. The objective of the study was explained to zonal and each district health officials and the study participants, at the time of sample collection and survey. Letter of support from the health department of South Omo Zone was obtained. Every sample was taken when the participant agreed to give the sample following informed consent. The ultrasound examination was conducted by the expert in the field of ultrasonography. Participants who were positive for cystic echinococcosis, were treated free of charge. Any information obtained from the participants remained confidential. Competent translator (s) were employed to overcome language barrier.

6 RESULTS

6.1 Ultrasound survey

A total of 2,838 people, aged 1 to 75 years, from 9 study sites (Achemusa, Borea and Turmi from Hamer District; Aipa, Kangaten and Kibish from Nyangatom District; Delegnemore, Torongole and Omorate from Dasenech District) of the three districts were willing and registered to participate in the ultrasound scanning survey from May 2 to 28, 2014. The overall number of female participants was slightly higher (51.6%). The major ethnic groups that participated in this survey were Hamer, Nyangatom, Murule and Dasenech. There were also few participants from other ethnic groups. Fewer participants were registered from Hamer District, especially from Borea study site (Table 2).

Table 2. Distribution of the study participants in different districts and study sites, South Omo, 2014.

DISTRICT	STUDY SITE	SEX		TOTAL
		F	M	
DASENECH	Delgnemor	113	172	285
	Omorate	246	234	480
	Toromgole	103	96	199
	Total	**462**	**502**	**964**
HAMMER	Achimusa	175	150	325
	Boria	60	60	120
	Turmi	92	97	189
	Total	**327**	**307**	**634**
NYANGATOM	Aipa	152	124	276
	Kangaten	204	225	429
	Kibish	320	215	535
	Total	**676**	**564**	**1240**
TOTAL		**1465**	**1373**	**2838**

The ultrasound examination revealed that the overall prevalence of cystic echinococcosis was 2.0% (56/2838). The infection seemed to be more prevalent in females (2.8%) than in males (2.5%); but this difference was not statistically significant. The age of CE patients ranged from 5 to 65 years. Distribution of CE in Hamer district was found to be high when compared to the other two districts (Table 3). Fewer numbers of participants were registered in this district; however, the prevalence was observed to be significantly high when compared with the other two districts. Especially, in Borea site of Hamer district, the prevalence of CE was exceptionally high (7.5%). Other study sites like Delegnemore (3.5%) of Dasenech, Achimusa (3.4%) of Hamer and Kibish (3.2%) of Nyangatom were observed to have relatively higher prevalence of CE. Lowest prevalence was observed at Omorate (1%) study site of Dasenech district (Table 3).

Table 3. Prevalence of CE based on abdominal ultrasound scanning in different districts and study sites of South Omo, 2014.

DISTRICT	STUDY SITE	CE CASES No. (%)	TOTAL EXAMINED
DASENECH	Delgnemor	6 (2.1)	285
	Omorate	3 (0.6)	480
	Toromgole	2 (1.0)	199
	Total	**11 (1.1)**	**964**
HAMMER	Achimusa	8 (2.5)	325
	Borea	9 (7.5)	120
	Turmi	4 (2.1)	189
	Total	**21 (3.3)**	**634**
NYANGATOM	Aipa	5 (1.8)	276
	Kangaten	7 (1.7)	429
	Kibish	12 (2.2)	535
	Total	**24 (1.9)**	**1240**
TOTAL		**56 (2.0)**	**2838**

Age-prevalence increases from 0.3% in 5 years or less patients to 7.5% in 55-65 years patients. Prevalence of CE1 was observed to increase as the age increases (Figure 6). Generally, older people were found to be more infected than the youngsters.

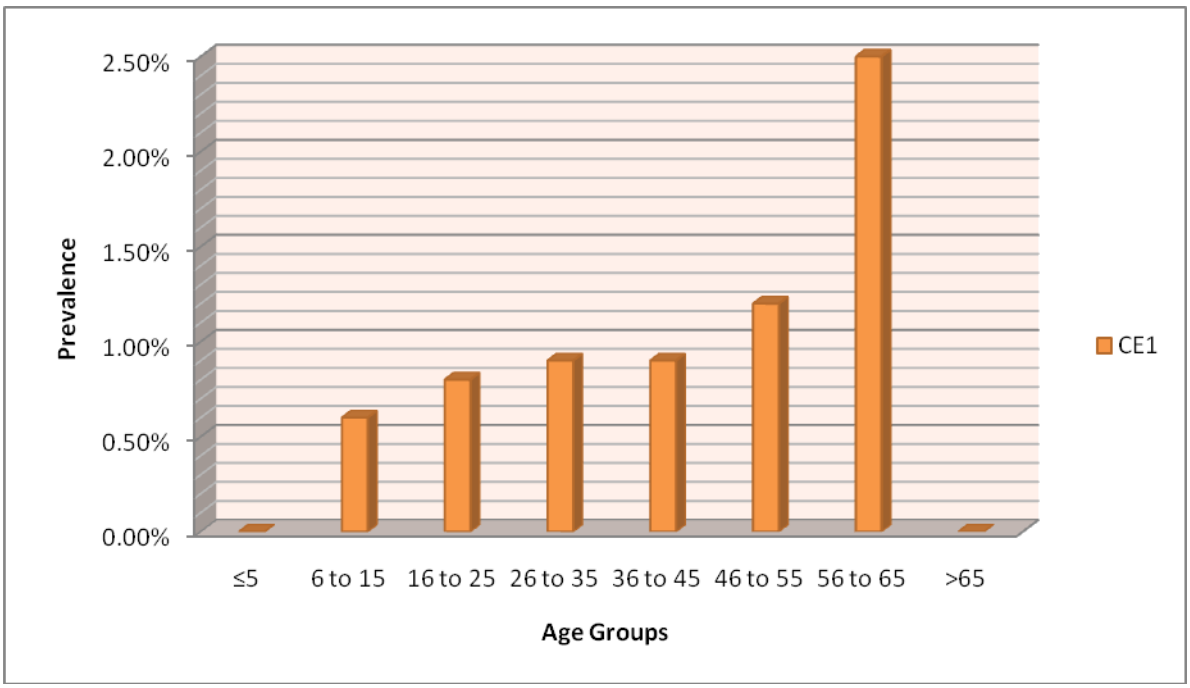


Figure 5. Prevalence of CE1 based on abdominal ultrasound scanning among different age groups, South Omo, 2014.

The cysts were detected in liver, kidney, abdomen, spleen, heart and other anatomical sites. Liver was found the most frequently affected organ in this infection (Figure 9) and the right liver was found to be highly affected. It comprises 53.6% (30/56) of all human CE cases. Abdomen was the next most affected body site, in which 12 (21.4%) CE cysts were detected. CE infection with multiple organ involvement, liver/heart, liver/ovary liver/spleen and abdomen/ovary, was observed in 4 cases. Cyst was not detected the lung in any participant in this survey by using ultrasound examination. No other imaging or serological methods were used to detect pulmonary CE in this study. Calcified cysts (CE4) were observed in unusual organs/tissues like breast and larynx. Size of cysts ranged from 1cm to 13.4cm. Small cysts

(<5cm) are highly frequent. Medium and larger CE cysts were observed in few pateints (Figure 7 & 8).

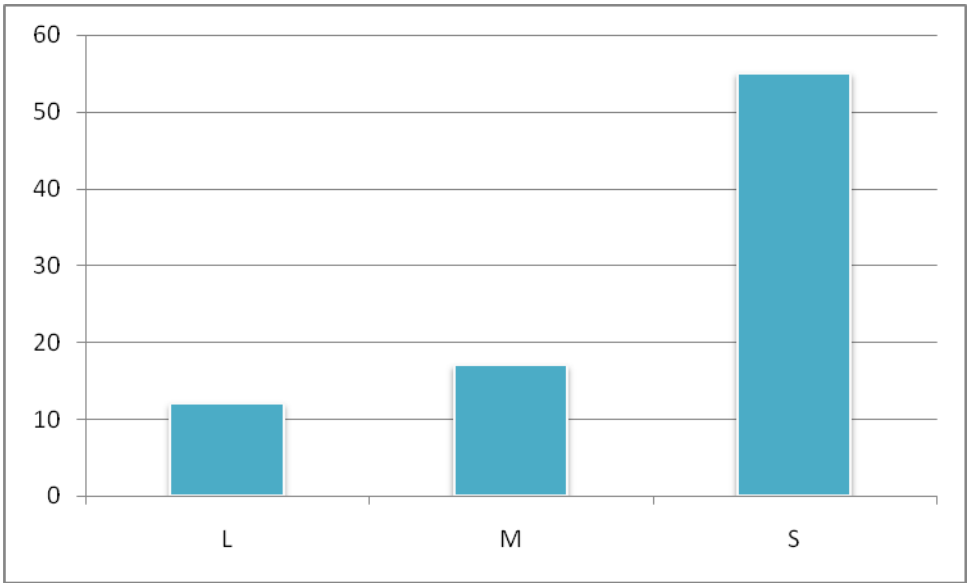


Figure 6. Frequency of CE cyst size based on abdominal ultrasound scanning; L=large (>10cm), M=medium (5-10cm) and S=small (<5cm), South Omo, 2014.

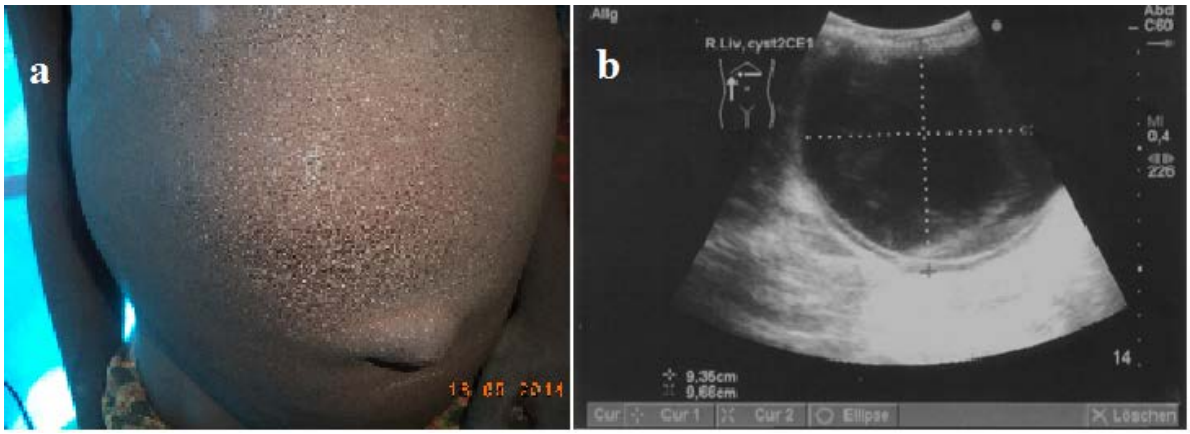


Figure 7. Hepatic CE in a 9 years old boy, abdominal appearance (a) and the ultrasound picture (b), South Omo, 2014.

