



## Research paper

Molecular characterization of *Echinococcus granulosus* s.l. cysts from cattle, camels, goats and pigs in Ethiopia

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## ABSTRACT

Cystic Echinococcosis (CE) caused by *Echinococcus granulosus* sensu lato (s.l.) is a neglected helminth zoonosis affecting humans and various animal species. Human CE has been reported in almost all countries of sub-Saharan Africa but its prevalence and public health impact are subject to large geographical variations. The reasons for these differences are not well understood; among other factors, occurrence of different species/genotypes of *E. granulosus* s.l. has been suggested. CE is very common in all livestock species in Ethiopia; human CE is poorly documented in the country. The aim of this study was to assess the fertility and molecularly characterize hydatid cysts collected from cattle, camels, goats and pigs from different parts of the country. From the 137 samples characterized by PCR-RFLP and sequencing, 115 (83.9%) were identified as *E. granulosus* s.s. (G1, common sheep strain), 6 (4.4%) as *Echinococcus ortleppi* (G5, cattle strain) and 16 (11.7%) as *Echinococcus intermedius* (G6/7, camel strain). In cattle, *E. granulosus* s.s. and *E. ortleppi* were found; in camels and goats, *E. granulosus* s.s. and *E. intermedius*; two cysts found in pigs were identified as *E. granulosus* s.s. and *E. ortleppi*, respectively. All cysts recovered from goats and pigs were sterile, while fertility was 34% and 50% in cysts from cattle and camels, respectively. In cattle, 31% of *E. granulosus* s.s. cysts were fertile, showing the importance of cattle in the transmission of the “sheep strain”. Next to *E. granulosus* s.s., *E. intermedius* (camel strain) was the predominant species: 34.4% of the cysts collected from camels and 62.5% from goats were identified as *E. intermedius*. These animals originated from the drier Central, Eastern and Southern parts of the country. For the first time, we showed the presence of CE in pigs in Ethiopia. The presence of these strains and especially the fact that the zoonotic *E. granulosus* s.s. and *E. intermedius* are dominant, make CE an important public health concern in Ethiopia.

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## 1. Introduction

Cystic Echinococcosis (CE) caused by *Echinococcus granulosus* sensu lato (s.l.) is a neglected helminth zoonosis affecting humans and various animal species (Moro and Schantz, 2006). While CE has a worldwide distribution, both the prevalence in livestock and the number of human cases are very unevenly distributed. Human CE has been reported in almost all countries of sub-

Saharan Africa where its occurrence goes from very occasional to exceptionally high (Romig et al., 2011). Various factors have been suggested to explain these differences, which are related to livestock husbandry types, number of dogs, but also to factors related to human behavior, genetic predisposition and malnutrition-related immunosuppression (Macpherson et al., 1989b; Romig et al., 2011). Risk factors for infection are typically found in traditional pastoralism conditions where the prevalence of human CE can be very high, such as in the Turkana region in Kenya and neighboring regions in South Sudan, southwestern Ethiopia and northeastern Uganda (Romig et al., 2011). However, in other African regions where similar conditions prevail, CE is rare, suggesting that other factors are involved in the transmission. Among these, the presence and distribution of different species/genotypes of *Echinococcus granulosus*

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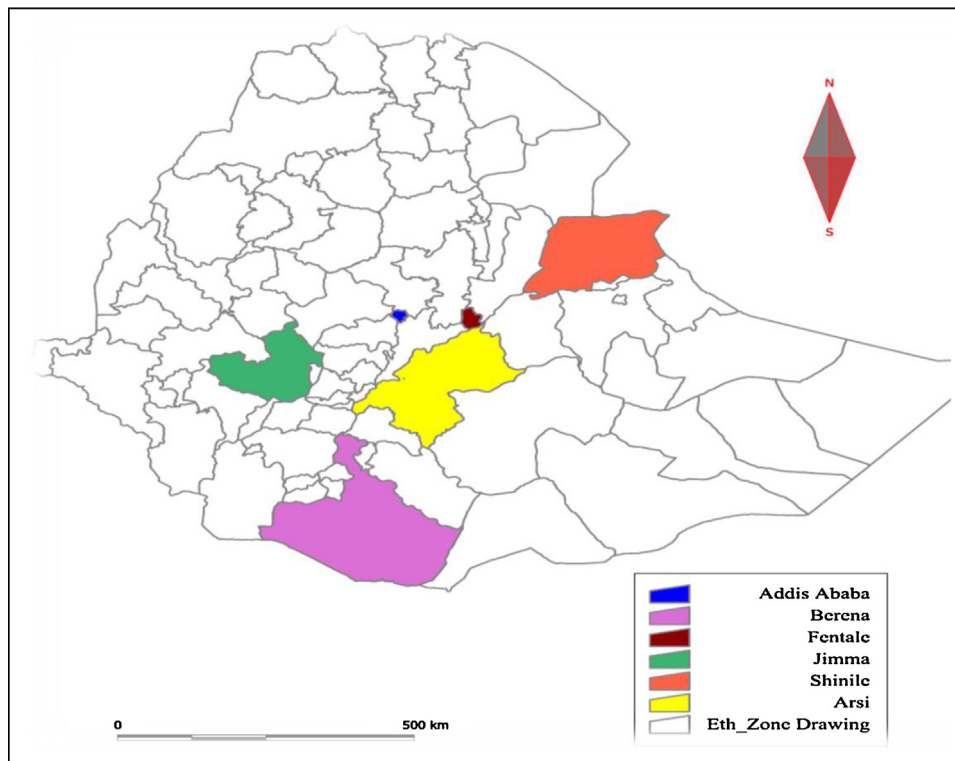