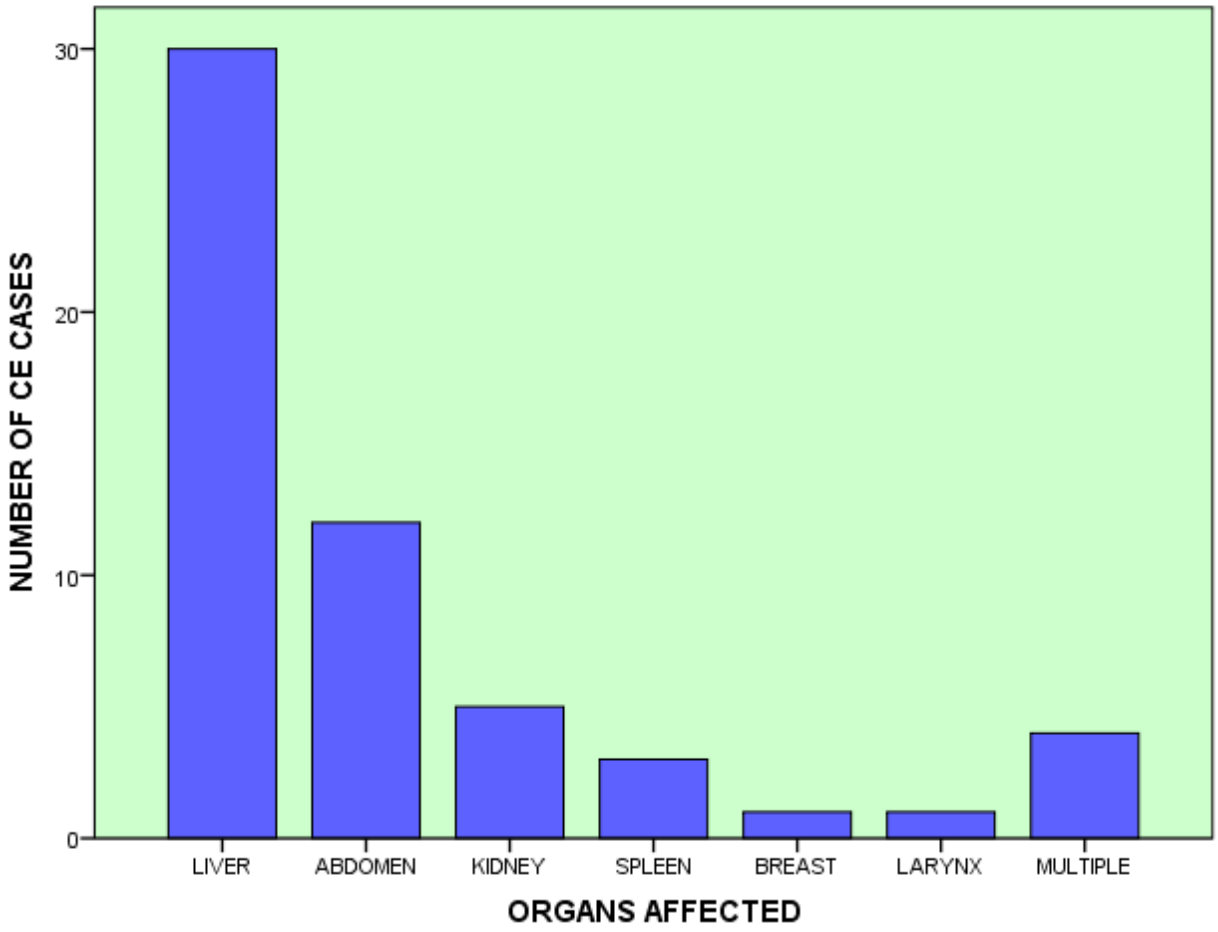
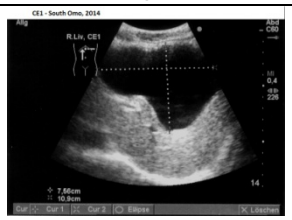
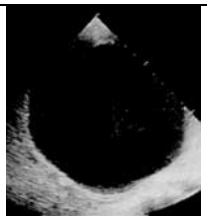
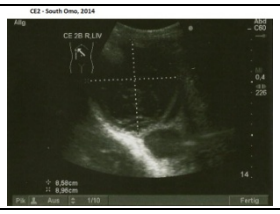
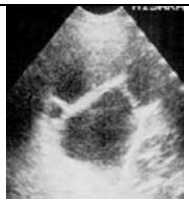
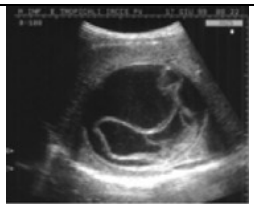
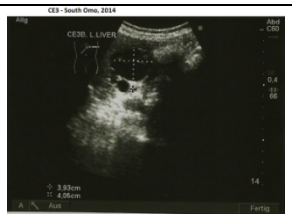
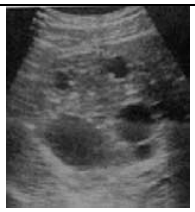
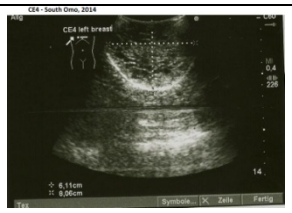
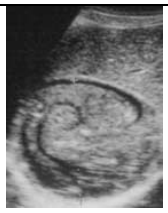
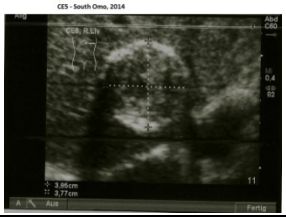
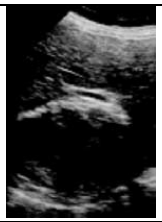


Figure 8. Distribution of organs involved in CE cases, South Omo, 2014.

The number of active cysts (CE1-CE2) and inactive cysts (CE4-CE5) were equal and higher, each of which constituted 44.6% of all the cases. However, from active cysts, the number CE2 stage was much less and found only in 2 patients (Table 4). No CE3A cyst stage was observed in this ultrasound examination. However, CE3B was detected in considerable number of cases. Multiple CE cyst stages (more than one CE stage per case, i.e CE1/CE3) were observed in 7 (12.5%) cases (Table 4). Suspected cysts (CL) were found in high frequency. It is observed in 21 study participants mostly on liver.

Table 4. Frequency of cyst stages among human CE cases based on abdominal ultrasound examination, South Omo, 2014.

CE – Stage	CE US Picture in this study	WHO Reference	Frequency	Multiple CE
CE1			**23**	**3** **CE1/CE1** **CE1/CE3B** **CE1/CE4**
CE2			**2**	**1** **CE2/CE3B**
CE3A			**0**	
CE3B			**9**	**1** **CE3B/CE3B**
CE4			**16**	**1** **CE4/CE4**
CE5			**9**	**1** **CE5/CE5**
Total			**56**	**7**

6.2 Questionnaire Survey Data

Interview questionnaire was randomly introduced on 311 (11% of the total US survey participants) consenting individuals among the ultrasound survey participants to assess their

knowledge, attitude about the disease, live stock and dog ownership that might contribute for the transmission of the disease. 52.7% (164) of the participants were females. About half of the respondent, 47.9% (149) were in the age group 26-45 years old. The overall prevalence of CE in these participants was 5.5% (17). The majority of the study participants were pastoralists and non-literate (Table 5).

Each potential risk was evaluated in terms of the prevalence of CE (Table 5). The risk of having CE was associated with occupation (being pastoralist), literacy (being non-literate), home slaughtering practice, disposal of infected offal, lack of awareness of CE, ownership and feeding infected offal to dogs, sharing of water source with livestock and dogs and poor personal hygiene (fail to wash hands before meal). There was no statistically significant association with the potential risk factors. Relatively strong association was observed in sharing of water source with animals. Based on the response of most participants, livestock and dogs had access to their water sources. This situation seemed significantly associated with the increased risk of CE.

Regarding the awareness of the studyparticipants about the infection, most of the respondents had no idea about hydatid disease, its cause, and the way the disease is transmitted. Only few participants respond that they had heard and read about the disease. But when they were shown the picture, they said that they had seen the disease in human and the cyst in organs of slaughtered animals.

Table 5. Assessment of Potential Risk factors of CE, South Omo, 2014.

VARIABLES	RESPONSE	N (%)	CE (%)	OR	*P*
Sex	Female	165 (53.1)	12 (7.3)	0.452	0.136
	Male	146 (46.9)	5 (3.4)		
Occupation	Pastoralist	251(80.7)	14 (5.6)	1.122	0.860
	Non-Pastoralist	60 (19.3)	3 (5.0)		
Literacy	Literate	90 (28.9)	3 (3.3)	0.510	0.291
	Non-Literate	221 (71.1)	14 (6.3)		
CE Awareness	Yes	27 (8.7)	1 (3.7)	0.644	0.673
	No	284 (91.3)	16 (5.6)		
Home Slaughtering	Yes	295 (94.9)	16 (5.4)	0.860	0.887
	No	16 (5.1)	1(6.2)		
Proper Disposal Of Infected	Yes	59 (19.0)	1(1.7)	0.254	0.157
Offal	No	252 (81.0)	16 (6.3)		
Sharing Water With Animals	Yes	229 (73.6)	15(6.6)	2.804	0.160
	No	82 (26.4)	2 (2.4)		
Dog Ownership	Yes	259 (83.3)	15 (5.8)	1.537	0.573
	No	52 (16.7)	2 (3.8)		
Feeding Dogs Infected Offal	Yes	247 (79.4)	15 (6.1)	2.881	0.143
	No	64 (20.6)	2 (3.1)		
Washing Hands Before Meal	Yes	62 (19.9)	3 (4.8)	0.808	0.854
	No	249 (80.1)	14 (5.6)		

The diseases in human has different name in different district. In Hamer, it is called ‘Gacha’ or ‘Wotre’. According to the respondent, somebody may acquire ‘Gacha’ or ‘Wotre’ if he/she steals some other body’s property. And it has no cure. The only solution is that someone should not steal in the first place. In Nyangatom, it is called ‘loteybwo’ or ‘ngikapespes’. The same names are used in Turkana, Kenya, for this disease. Respondents indicated that ‘Loteybwo’ or ‘ngikapespes’ is believed to be acquired when a Nyangatom kills ‘Champa’ or a person from central Ethiopia. Some others believe that the disease may occur if someone

eats ill or dead animals/goats or by drinking well water. Few respondents stated that it might be acquired from dog but they didn't know how. In Dasenech, it is called 'Loruwa' meaning 'water pregnancy'. The cyst in the organ of slaughtered animals has a local name, 'nakarsha'. Most respondents in Dassenech districts agreed that the disease was acquired through dogs. But they didn't clearly indicate how it is acquired; in other words, they didn't show the connection between 'loruwa' and 'nakarsha'. Some said that the infection was acquired through sharing of water with dogs while others said it is through dog bite. Still some believed that it might be acquired if one of the tribe members killed a person from his tribe. That person who commited the crime should compensate the family of the victim in terms of cattle for him to be cured from the disease. Almost all of the participants in this survey reported that they slaughter cattle or small ruminants at home. Small ruminants (sheep and goats) were frequently slaughtered but oxen were rarely slaughtered, when there was a rituals ceremony. Cows were never slaughtered according to most pastoralist respondents. Cows might be eaten if they die accidentally or due to old age. Almost all of the respondents that practiced slaughtering at home did not properly dispose the infected or condemned offal (Table 5). The water sources of most of the pastoralists were rivers and ponds. These water sources were also utilized by their livestock and dogs. For their consumption, they did not boil or use any material to make the water safe and clean. Every pastoralist and few non-pastoralists owned one or more dogs. Dogs were kept for the purpose of guarding and alerting mostly the pastoralists when someone came to steal their livestock. These dogs were fed condemned offal according to the responses of their owners. If the liver or any internal organ that was not edible due to extensively damage or found to harbor strange bodies filled with fluid or calcified ones, were thrown to dogs.

The prevalence of CE seemed to increase as the number of dogs owned increased; however, this trend was not consistent as the number of dog exceeded three. Moreover, the association was not significant (Table 6).

Table 6. Association of CE prevalence with number of dogs owned, South Omo, 2014.

Number of dogs	Participants % (No.)	CE case % (No.)	X^2-Value	P-Value
0	49 (15.8)	2 (4.1)	4.144	0.657
1	121 (38.9)	4 (3.3)		
2	66 (21.2)	4 (6.1)		
3	59 (19.0)	6 (10.2)		
4	13 (4.2)	1 (7.7)		
5	2 (0.6)	0 (0)		
6	1 (0.3)	0 (0)		

Stray dogs were observed in each village and the hygienic conditions of these villages were very poor. Though only in one village in Dasenech District during the selection of the survey sites, it was observed that some people were eating poorly fried meat in the open, where dogs were around.

6.3 Cysts from Slaughter House Survey

A total of 1338 (1191 from Arba Minch and 147 from Jinka Abattoirs) slaughtered cattle and 4312 (3254 goat and 1058 sheep) slaughtered small ruminants from HELMEX plc were inspected for the presence of hydatid cyst. The internal organs like the liver, lung, heart, kidney, spleen, lower abdomen of these animals were carefully inspected. A total of 75 cattle and 23 small ruminants originated from Babile and Borena, 20 goats and 3 sheep were found infected with cystic echinococcosis. 44 (11 from Jinka abattoir) cattle were infected in their liver; 46 (5 from Jinka abattoir) cattle were infected in their lung and 21 (2 from Jinka abattoir) cattle were infected in both their liver and lung. 6 cattle, all from Arba Minch Abattoir, were infected and cysts were found in other organs like heart, kidney and spleen.

Lung CE was more common in cattle slaughtered at Arba Minch abattoir; however, liver CE was the major infection in cattle slaughtered at Jinka abattoir. CE in other organs like heart, kidney and spleen and multiple organs infection were predominantly observed in those cattle slaughtered at Arba Minch Abattoir. Multiple infections, infection of more than two organs, were observed in three cattle (Table 7).

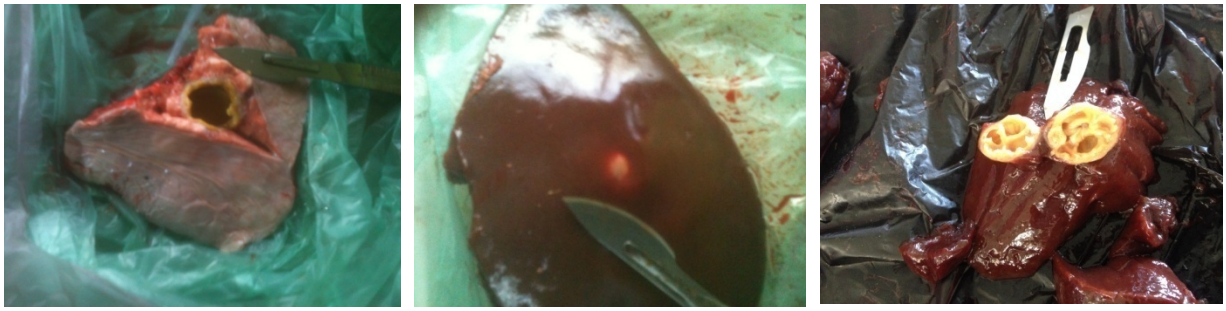
Table 7. Prevalence and affected organs of CE among cattle in Jinka and Arba Minch abattoirs, 2013 and 2016.

Abattoir	**Inspected**	**Infected No. (%)**	**Liver**	**Lung**	**kidney**	**Heart**	**Spleen**	**others**
Jinka	147	14 (9.5)	11	5	0			
Arba Minch	1191	61 (5.1)	33	46	3	1	1	1(tongue)
Total	1338	75 (5.6)	44	51	6			

About half of the cysts were transitional and/or calcified. Active cysts were observed in the majority of the 62 (9 from Jinka) cattle at both abattoir houses (Table 8). The majority of the cysts were mostly active (Figure 4A) and few were calcified ones (Figure 4C).

Table 8. Type of hydatid cyts in different organs of cattle slaughtered at Jinka and Arba Minch Abbattors, 2013 and 2016.

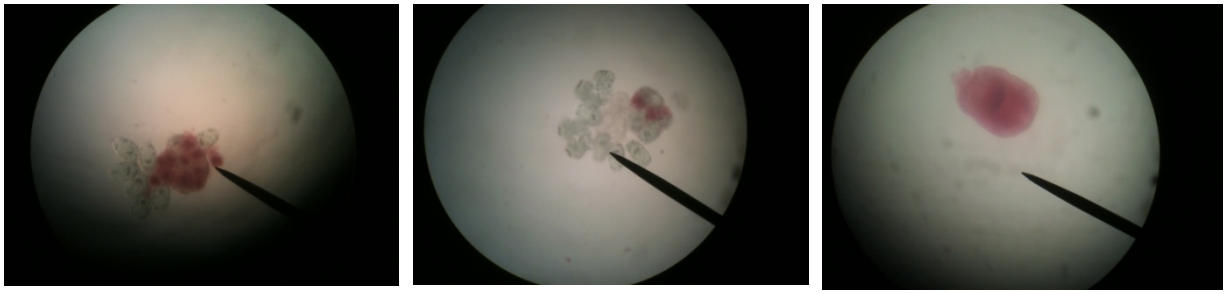
Abattoir	**Fertile**		**Sterile cyst**	**Calcified cyst**
	Motile	**Non-motile**		
Jinka	2	7	0	5
Arba Minch	5	48	18	17
Total	7	55	18	22



A B C

Figure 9. Hydatid cysts from different organs; lung (A) and liver (B and C), of cattle slaughtered at Arba Minch Abattoir, 2016.

Few motile cysts were fertile, in which live protoscolices were observed under the microscope (Figure 11A and 11B). Eosin Red staining showed that these protoscoleces resisted to take the dye. Turbid movement was also observed in these cysts (Figure 11B). Dead protoscolices, which retained Eosin Red, were also observed (Figure 11C).



A **B** **C**

Figure 10. Protoscoleces, non-viable (A and C) and fertile (B) under light microscope, 4x10 (A & B) and 10x10 (C) from infected liver at Jinka Abattoir House, South Omo, 2013.

4312 small ruminants were inspected at HELMEX Export Abattoir plc, meat processing center for export in Bishoftu town, Oromia Region. From these, 751 were sheep and all of them were purchased from Borena. All of the small ruminants were male. 1512, 1781 and 968 goats were purchased from Babile, Borena and South Omo, respectively. 20 goats (7 from Babile) and 3 sheep (all from Borena) were positive for CE. None of the goats from South Omo were positive for CE.

6.4 Cysts from Surgically Operated Patient

One cyst isolate was obtained from a 55 year old male patient seeking treatment before the surgical campaign, at Turmi Health Center, Hamar District. Physical examination revealed a 20x20cm non-tender mass that was palpable at the medial of the distal part of the right thigh. Ultrasonography showed a thick-walled structure with diagnostic features of a CE2 (multivesicular) *Echinococcus* cyst (Figure 3). Thorough ultrasound examination of the abdomen was also performed and no other cystic structures could be visualized. After surgical removal, the cyst was found to contain more than 1000 small daughter cysts and vesicles of different sizes.

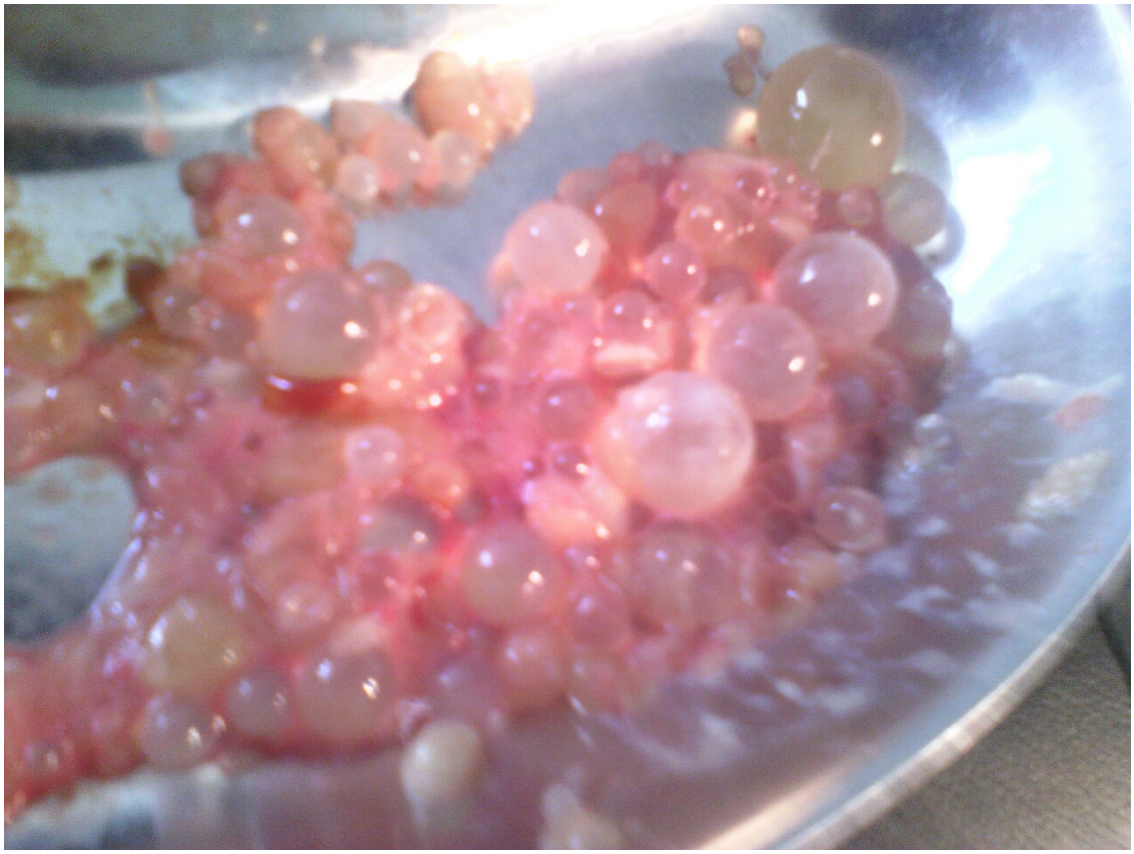


Figure 11. Hydatid cysts surgically removed from treated human patient (right thigh) at Turmi Health Center, South Omo, 2015. (Courtesy of Dr. Banchiwosen Mechal)

The other cyst isolates were extracted from patients who were consenting to participate in the study and surgically operated Jinka General Hospital, South Omo Zone. During the US survey, 30 positive cases were expected to be surgically treated. Two patients died before the treatment campaign. At the time of treatment campaign 16 patients were available at Jinka General Hospital while other participants could not come to the hospital due to several reasons. 6 of the patients were eligible for surgical treatmet, while the other 10 were not due to the stage of the cysts based on physical and ultrasound re-examination at the hospital. 12 participants were lost follow up due to mobilization and lack of concent.

Large numbers of cysts were removed from the right liver of a 39 years male participant from Nyangatom District. The previously diagnosed single big CE1 cyst clearly transformed to CE2 stage with in 3 and half years (Figure 13). Many daughter cysts were recovered from an enclosed big cyst.

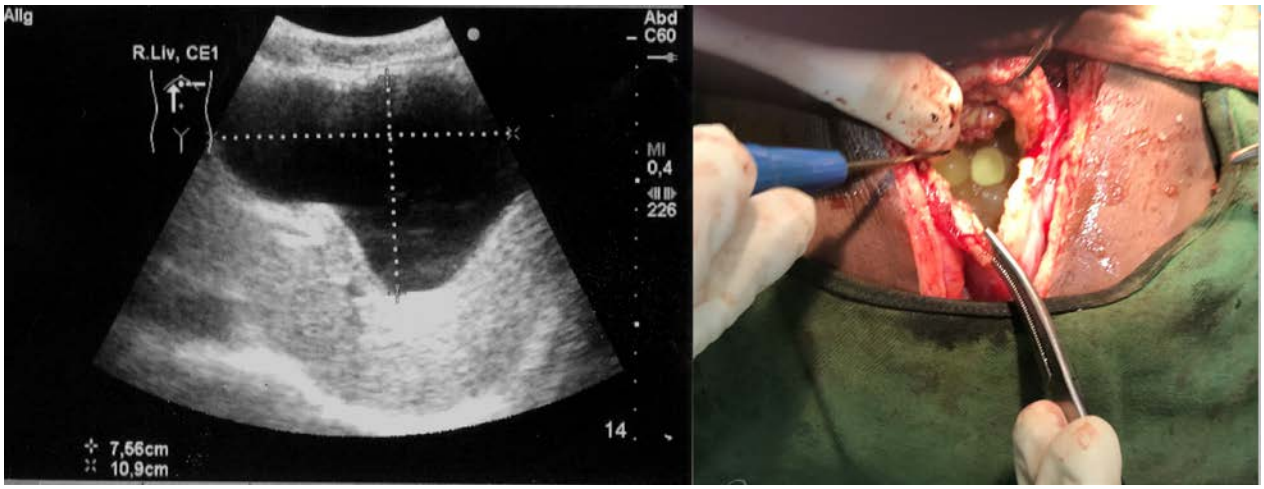


Figure 12. Ultrasound picture (CE1) of a cyst and daughter cysts (CE2) after surgery of a 39 years old participant from Nyangatom, South Omo, 2014 and 2018. (Courtesy of Dr. Eberhard Zeyhle, Dr. Bjarte T. Andersen and Dr. Yared Agidew)

One case from Hamer was diagnosed CE during the ultrasound survey and re-examination at Jinka Hospital. At surgery, a big ovarian cyst was removed and it was a mis-diagnosis (Figure 14).

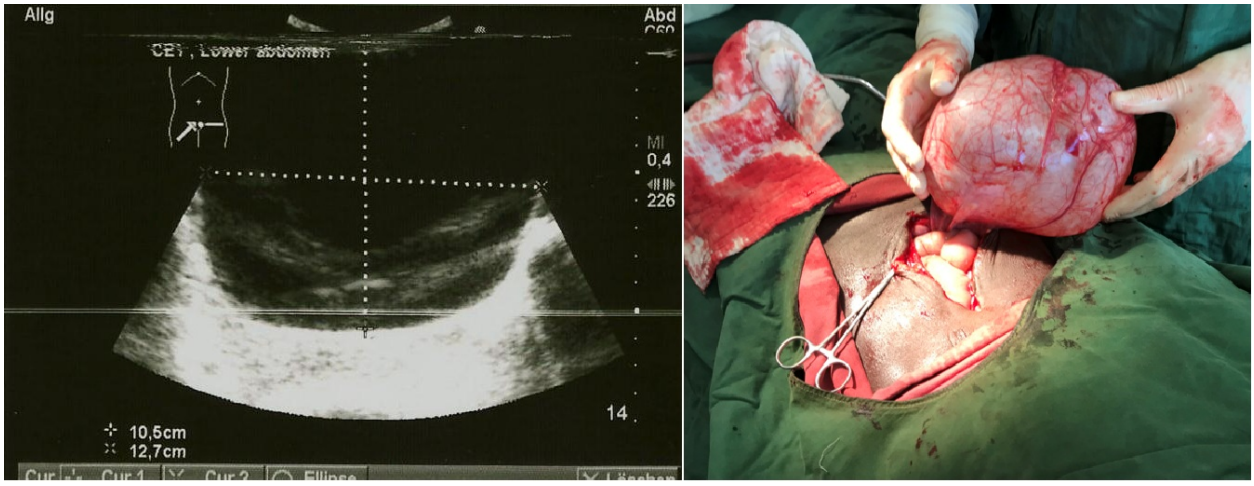


Figure 13. Ultrasound picture of misdiagnosed CE and ovaian cyst after surgery of a 43 years old woman participant from Hamer, South Omo, 2014 and 2018. (Courtesy of Dr. Eberhard Zeyhle, Dr. Bjarte T. Andersen and Dr. Yared Agidew)

6.5 Molecular Characterization

6.5.1 Results from PCR/RFLP

A total of 40 cyst samples underwent molecular characterization. 23 of the cyst samples were taken from small ruminants; 16 were from cattle and one from human. Nested-PCR was conducted targeting nad 1 gene after DNA extraction. After the gel electrophoresis, single band was observed in 15 cyst samples from cattle, 5 from small ruminants and one from human, were confirmed positive for taeniid species (Figure 13A, B, C & E). Those negative cyst samples were re-examined using another target gene (cox1). Except one sample from a small ruminant, all of them were negative after re-examination.

Those nested-PCR positive samples were further analysed using restricted fragment length polymorphism (RFLP). Four cyst samples from small ruminants and 13 from cattle showed

three bands after gel electrophoresis, which confirmed the G1 strains (Figure 10 D and F). One strain from small ruminants and two from cattle were observed to have two bands, which confirmed *Taenia hydatigena*. Vague banding patterns were observed after RFLP analysis of the cyst from the human sample.

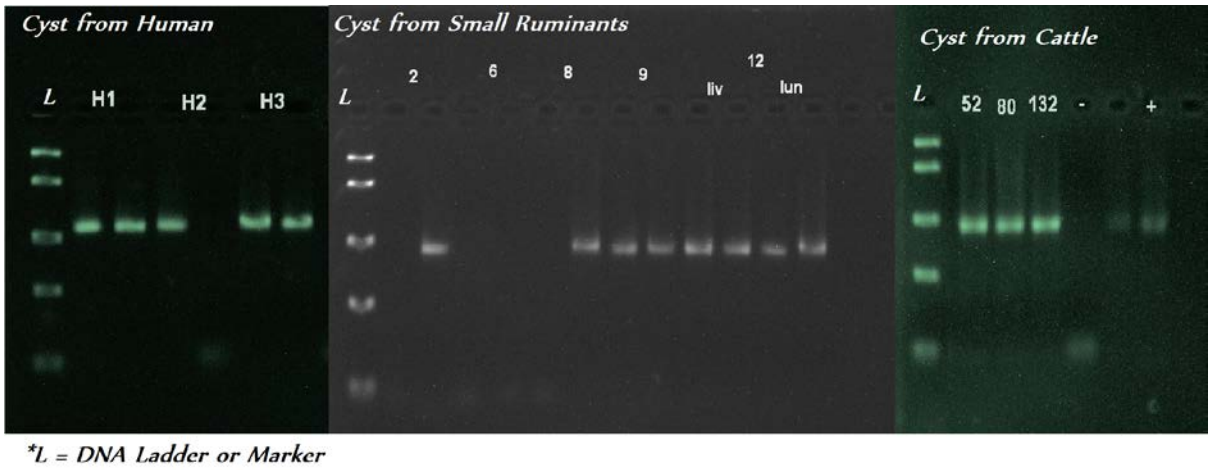


Figure 14. Nested-PCR analysis of protoscoleces and/or germinal layer of representative CE cysts (from human, small ruminants and cattle) targeting nad 1 gene, South Omo, 2015.

Figure 15. RFLP analysis of protoscoleces and/or germinal layer of representative CE cysts (from human, small ruminants and cattle) targeting nad 1 gene, South Omo, 2015.

6.5.2 Sequence Analysis

The taxonomic status of the CE cyst sample from the human was unclear after the RFLP analysis and appeared vague after partial DNA sequencing. A complete mitochondrial gene sequence was conducted and the result demonstrated that the cyst was a unique strain. The phylogenetic tree of the 12 protein coding mitochondrial gene sequences placed this isolate within the *E. felidis/E. granulosus* s.s. clade as a sister taxon to the latter (Figure 14). Until further information on this taxon becomes available, this isolate was categorized within *E. granulosus* s.s. as genotype G_{Omo} (after its origin from South Omo Zone of Ethiopia). Regarding only the *E. felidis/E. granulosus* s.s. clade, it fitted with the 12 gene tree that G_{Omo} is in sister position to *E. granulosus* s.s., but clearly distant from the G1–G3 cluster.

Hence, the sequence data was deposited on the Gen-Bank/DDBJ/EMBL database under accession numbers KU601616, KX03720 and KX03721 (ANNEX 9).

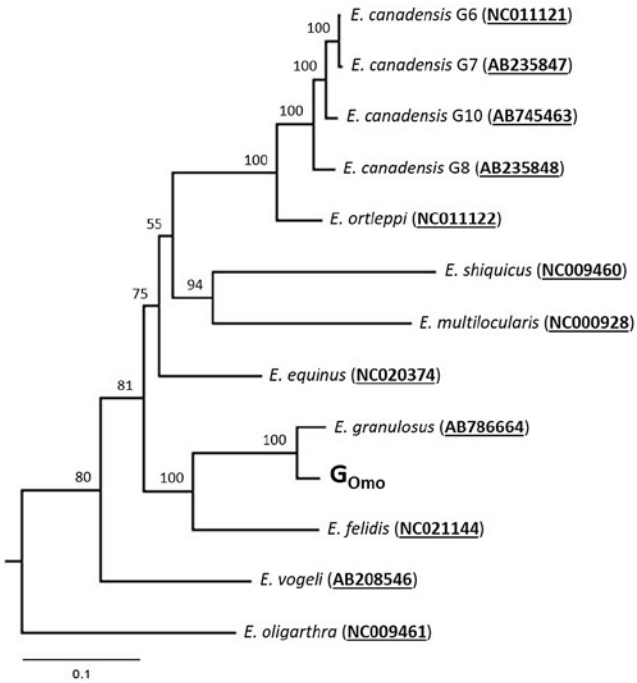


Figure 16. Phylogenetic tree for *Echinococcus* spp. based on ML analysis of the sequences from the 12 mitochondrial protein coding genes (10,912 bp). The outgroup (*T. solium*) was omitted from the tree. The bootstrap proportions (%) of ML are labelled on the nodes.

7 DISCUSSIONS

Southeastern South Sudan, southwestern Ethiopia and northwestern Kenya are adjacent regions of East Africa, which are believed to be endemic foci for CE (Romig *et al.,* 2011). The present study generally confirmed this notion. Many areas with high prevalence of human CE were observed in the ultrasound survey. Borea (7.5%) and Achemusa (2.5%) from Hamer, Delegnmore (2.1%) from Dassenech and Kibish (2.2%) from Nyangatom districts were areas having relatively higher CE prevalence. Some of these areas have prevalence rates much higher than the prevalence in previously known hyper-endemic areas like Turkana. The overall prevalence of CE in the three districts showed clear difference even from the prevalence in the previous similar survey in these districts. Ultrasound surveys' results in Hamer in 1989 and 1993 by Macphersen *et al.* and Klungsoyr *et al.,* respectively, showed that the prevalence was 0.7% in both surveys, which is much lower from that of the present finding (3.3%) (Macpherson *et al.,* 1989; Klungsoyr *et al.,* 1993). Moreover, there was zero prevalence in Dassanetch according to the ultrasound survey done by Macpherson and colleagues (1989), which is still much lower than the present finding (1.1%). But the prevalence in Nyangatom was 2.9% according to Macpherson and colleague (1989), which is higher than that of the present study (1.9%). The possible explanation for such differences may be the patchy nature of CE distribution and/or the difference in sample size and the number of villages/sites involved. In this study, at least three major villages were included from each district.

Even though it is not statistically significant, the prevalence of CE in female is slightly higher than that in male and it seemed to increase with age in this survey. This finding is in agreement with several previous surveys in the East African regions (Macpherson *et al.,* 1989; Carmona *et al.,* 1998). It is generally believed that females in these areas are exposed

to CE due to their activities and frequent contact with dogs (Macpherson *et al.*, 1989). An increased prevalence of cystic echinococcosis with age may indicate that infection pressure is low such that protective immunity may not develop in the community (Carmona *et al.*, 1998).

Regarding the organ involvement of human CE, the liver was the most frequently affected organ in this study. This finding is in line with the general truth that CE affects the liver in majority of the cases. The right liver is primarily affected because this part of the liver is the first site that traps the hatched oncosphere after it leaves the small intestine and enters the hepatic portal vein. There was no single case that involves the lungs in this survey. This contradicts with the general belief that lung the most affected organ next to liver. This discrepancy may be due to the ultrasound method used for the diagnosis. Ultrasound is not effective method of diagnosis for pulmonary CE (Wahlers *et al.*, 2012). Since chest X-ray was not used in this field survey, it is inconvenience to conclude the absence of pulmonary CE in this study.

High frequency of CE1 stages in the present study may show active transmission of cystic echinococcosis in the study area. Availability of relatively younger cysts in such higher frequency is suggestive of recent infections. Moreover, equivalent abundance of suspected lesion (CL) largely on the liver indicates that the potential occurrence of the infection in the area. Since the present study area is CE endemic, some of these suspected lesions might be changed into CE.

All of the potential risk factors assessed seem to have no association with CE cases in this study. For instance, those practices like washing hands, proper disposal of infected offal and feeding dogs condemned offal were shown to have no significant association with CE infection. This contradicts with the general fact that these are highly risky practices in many

CE endemic areas (Macpherson, 2001). The discrepancy may be the translator and/or respondent biases. The translators used during the survey were health workers. The participants might not respond properly because they fear the blame that they failed to practice what the health workers taught them. Sometimes, these translators might provide the questionnaire in a way that it might lead the participant not to respond the proper answer. The other plausible explanation for insignificant association of failure of washing hand and CE infection may be the hygienic status of the water used for washing. In the study area, humans share the same water sources with animals. Hence, washing with such water may not helpful in avoiding the infection.

However, those potential risk factors related to awareness and attitude, ownership of livestock and dogs, practices like home slaughtering and disposal of condemned offal are common in the area. The belief that this infection affects individuals who steal others property and has no cure in Hamer, is potentially highly risky attitude since the patient may not try to get treatment and instead hide him/herself to avoid stigma. Potentially risky factors such as sharing of water sources were expected to have significant association with CE infection in this study; however, they didn't show any relation with the infection. This finding contradicts the result of the previous study in Uruguay (Carmona *et al.,* 1998). Carmona and colleagues confirmed that use of rural water has significant association with CE infection. Rural water may be shared by various animals, especially dogs, and contaminated by infective *Echinococcus* eggs. Existence of stray dogs in all study sites should strengthen this assumption. The possible reason for this difference may be the low number of participants in the questionnaire survey. The number of dogs owned seems to have some association with CE infection even though it was not consistent and significant. Moderate association was observed between inappropriate disposal of condemned offal and CE infection. This result

suggests that dogs may have access to this offal and get infected. These infected dogs then become source of infection to the community.

Distribution of cystic echinococcosis is not uniform in humans and animals. In the present study the prevalence of bovine CE was 9.5% and 5.1% in Jinka and Arba Minch, respectively. The surveys had been done at different periods and the sample size of the survey at Arba Minch was much higher than that of Jinka. The slaughtered cattle at Jinka abattoir mostly came from pastoralists where as the slaughtered cattle at Arba Minch Abattoir came from non pastoralists. The potential risk factors like dog ownership and number of cattle and dogs are different in these two areas. These might contribute for this difference. The prevalence of CE at Arba Minch and Jinka Abattoirs is higher than the prevalence in Uruguay, which is 3.90% (Pavletic *et al.,* 2017), in Sudan, 2.7% (Ibrahim *et al.*, 2011), in Tanzania, 4.2% (Nonga and Karimuribo, 2009) and much lower than that of Kenya, 25.8% (Addy *et al.,* 2012). The prevalence of CE in Mauritius, 5.5% (Salem *et al.,* 2011) and Sudan, 6.1% (Omar *et al.,* 2010) is less than that of Jinka but higher than that of Arba Minch. The findings of the survey at these abattoirs in the present study highly contradict a number of results from different localities in Ethiopia, which is 16.0% and 16.8% at Wolaita Sodo (Kebede *et al.,* 2009; Bekele and Butako, 2011), 15.2% at Birre-Sheleko (Kebede *et al.,* 2011), 20.5% at Gondar and Arba Minch (Abebe *et al.*, 2014; Tilahun and Terefe, 2013), 32% at Shire (Asfaw and Afera, 2014), 46.8% at Adama (Getaw *et al.,* 2010), 31.4% at Jimma (Tolossa *et al.,* 2009) and 60.9% at Hawassa (Regassa *et al.,* 2010). These differences may be due to the nature of the studies, largely being cross sectional. For bovine CE, cross sectional study may not reveal the true prevalence due to the patchy nature of CE distribution. The prevalence may vary through time and locality. Hence longitudinal study is needed to acquire a more reliable prevalence. Such studies in South American countries like Uruguay

generated data ranging from 3.9% to 7.05%, Peru, 3.61% to 6.12% and Chile, 12.6% to 15.2% (Pavletic *et al.,* 2017). Other explanation for the lower prevalence may be due to the age of host animal. In this study most of the cattle (59.9%) were young and adult. Based on the findings of previous studies, the prevalence of CE increases with age of the cattle (Deplaze *et al.,* 2017). Prevalence of active cysts was relatively very high in this study. The findings of this study suggest active transmission since the occurrence of more active cysts implies existence of relatively viable cysts indicating recent infections. Observation of few fertile cysts, even though the prevalence of active cysts was high, is in congruent with the general fact that *E. granulosus* cysts in cattle are non-fertile (Deplaze *et al.,* 2017). Regarding the organ involved in this study, lung was observed to be the most frequently affected organ in the Arba Minch abattoir survey, while liver was the main organ during Jinka slaughter house survey. One of the previous abattoir surveys in Shire showed that lungs (15.9%) of slaughtered cattle were found to be more commonly infected than livers (14.8%) (Asfaw and Afera, 2014), which is in agreement with the present study. However, this result contradicts the general belief that liver is the most affected organ. The most probable explanation may be due to calcification of the cyst at early stage in liver than in the lung, which has softer parenchyma. Those small calcified cysts may be missed in the liver during inspection.

None of the infected small ruminants slaughtered at HELMEX plc originated from South Omo Zone. They are all from Babile and Borena. All of the small ruminants from South Omo were goats. Goats are browsers unlike cattle and sheep, which are grazers. This feeding behavior might contribute for the low or zero prevalence of CE. The other possible explanation is that the animals were inspected during the purchase. According to the marketing manager, the veterinary doctor physically inspects through palpation and visual

examination. Moreover, the animals were given anti-helminthic drug. These all might have contribution for the lower prevalence.

The predominant causative agent of cystic echinococcosis of cattle and small ruminants from the study area and other areas, respectively, was found to be G_1 strain of the species *Echinococcus granulosus* **ss**. This finding is in agreement with the worldwide findings since most of the CE cases are due to the G_1 strain. The confirmation of *Taenia hydatigena* during molecular characterization may be due to possible morphological similarity of the cysts during visual inspection of the carcass. The cysts were found embedded in the liver and lung; they were not transparent but whitish just like hydatid cysts. *Cysticircus tenuicollis* cyst (cyst of *T. hydatigena*) is mostly transparent and it is not embedded in the tissues like liver. It is mostly found in the abdominal areas. So, visual inspection of carcass for hydatid cyst requires experience in the field since it might be confused with *Cysticircus tenuicollis*.

The cyst isolate obtained from the human patient after surgical operation from Hamer was very interesting. The morphological appearance of the daughter cysts looks like a bunch of grapes, which is unusual. Moreover, the isolate was found in an unusual but possible anatomical site, the thigh. This poses question on susceptibility of human for this isolate since most of the cases in this area involve internal organs, like liver. According to its mt gene sequence, the described genotype is phylogenetically positioned in the *E. granulosus* **ss**/*E. felidis* clade, but cannot easily be allocated to either species. This variant, named G_{Omo}, clearly represents a phylogenetic unit distinct from *E. granulosus* **ss** (Romig *et al.,* 2015).

The discovery of G_{Omo} poses interesting questions on the biogeographical origin or the *E. granulosus* **ss**/*E. felidis* clade. With the two basal members being restricted to sub-Saharan Africa (assuming that this is the case for G_{Omo}), there is some likelihood for an ‘‘out-of-

Africa" origin of the ancestral taxon of *E. granulosus* **ss**. This is an apparent paradox, since at present the Middle East and western Asia are recognised as the centre of *E. granulosus* **ss** diversity (Nakao *et al.,* 2010; Casulli *et al.,* 2012; Yanagida *et al.,* 2012). Especially, the Middle East is considered as the origin of human CE as domestication of small ruminants is believed to have started in this area (Zeder, 2008). However, the findings of the present study may challenge this belief since the Omo Valley is also an area where the relatively oldest human fossil is discovered (Leakey, 1969). The same argument may be provided to justify that human CE might have been existed in this area earlier than in the Middle East.

8 CONCLUSIONS

From this study, the following major findings are obtained:

1. Cystic echinococcosis (CE) is common in humans and is a public health problem,
2. CE is common in cattle and could cause significant economic impact in the area,
3. The community has low level of awareness and wrong attitude about CE and practices potentially risky activities that can expose to the infection,
4. The predominant species or genotype that causes CE in cattle is *Echinococcus granulosus* **ss** G1 genotype,
5. One unique strain, designated as G_{Omo}, closely related to *Echinococcus granulosus* ss, is detected from one individual.

9 RECOMMENDATIONS

Based on the major findings of the present study, the following recommendations are forwarded:

1. Health education is very crucial to control CE since the community attitude and awareness about it is wrong and very low. More-over, the “one health” approach of combating the disease by integrating health, agricultural and other sectors will very relevant.
2. Further study on molecular characterization of the parasite in humans, dogs and wild carnivores is important to elucidate the life cycle of the newly detected strain of the parasite.

10 LIMITATIONS OF THE STUDY

The major limitations of this study were:

1. Treatment of patients who participated in the study took a very long time due to financial cconstraint.
2. Unable to conduct and document extensive molecular characterization of cysts from humans as a result of which only a single cyst was isolated was characterized.
3. Unable to get cyst samples from small ruminants from the study area; all the cyst isolates were obtained from small ruminants brought for slaughter from other areas like Borena and Babile.

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ANNEXES

ANNEX 1 – QUESTIONNAIRE

1. General Demographic Data

1.1. Sex:

1.1.1. Male 1.1.2. Female

1.2. Age Groups:

1.2.1. 0-5 y 1.2.2. 6-15y 1.2.3. 16 - 25y 1.2.4. 26-45y 1.2.5. 46-60y 1.2.6. >60y

1.3. Marritual status:

1.3.1. married 1.3.2. single

1.4. Family size :

1.4.1. number of wives 1.4.2. number of children

1.5. Residence (Present):

1.5.1. HM 1.5.2. ER 1.5.3. BN 1.5.4. TS 1.5.5. DS 1.5.6. NY

1.6. Birth place:

1.6.1. HM 1.5.2. ER 1.5.3. BN 1.5.4. TS 1.5.5. DS 1.5.6. NY

1.7. Occupation: (Please Choose from the following)

1.7.1. Pastoralist (Herder) 1.7.3. Pastoralist (Herder with farming)

1.7.2. Pastoralist (Housewife) 1.7.4. Other

1.8. Educational Level: (Please Choose from the following)

1.8.1. College degree or diploma

1.8.2. Secondary School complete (10 or 12 Grade)

1.8.3. Elementary School complete (6 or 8 Grade)

1.8.4. Basic Education (capable of reading and writing)

1.8.5. Non-literate

2. Knowledge or Awareness about (CE)

2.1. Have you ever heard of hydatid disease?

2.1.1. Yes 2.1.2. No

2.2. Do you have a local name for the disease:

2.2.1. yes (name it) 2.2.2. no

2.3. symptoms of the disease in humans:

2.3.1. sicklish looking person 2.3.3. thin/wasted

2.3.2. swollen abdomen 2.3.4. no symptoms

2.4. symptoms in slaughter animals

2.4.1. Thin/wasted 2.4.3. Whitish looking blisters in liver/lungs

2.4.2. no symptoms

2.5. Do you know people with the disease?

2.5.1. Yes 2.5.2. No

2.6. Have you seen hydatid cysts in viscera of slaughtered livestock?

2.6.1. Yes 2.6.2. No

2.7. Do you know how humans contract the disease (Where the disease comes from)?

2.7.1. comes from God 2.7.3. through bewitching

2.7.2. transmitted by insects 2.7.4. Transmitted by animals:

2.8. If 2.9.4., by which animals?

2.8.1. Livestock 2.8.2. Wild animals 2.8.3. birds 2,8.4. dogs

2.9. Do you think the disease can be cured?

2.9.1. No 2.9.3. In Hospital

2.9.2. by witch doctor 2.9.4. traditional herbs (name it)

3. Risk Factors

3.1. Do you have livestock?

3.1.1. Sheep 3.1.2. Goats 3.1.3. Cattle 3.1.4. Hoofed animals

3.2. Do you slaughter at home?

3.2.1. No 3.2.2. Yes

3.3. If Q3.2 is yes, how often?

3.3.1. Sometimes 3.3.2. Frequent 3.3.3. regular

3.4. Which animals do you slaughter?

3.4.1. No 3.4.3. Sheep 3.4.5. goats 3.4.7. sheep/goats

3.4.2. Cattle 3.4.4. Sheep/goats/cattle 3.4.6. donkeys 3.4.8. camels

3.5. What do you do with slaughter offal (bad meat/infected organs

3.5.1. throw away to open ground 3.5.3. feed to dogs

3.5.2. burry 3.5.4. throw into fire

3.6. Do you share water sources with your livestock and dogs:

3.6.1. yes 3.6.2. no

3.7. Do you boil your water?

3.7.1. yes 3.7.2. no

3.8. Do you have dogs?

3.8.1. no 3.8.2 Yes

3.9. If your answer for Q 3.8 is yes, how many dog?

3.9.1. one 3.9.2. two 3.9.3. more than 2

3.10. Dogs are kept for:

3.10.1. Pets 3.8.2. Guarding 3.8.3. Herding 3.8.4. hunting

3.11. Do you feed your dogs?

3.11.1. No 3.9.3. yes

3.12. If your answer for Q3.11 is yes, what do youfeed?

3.12.1. food leftovers

3.12.2. Slaughter offal 3.12.3. dogs look for their own food (roam around)

3.13. Dogs are kept

3.13.1. Chained up in and around the house? 3.13.3. free roaming

3.13.2. free in and around the house

3.14. Are there stray dogs:

3.14.1. yes 3.11.2. no

3.15. What do you do with excess dogs (puppies)/ stray dogs?

3.15.1. Let them just be there 3.12.2. kill them

3.16. Do you touch your dogs?

3.16.1. Yes 3.13.2. no

3.17. Do you wash your hands thereafter?

3.17.1. yes 3.14.2. no

3.18. Do all family members have contact with dogs?

3.18.1. Yes 3.15.2. No

3.19. Do your dogs have access to your kitchen utensils?

3.19.1. Yes 3.17.2. no

3.20. Do you wash your hands before taking food:

3.20.1. Yes 3.17.2. no

3.21. Do you treat your dogs?

3.21.1. No 3.18.2. Occasionally 3.18.3. regularly

3.22. Do you feed dogs condemned offal?

3.22.1. Yes 3.19.2. No

3.23. Do you use dog faeces for any traditional medicine?

3.23.1. Yes 3.20.2. No

ANNEX 2 – CONCENT FORM

You are invited to participate in a study of Epidemiology of Cystic Echinococcosis (hydatidosis) in South Omo Zone, SNNPR, Ethiopia. The purpose of the study is to determine prevalence and public health significance of cystic echinococcosis or hydatidosis in this zone. It is also important to identify the strain of the parasite that is common in the area. Cystic echinococcosis is a devastating and neglected zoonosis that is not well known in the community. Some previous studies showed that the disease is very common in the pastoral community. Your participation in this study is very important in the control effort of this disease.

The study is being conducted by Ato Daniel Woldeyes Habtie from Addis Ababa University. And he is a PhD student of Biomedical Sciences in the Department of Microbial, Cellular and Molecular Biology.

If you decide to participate, you will be asked for ultrasound examination, which is done by an ultrasonography expert, and if hydatidosis is suspected you may be asked to give about 10ml blood through venupuncture by a laboratory technician using sterile and disposable syringe. During the activity of blood taking, there might be some sort of discomforts and pain on your hand. But there is no anticipated risk of ultrasound examination.

If you are found infected by cystic echinococcosis or hydatidosis, you will be treated based on the severity of the disease, from simple chemotherapy to surgery, free of charge. Any information or personal details gathered in the course of the study are confidential. No individual will be identified in any publication of the results.

If you decide not to participate, you are free to withdraw from further participation in the research at any time without having to give a reason and without consequence.

I, _____, the resident of South Omo Zone, _____ District, have read (have had read and/or translated to me) and understand the information above and any questions I have asked have been answered to my satisfaction. I agree to participate in this research, knowing that I can withdraw from further participation in the research at any time without consequence, that I might experience some sort of discomforts and pain when I give blood, that I would be treated free of

charge if I am found infected cystic echinococcosis or hydatidosis, and that my information is confidential. I have been given a copy of this form to keep.

Participant's Name: _____ (Block letters)

Signature: _____ Date:_____

Investigator's Name: DANIEL WOLDEYES

Signature: _____ Date: _____

The ethical aspects of this study have been approved by the Ethical Committee of College of Natural Sciences, AAU, and National Ethical Committee. If you have any complaints or reservations about any ethical aspect of your participation in this research, you may contact the committee through its secretary. Any complaint you make will be treated in confidence and investigated, and you will be informed of the outcome.

Ethical Committee of College of Natural Sciences

Addis Ababa University

P. O. Box: 1176

Tel:

Fax:

Addis Ababa

የጥናት ተሳታፊ ስምምነት

በደቡብ ኦሞ ዞን በሚደረገው የሲስቲክ ኤካይኖኮከሲስ (ሃይዳቲዶሲስ) ስርጭት ጥናት ላይ እንዲሳተፉ ተጋብዘዋል፡፡ የዚህ ጥናት ዋና አላማ የሃይዳቲዶሲሰ ስርጭትና በህብረተሰቡ ጤና ላይ ያለውን ተፅዕኖ በምን ደረጃ ላይ እንዳለ ማረጋገጥ ነው፡፡ ጥናቱ ለበሽታው መንስኤ የሆኑ ንኡስ ዝርያዎችን ለመለየትም ይጠቅማል፡፡ ሃይዳቲዶሲስ ከእንስሳት ወደ ሰው የሚተላለፍ አስከፊ በሽታ ሲሆን፤ በቂ ትኩረት ያላገኘ እና በህብረተሰቡ ዘንድ የማይታወቅ በሽታ ነው፡፡ በሽታው በአርብቶ አደሩ ዘንድ በስፋት እንደሚገኝ አንዳንድ ቀደም ያሉ ጥናቶች ያመልክታሉ፡፡ የእርስዎ በዚህ ጥናት ላይ መሳተፍ በሽታውን ለመቆጣጠር ወደፊት ለሚደረገው ጥረት ከፍተኛ አስተዋፅኦ ይኖረዋል፡፡

ጥናቱ የሚከናወነው በአዲስ አበባ ዩኒቨርሲቲ የባዮሜዲካል ሳይንስ የድህረ ምረቃ ተማሪ በሆነው በአቶ ዳንኤል ወልደየስ ሀብቴ ነው፡፡

በዚህ ጥናት ለመሳተፍ ከወሰኑ በአልትራሳውንድ ማሽን በመስኩ በሰለጠነ ባለሙያ እንዲመረመሩ እና በሽታው እንዳለበዎት የሚጠቁም ሁኔታ ካለ 10 ሚሊ ሊትር ደም ከክንድዎ ላይ እንዲሰጡ ሊጠየቁ ይችላሉ፡፡ ከክነድዎ ላይ ደም የሚወሰደው በህክምና ላቦራቶሪ በሰለጠነ ባለሙያ፤ አንድ ሰው ብቻ ሊገለገልበት እና ሊወገድ በሚችል ንፁህ መርፌ ነው፡፡ የአልትራሳውንድ ምርምራው ምንም ጉዳት አያስከትልብዎትም፡፡ ነገር ግን ከክንደዎ ላይ ደም ሲወሰድ መጠነኛ የሆነ የህመም ስሜት ሊኖር ይችላል፡፡

በሃይዳቲዶሲስ በሽታ መያዘዎት ከተረጋገጠ እንደ በሽታው ጥንካሬ (ከቀላል ህክምና እስከ ቀዶ ጥገና) በነፃ ይታከማሉ፡፡ ማንኛውም በጥናቱ ወቅት ከእርስዎ የተገኘ መረጃ ሚስጥራዊነቱ የተጠበቀ ነው፡፡ የማንም የጥናት ተሳታፊ ስም በማናቸውም የዚህ ጥናት የህትመት ስራ ላይ አይገለጽም፡፡

በጥናቱ ለመሳተፍ ካልፈለጉ ተሳትፎዎን በማንኛውም ጊዜ የማቋረጥ መብት አለዎት፡፡ ተሳትፎዎን በማቋረጥዎ በእርስዎም ሆነ በጥናቱ ላይ የሚደርስ ችግር የለም፡፡

እኔ _____ በደቡብ ኦሞ ዞን የ _____ ወረዳ ነዋሪ ከላይ የተዘረዘረውን መረጃ አንብቤ (ተነቦልኝ ወይም ተተርጉሞልኝ) እና ገብቶኝ

እንዲሁም ለጠየቅኳቸው ጥያቄዎች አርኪ መልስ ስላገኘሁ በዚህ ጥናት ለመሳተፍ ወስኛለሁ፡፡ በዚህ ጥናት ላይ ለመሳተፍ ስወስን የጥናቱ አላማ ገብቶኝ፤ በማናቸውም ግዜ ለማቋረጥ እንደምችል በመገንዘብ፤ የደም ናሙና በምሰጥበት ወቅት መጠነኛ የህመም ስሜት ሊኖር እነደሚችል በማወቅ፤ ሃይዳቲዶሲስ በሽታ በምርመራ ቢገኝብኝ በነፃ መታከም እንደምችል በመረዳት፤ እንዲሁም በምስጢራዊነቱ ተማምኜ ነው፡፡ የዚህ ስምምነት ቅጽ አንድ ቅጅ ተሰጥቶኛል፡፡

የተሳታፊ ስም፡- _____

ፊርማ፡- _____ ቀን፡- _____

የተመራማሪው ስም፡- ዳንኤል ወልደየስ

ፊርማ፡- _____ ቀን፡- _____

የዚህ ጥናት የስነ ምግባር ነክ ጉዳዮች በአዲስ አበባ ዩኒቨርሲቲ የተፈጥሮ ሳይንስ ኮሌጅ እና በአገር አቀፍ ኤቲካል ኮሚቴዎች ታይቶ የፀደቀ ነው፡፡ በዚህ ጥናት ላይ ሲሳተፉ ለሚኖረዎት ማንኛውም ስነ ምግባር ነክ ቅሬታዎች ለኤቲካል ኮሚቴው ከታች በተጠቀሰው አድራሻ ቅሬታዎን ማቅረብ ይችላሉ፡፡ ማንኛውም ቅሬታዎ በአግባቡ ተመርምሮ ይስተናገዳል፡፡ የተወሰደውንም ርምጃ እንዲያውቁት ይደረጋል፡፡

የተፈጥሮ ሳይንስ ኮሌጅ ኤቲካል ኮሚቴ፤ አዲስ አበባ ዩኒቨርሲቲ
ፖ.ሣ.ቁ. 1176
ስልክ፡-
ፋክስ፡-
አዲስ አበባ

ANNEX 3 – ASSENT FORM

Your child is invited to participate in a study of Epidemiology of Cystic Echinococcosis (hydatidosis) in South Omo Zone, SNNPR, Ethiopia. The purpose of the study is to determine prevalence and public health significance of cystic echinococcosis or hydatidosis in this zone. It is also important to identify the strain of the parasite that is common in the area. Cystic echinococcosis is a devastating and neglected zoonosis that is not well known in the community. Some previous studies showed that the disease is very common in the pastoral community. Your child's participation in this study is very important in the control effort of this disease.

The study is being conducted by Ato Daniel Woldeyes Habtie from Addis Ababa University. And he is a PhD student of Biomedical Sciences in the Department of Microbial, Cellular and Molecular Biology.

If you let your child to participate, she/he will be asked for ultrasound examination, which is done by ultrasonography expert, and if hydatidosis is suspected she/he may be asked to give about 10ml blood through venupuncture by a laboratory technician using sterile and disposable syringe. During the activity of blood taking, there might be some sort of discomforts and pain on your child's hand. However, there is no anticipated risk of ultrasound examination.

If your child is found infected by cystic echinococcosis or hydatidosis, she/he will be treated based on the severity of the disease, from simple chemotherapy to surgery, free of charge. Any information or personal details gathered from your child in the course of the study are confidential. No individual will be identified in any publication of the results.

If you decide the child not to participate, you are free to withdraw your child from further participation in the research at any time without having to give a reason and without consequence.

I, _____, the resident of South Omo Zone, _____ District, have read (have had read and/or translated to me) and understand the information above and any questions I have asked have been answered to my satisfaction. I agree to let my child participate in this research, knowing that I can withdraw her/him from further participation in the research at any time without

consequence, that she/he might experience some sort of discomforts and pain when she/he gives blood, that she/he would be treated free of charge if she/he is found infected cystic echinococcosis or hydatidosis, and that her/his information is confidential. I have been given a copy of this form to keep.

Participant's Parent or Guardian Name: _____ (Block letters)

Signature: _____ Date:_____

Participant's Parent or Guardian Name: _____ (Block letters)

Signature: _____ Date:_____

Investigator's Name: DANIEL WOLDEYES

Signature: _____ Date: _____

The ethical aspects of this study have been approved by the Ethical Committee of College of Natural Sciences, AAU, and National Ethical Committee. If you have any complaints or reservations about any ethical aspect of your participation in this research, you may contact the committee through its secretary. Any complaint you make will be treated in confidence and investigated, and you will be informed of the outcome.

Ethical Committee of College of Natural Sciences

Addis Ababa University

P. O. Box: 1176

Tel:

Fax:

Addis Ababa

የጥናት ተሳታፊ ወላጅ/አሳዳጊ ስምምነት

በደቡብ ኦሞ ዞን በሚደረገው የሲስቲክ ኤካይኖኮከሲስ (ሃይዳቲዶሲስ) ስርጭት ጥናት ላይ ልጅዎ እንዲሳተፍ ተጋብዛለች/ዋል፡፡ የዚህ ጥናት ዋና አላማ የሃይዳቲዶሲሰ ስርጭትና በህብረተሰቡ ጤና ላይ ያለውን ተፅዕኖ በምን ደረጃ ላይ እንዳለ ማረጋገጥ ነው፡፡ ጥናቱ ለበሽታው መንስኤ የሆኑ ንኡስ ዝርያዎችን ለመለየትም ይጠቅማል፡፡ ሃይዳቲዶሲስ ከእንስሳት ወደ ሰው የሚተላለፍ አስከፊ በሽታ ሲሆን፤ በቂ ትኩረት ያላገኘ እና በህብረተሰቡ ዘንድ የማይታወቅ በሽታ ነው፡፡ በሽታው በአርብቶ አደሩ ዘንድ በስፋት እንደሚገኝ አንዳንድ ቀደም ያሉ ጥናቶች ያመልክታሉ፡፡ የእርስዎ ልጅ በዚህ ጥናት ላይ መሳተፍ በሽታውን ለመቆጣጠር ወደፊት ለሚደረገው ጥረት ከፍተኛ አስተዋፅኦ ይኖረዋል፡፡

ጥናቱ የሚከናወነው በአዲስ አበባ ዩኒቨርሲቲ የባዮሜዲካል ሳይንስ የድህረ ምረቃ ተማሪ በሆነው በአቶ ዳንኤል ወልደየስ ሀብቴ ነው፡፡

ልጅዎ በዚህ በዚህ ጥናት እንትሳተፍ/እንዲሳተፍ ከወሰኑ በአልትራሳውንድ ማሽን በመስኩ በሰለጠነ ባለሙያ እንድትመረመር/እንዲመረመር እና በሽታው እንዳለባት/እንዳለበት የሚጠቁም ሁኔታ ካለ 10 ሚሊ ሊትር ደም ከክንዱ/ዋ ላይ እንድትሰጥ/እንዲሰጥ ልትጠየቅ/ሊጠየቅ ትችላለች/ይችላል፡፡ ከክነዱ/ዋ ላይ ደም የሚወሰደው በህክምና ላቦራቶሪ በሰለጠነ ባለሙያ፤ አንድ ሰው ብቻ ሊገለገልበት እና ሊወገድ በሚችል ንፁህ መርፌ ነው፡፡ የአልትራሳውንድ ምርምራው ምንም ጉዳት አያስከትልባትም/አያስከትልበትም፡፡ ነገር ግን ከክንዱ/ዋ ላይ ደም ሲወሰድ መጠነኛ የሆነ የህመም ስሜት ሊኖር ይችላል፡፡

በሃይዳቲዶሲስ በሽታ መያዙ/ዋ ከተረጋገጠ በነፃ ትታከማለች/ይታከማል፡፡ ማንኛውም በጥናቱ ወቅት ከልጅዎ የተገኘ መረጃ ሚስጥራዊነቱ የተጠበቀ ነው፡፡ የማንም የጥናት ተሳታፊ ስም በማናቸውም የዚህ ጥናት የህትመት ስራ ላይ አይገለጽም፡፡

ልጅዎ በጥናቱ እንዲሳተፍ ካልፈለጉ ተሳትፎዋ/ውን በማንኛውም ጊዜ የማቋረጥ መብት አለዎት፡፡ ተሳትፎዋ/ውን በማቋረጥዎ በእርስዎም ሆነ በጥናቱ ላይ የሚደርስ ችግር የለም፡፡

እኔ _____ በደቡብ ኦሞ ዞን የ _____ ወረዳ ነዋሪ ከላይ የተዘረዘረውን መረጃ አንብቤ (ተነቦልኝ ወይም ተተርጉሞልኝ) እና ገብቶኝ እንዲሁም ለጠየቅኳቸው ጥያቄዎች አርኪ መልስ ስላገኘሁ ልጄ በዚህ ጥናት እንድትሳተፍ/እንዲሳተፍ ወስኛለሁ፡፡ ልጄ በዚህ ጥናት ላይ እንድትሳተፍ/እንዲሳተፍ ስወስን የጥናቱ አላማ ገብቶኝ፤ በማናቸውም ግዜ ለማቋረጥ እንደምችል በመገንዘብ፤ የደም

ናሙና በሚሰጥበት ወቅት መጠነኛ የህመም ስሜት ሊኖር እነደሚችል በማወቅ፤ ሃይዳቲዶሲስ በሽታ በምርመራ ቢገኝብኝ በነፃ መታከም እንደሚቻል በመረዳት፤ እንዲሁም በምስጢራዊነቱ ተማምኜ ነው፡፡ የዚህ ስምምነት ቅጽ አንድ ቅጅ ተሰጥቶኛል፡፡

የተሳታፊ ወላጅ/አሳዳጊ ስም፡- _____

ፊርማ፡- _____ ቀን፡- _____

የተሳታፊ ስም፡- _____

ፊርማ፡- _____ ቀን፡- _____

የተመራማሪው ስም፡- ዳንኤል ወልደየስ

ፊርማ፡- _____ ቀን፡- _____

የዚህ ጥናት የስነ ምግባር ነክ ጉዳዮች በአዲስ አበባ ዩኒቨርሲቲ የተፈጥሮ ሳይንስ ኮሌጅ እና በአገር አቀፍ ኤቲካል ኮሚቴዎች ታይቶ የፀደቀ ነው፡፡ በዚህ ጥናት ላይ ሲሳተፉ ለሚኖረዎት ማንኛውም ስነ ምግባር ነክ ቅሬታዎች ለኤቲካል ኮሚቴው ከታች በተጠቀሰው አድራሻ ቅሬታዎን ማቅረብ ይችላሉ፡፡ ማንኛውም ቅሬታዎ በአግባቡ ተመርምሮ ይስተናገዳል፡፡ የተወሰደውንም ርምጃ እንዲያውቁት ይደረጋል፡፡

የተፈጥሮ ሳይንስ ኮሌጅ ኤቲካል ኮሚቴ፤ አዲስ አበባ ዩኒቨርሲቲ

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ANNEX 4 - RESEARCH SUBJECT INFORMATION SHEET

Title: Epidemiology of Human Cystic Echinococcosis in Selected Districts of South Omo Zone, SNNPR, Ethiopia

Sponsor: Addis Ababa University, Office of Research and Technology Transfer,

Investigator: Daniel Woldeyes Habtie, Addis Ababa, Arada Sub-city, Ethiopia

Study Sites: Hamer, Bena-Tsemai and Nyangatom Districts of South Omo Zone, South Nations Nationalities and Peoples Region, Ethiopia

Phone Numbers: +251-911-153066, +251-913-188950

E-mail: dannybiomed@yahoo.com, daniel.woldeyes@aau.edu.et

Invitation: You are invited to take part in this research study. Before you decide to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. If you have any questions please feel free to ask. You do not have to take part in this study if you do not want to. If you decide to take part you may withdraw at any time without having to give any reason. Your decision will not result in any penalty or loss of benefits to which you are entitled.

What the study is about? This study is required for the fulfillment of Doctoral Degree in Biomedical Sciences. The study will try to assess the prevalence and risk factors of cystic echinococcosis (hydatidosis). Cystic echinococcosis (CE) or hydatidosis is one of the most neglected and emerging parasitic zoonosis of worldwide occurrence. It is caused by larval stage (hydatid) of the cestode, *Echinococcus granulosus*. Human CE is acquired through accidental ingestion of the eggs that are released with faeces of infected dogs. Although liver and lungs are the most affected organs, CNS, kidney, bone… are also affected. Organ malfunctioning and anaphylaxis due to slowly growing cysts and ruptured cysts, respectively, are the most common pathological consequences of the infection. Currently, diagnosis, treatments and control of the infection are difficult tasks. CE is significant public health problem among pastoralists in East

Africa. It is found in every corner of the country, Ethiopia. However, the current status and the causative strains of human CE in the country have not been assessed. Camel and cattle strains are identified in Kenya and Sudan. Other strains are also expected to be identified in the present study area. The general objective of this study is to assess the prevalence of the infection among the pastoralist and semi-pastoralist and to determine the strain of the parasite in selected districts of South Omo Zone. Imaging, serologic and molecular techniques will be employed to assess the prevalence of infection and to determine the strains of the parasite in the study area. Appropriate statistical methods will be used to analyze the data. The result of study will help to have basic information about the current status of the infection and identify the species and/or strain of the parasite involved. This information is important to devise appropriate control mechanism against the infection.

What you will be asked to do? If you agree to be in this research, you will participate in an interview about the disease and the ultrasound examination. If you are found ultrasound positive, you may be asked to give about 10ml of venous blood.

Risks: There are no anticipated risks to you by participating in the interview and ultrasound examination. However, there may experience some sort of pain and discomfort in case of blood examination.

Benefits: You will get ultrasound diagnosis and treatment of hydatidosis free of charges, if you are found positive to hydatidosis. By participating in this research, you may help researchers, policy makers in the health sectors, in the future, to deal with problems associated with this disease.

Payment for participation: You will not be paid for taking part in this study.

Alternatives: Your alternative is to not be in this study.

Source of Funding: Funding for this research study will be provided by Office of Research and technology transfer of Addis Ababa University.

Your information will be confidential: The information collected and the result of the ultrasound examination will be kept confidential. They will not be shared with other persons

other than the researcher and research team. No individual's name will be attached to comments offered when presenting the study results. The interview and the Ultrasound survey data will be stored in a secure location.

If you have questions or want a copy or summary of the study results: Contact Daniel Woldeyes at +251-913-188950, if you have questions, concerns or complaints or if you want a copy or summary of the study results.

If you have any questions about your rights as a research subject or you have any questions, concerns or complaints about the research, contact:

Ethical Committee of the College of Natural Science (ECCNS)
Addis Ababa University, P.O. Box 1176
Addis Ababa, Ethiopia
Telephone:
E-mail:

ECCNS is a group of people who perform independent review of research and gives ethical approval. You may contact ECCNS if the research staff cannot be reached or if you wish to talk to someone other than the research staff.

ANNEX 5 – INSTITUTIONAL ETHICAL CLEARANCE

Date: Monday, May 27, 2013

Professor Negussie Reta
Dean, College of Natural and Computational Sciences
Addis Ababa University

Re: College Research Ethics Review Committee Decision

Dear Professor Negussie,
College of Natural and Computational Sciences research ethics review committee evaluated Ato Daniel Woldeyes's PhD thesis project proposal titled ***Epidemiology of Human Cystic Echinococcosis in Selected Districts of South Omo Zone, SNNPR, Ethiopia*** from Department of Microbial, Cellular and Molecular Biology, Addis Ababa University for ethical soundness. The proposal was cleared at institutional level and referred to national ethics review committee for final approval.

Thus, this is to request your good office to communicate the committee's decision to the concerned Department.

Attached please find the minutes.

With best regards,

Chairperson
Hassen Mamo (PhD)

CC. Daniel Woldeyes's

ANNEX 6 – NATIONAL ETHICAL CLEARANCE

The Federal Democratic Republic of Ethiopia
Ministry of Science and Technology

MOST

Ref. No. 3:10/162/2016
Date: Nov 4-2016

To: Addis Ababa University Collage of Natural Sciences
Addis Ababa

Re: Epidemiology of Human Cystic Echinococcosis in selected Districts of South Omo Zone, SNNPR, Ethiopia

Dear Sir/Madam /Mr./Mrs./Dr.

We are writing this letter in reference to your renewal request letter ref: CNSDO/092/09/2016 dated 2nd November -2016.

After having in depth review of your request, the National Research Ethics Review Committee has accepted your renewal request for one year from **(November 4, 2016- November 3, 2017).** This is, therefore, to notify that the ethical approval is renewed and your group can proceed in accordance to the latest approved documen2t. Please ensure that you submit a biannual report and an annual renewal application 30 days prior to expire date. We are confident that you as PI of the project and your esteemed organization will monitor the ethical implication of the project as it is stipulated in the latest approved document.

With regards,

Yohannes Sitotaw
Secretary of NRERC

CC: _ **Chairperson, NRERC**
Mr. Daneil Woldeyes (PI)

Website : www.most.gov.et
Tel : +251 114-674-353
E-Mail : most@ethionet.et
Fax : +251 114-660-241
P.O.Box : 2490
Addis Ababa Ethiopia

From Facilitator to Main Actor

ANNEX 7 – LETTER OF EXTERNAL COLLABORATION

UNIVERSITÄT HOHENHEIM

FACHGEBIET PARASITOLOGIE 220 B

Leiterin: Prof. Dr. Ute Mackenstedt

Universität Hohenheim (220 B) · D - 70593 Stuttgart

Daniel Woldeyes
Addis Ababa University
Department of Biology (Biomedical Sciences)

Emil-Wolff-Str. 34
D - 70599 Stuttgart

Dr. Thomas Romig

Telefon: 0711 / 459 - 23076
Telefax: 0711 / 459 - 22276
Thomas.Romig@uni-hohenheim.de
Internet: www.uni-hohenheim.de

Hohenheim, 20. Juli 2012

Letter of Collaboration

Dear Mr. Woldeyes

I confirm that I am willing to collaborate with you in the context of your PhD proposal named ‚Epidemiology of Human Cystic Echinococcosis in Selected Districts of South Omo Zone, SNNPR, Ethiopia'.

Specifically, I will organize and fund a research stay and training course for you at our institute at the University of Hohenheim, with the purpose for molecular characterization of *Echinococcus* samples obtained in your study. Results from this particular part of the study will be published in mutual agreement and authorship, with you as first author. Your stay will take place during 2013, specific dates have to be agreed on.

Further, I will facilitate your ultrasound survey by linking you up with our collaboration partner in Kenya, Eberhard Zeyhle of the African Medical & Research Foundation, Nairobi. The time period for this survey has to be agreed on by all partners.

Yours sincerely

Dr. Thomas Romig

ANEEX 8 – MATERIAL TRANSFER AGREEMENT (MTA)

MATERIAL TRANSFER AGREEMENT

This Material Transfer Agreement (MTA) has been prepared for use by The Department of Microbial Cellular and Molecular Biology (MCMB), Addis Ababa University and FG Parasitology Laboratory, The University of Hohenheim in all transfer of *Research Material* (samples, derivatives, and specimens) related to the research project title:

"Epidemiology of Cystic Echinococcosis in Selected Districts of South Omo Zone, SNNPR, Ethiopia"

Provider

Addis Ababa University,
Department of MCMB,
P.O. Box 1176
Phone +251-0118959216
Addis Ababa, Ethiopia

Recipient

University of Hohenheim,
FG Parasitologie 220B
phone +49711 45923076
fax +49711 45922276
70599 Stuttgart, Germany

Provider and Recipient here with acknowledge the following conditions: transfer of 729 tubes of *Echinococcous granulosus* cyst samples, for the use of analysis of the mentioned samples from Ethiopia collected within the collaborative project:

> **Epidemiology of Cystic Echinococcosis in Selected Districts of South Omo Zone, SNNPR, Ethiopia (PI:** Daniel Woldeyes Habtie**)** funded by Addis Ababa University, College of Natural Sciences, Addis Ababa, Ethiopia; and University of Hohenheim, Stuttgart, Germany.

The named samples and any biological materials derived from them, together with the information provided therewith, shall be referred to as *Research Material* in this agreement.

1. The *Research Material* is and shall remain the sole property of the provider and will be used within or by the Recipient's immediate research group and will not be supplied to other laboratories within or outside the Recipient's research institute.
2. The *Recipient* will be solely responsible for his/her use of the *Research Material* and all other undertakings provided hereunder:
   i) that, unless the *Recipient* and *Provider* agree otherwise in writing, the *Recipient* will not reconstitute, analyze, reverse engineer or modify the *Research Material* and
   ii) that the *Recipient* will keep said information confidential in accordance with the applicable provisions of the Agreement concluded between the participants of the project referenced in Section 6 below.
3. The *Provider* shall not be responsible for the *Research Material* supplied to the *Recipient*, hereunder nor for any claims or liabilities resulting, thereafter.
4. The *Research Material* is experimental in nature and it is provided without warranty of merchantability or fitness for any particular purpose or any other warranty, expressed or implied. The *Provider* makes no representation or warranty that the use of the material will not infringe any patient or other proprietary right or that the use of the *Research Material* (or any derivatives) will not pose a health or safety risk.
5. The *Research Material* is available solely for non-clinical research purposes and will not be used on human subjects.

6. The *Research Material* will be used solely for non-commercial research purposes and will not be used in any studies other than the named project below:

   Epidemiology of Cystic Echinococcosis in Selected Districts of South Omo Zone, SNNPR, Ethiopia (PI: Daniel Woldeyes Habtie) funded by Addis Ababa University, College of Natural Sciences, Addis Ababa, Ethiopia; and University of Hohenheim, Stuttgart, Germany.

7. Any derivative of the *Research Material* made in the Recipient's laboratory will be made available to the Provider under conditions similar to those set forth in this Agreement.
8. If the *Research Material* is referred to in any publication, the correct reference will be made to the work of the *Provider*. All rights to publish by the *Provider* shall remain unaffected by the transfer of the *Research Material*.
9. The *Recipient* will use the *Research Material* in compliance with all laws and governmental regulations and guidelines applicable to the *Research Material* including, but not limited to, the safe use, handling, storage and disposal of the *Research Material*.
10. Any and all issues relating to intellectual property resulting from the use of the *Research Material by the Recipient* hereunder shall be governed exclusively by the *Intellectual Property Rights Agreement* to be concluded between the participants referenced in Section 6 above, and the *Recipient* hereby irrevocably submits to the provisions thereof.
11. This Agreement is part of the Agreement of the Project referenced in Section 6 above, and the provisions of the Agreement are applicable to the subject matter of this Agreement.

Agreed by:

RECIPIENT

Institution: **FG Parasitologie 220B,** ***UNIVERSITY OF HEHENHEIM***

Name of Authorized Institution Representative: ***Dr. Thomas Romig***

Position of Authorized Institution Representative: ***Researcher, Project Coordinator***

Signature of Authorized Institution Representative: _____

Fachgebiet PARASITOLOGIE (220 B)
(Institut f. Zoologie)
UNIVERSITÄT HOHENHEIM
Emil-Wolff-Straße 34
70599 Stuttgart

Date: ***17 April 2013***

PROVIDER

Institution: DEPARTMENT OF MCMB, COLLEGE OF SCIENCES, AAU

Name of Authorized Institution Representative: ***Dr. Fasil Assefa***

Position of Authorized Institution Representative: ***Head, Department of MCMB***

Signature of Authorized Institution Representative: _____

Date: ***16 April 2013***

ANNEX 9 – *Echinococcus granulosus* G_{Omo} (Accession No. KX037021)

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001 CTCTGGAAGT TCGAAACTCC GAAGTACCTC GTTACCATCA TCGACGCGCC
051 CGGTCATCGT GACTTTATTA AGAACATGAT TACGGGCACC AGCCAGGCGG
101 ACTGCGCAAT TCTGGTTGTT GCTGCTGGTA CTGGTGAGTT CGAGGCAGGT
151 ATTAGTAAGA ATGGTCAAAC GCGCGAGCAT GCGCTTCTCG CCTTCACTCT
201 GGGTGTGAAG AAGCTCATCA TTGCGGTCAA CAAAATGGAT GCAGTTGATT
251 ACAGTGAAAA GCGCTTCCAG GAGATTAGTT CCGAAATGAA GGCGTACATT
301 AAGAAGGTGG GATACAACCC CGATACTGTA AATATCGTCC CAATCTCCGG
351 TTGGGTCGGC GACAACATGC TGGAGCCCAG TCCTAACATG CCTTGGTACA
401 AGGGGCCCAC GCTTCTTGCC AGTATCGATC TTGTTGAGCC TCCAACTCGT
451 CCCGTGGACA AGCCCCTTCG ACTTCCTCTT CAGGTGAGGC CTTTTTAATG
501 GGGTTTATTG TCTAGGATGT GTTTAAAATT AGCGGTATTG GTACTGTTCC
551 CGTCGGCCGT GTCGAGACTG GCATAATGAA GCCGGGTATG ATCGTCACAT
601 TTGCTCCTGT CGGCATTTCA ACCGAAGTGA AATCAATTGA AATGCACCAT
651 GAAGCCCTAT CCGAGGCCGT CCCCGGAGAC AACGTTGGCT TCAACGTGAA
701 AAATATCTCT GTAAAGGACG TTCGTCGCGG TAACGTGGCT GGGGACTCAA
751 AGAATCACCC TCCTCGCGAG GCTGCAGAGT TCACCGCCCA AGTTATCGTC
801 CTCAATCATC CTGGCGAGAT TGGTGCCGGC TACACTCCCG TTCTGGACTG
851 TCATACTGCT CACATCGCCT GTAAGTTTGC CGAACTTAAG GAGAAAATCG
901 ATCGCCGTAC TGGTCAGGTC AAGGAGACTA ACCCTGCGAA GATCAAATCC
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ANNEX 10 – TITLES OF PUBLISHED ARTICLE AND CONFERENCE PAPER

1. Daniel Woldeyes, Beyene Petros, Getache Tilahun, Eberhard Zeyhle, Thomas Romig and Peter Kern (2015). Ultrasound Survey on Human Cystic Echinococcosis in Selected Districts of South Omo Zone, SNNPR, Ethiopia. Conference paper on the 26th Annual Conference of EPHA. Abstract code: 373.

2. Wassermann M., Woldeyes D., Gerbi B.M., Ebi D., Zeyhle E., Mackenstedt U., Petros B., Tilahun G., Kern P. and Romig T. (2016). A novel zoonotic genotype related to Echinococcus granulosus sensu stricto from southern Ethiopia. Int. J. Parasitol. 46:663–668.