Fig. 1. Map of Ethiopia showing the study areas.

have received most attention, because not all species/genotypes have the same infectivity or pathogenicity to humans. Molecular methods have allowed discrimination of different genotypes (G1–10 and the lion strain), some of which are now considered different species (Alvarez Rojas et al., 2014). There is now a widespread agreement based on morphological, molecular, and ecological criteria that *E. granulosus* s.l. should be split into the species *E. granulosus* sensu stricto (s.s.) (including the genotypes G1, G2, G3; sheep and buffalo strains), *Echinococcus equinus* (G4; horse strain), *Echinococcus ortleppi* (G5; cattle strain) and *Echinococcus canadensis* (G6–10). Recently, Lymbery et al. (2015) demonstrated based on similar criteria that the G6 (camel strain) and G7 (pig strain) genotypes represent a single species that is different from both the G8 and G10 genotypes (cervid strains). They suggested the names *Echinococcus intermedius* (G6, G7), *Echinococcus borealis* (G8) and *E. canadensis* (G10). In addition, *Echinococcus felidis* (lion strain) has been described in Africa (Nakao et al., 2007; Saarma et al., 2009). The G1 genotype has the widest geographical distribution and is responsible for 95% of human CE cases (Craig and Larrieu, 2006). The G6/7 genotypes (*E. intermedius*) are common causes of human CE in camel-producing areas and in Eastern Europe (Casulli et al., 2010; Omer et al., 2010). The other genotypes do cause rare cases of human CE (G5, G8, G10) or no cases at all (G4) (Alvarez Rojas et al., 2014).

Identification of the locally prevailing *E. granulosus* strains, their distribution and host specificity are needed to increase cost-effectiveness of control actions by targeting the locally most important transmission cycles for human health and animal production (Romig et al., 2011).

Similar to other East African countries, CE is endemic in Ethiopia and the abattoir-based prevalence of the parasite was reported to be as high as 60.8%, 35.4%, 29.3% and 16.0% in cattle, camels, sheep and goats, respectively (Erbeto et al., 2009; Getaw et al., 2010; Koskei et al., 2011; Haile, 2012). Maillard et al. (2007) identified G1 genotype cysts in four cattle and one sheep slaughtered in the

Addis Ababa area. Hailemariam et al. (2012) found 35 G1 (from sheep, cattle and camels) and five G6 (from camels and cattle) in cysts collected in abattoirs in Addis Ababa and East Ethiopia. Romig et al. (2011) reported an unpublished study by Romig and Dinkel who identified *E. granulosus* G1, *E. ortleppi* G5 and G6/G7 among 21 cysts from cattle in North Ethiopia. Human CE was reported from different parts of the country (Minas et al., 2007; Kebede et al., 2009; Macpherson et al., 1989a; Fuller and Fuller, 1981) but the overall public health impact is not known.

The aim of this study was to characterize hydatid cysts sampled from different intermediate host species, including cattle, camel but also goat and pig that had not yet been investigated, originating from different parts of Ethiopia, including the southern region of the country, to generate information on the strains of *E. granulosus* infecting animals in the country.

## 2. Materials and methods

### 2.1. Study area

From January 2010 till October 2011, cattle, camels, goats and pigs were inspected at slaughter for hydatid cysts in abattoirs in Jimma town and the Addis Ababa region.

Only cattle were sampled in the Jimma municipal abattoir; they originated from mixed crop–extensive livestock production systems in Jimma zone and its surrounding districts. Camels slaughtered in the Akaki Kality slaughterhouse in Addis Ababa were from the Shinele, Borena and Fentale areas, which are located in East, Central and South Ethiopia, respectively and are pastoral production zones. Samples from goats were collected from an export abattoir in Addis Ababa; they originated mainly from the Arsi Zone of Oromia regional state. Pigs were inspected in slaughterhouses in Jimma town and in the Addis Ababa area; they originated from commercial pig farms in the central part of the country. A map showing the main areas of origin of the animals is presented in Fig. 1.

**Table 1**

PCR and RFLP theoretical profiles for complete mitochondrial sequences of G1, G5, G6 and G7 strains of *Echinococcus granulosus* with primers ITM TnR/TaenF and restriction enzyme *AluI*.

<i>E. granulosus</i> strain	Isolation source	Accession number	Theoretical band with primers ITMTnR/TaenF (RFLP profiles with restriction enzyme <i>AluI</i> )
G1	Not specified	AF297617	889 bp (524, 200, 125, 40)
G5	Cattle	AB235846	898 bp (583, 201, 74, 40)
G6	Camel	AB206083	899 bp (813, 46, 40)
G7	Pig	AB235847	899 bp (813, 46, 40)

## 2.2. Sample collection

A total of 139 hydatid cysts were sampled from the liver and lungs of 97 adult cattle and 32 old culled camels, and from the liver of eight goats of various ages and two pigs in slaughterhouses in Jimma town and the Addis Ababa region. Only cysts that did not show degeneration by macroscopic inspection were collected and taken to the laboratory in a cool box. The fertility of the cysts was assessed by macroscopic and microscopic examination of the cyst wall and content. A fertile cyst was defined as a cyst that contained protoscolices in the content. The viability of the protoscolices was not assessed. Protoscolices (for fertile hydatid cysts) and germinal layers (for non-fertile cysts) were rinsed in physiological saline solution. Thereafter, the samples were transferred into sterile test tubes (each sample originated from a single cyst), fixed in 70% ethanol, stored and transported to the Institute of Tropical Medicine of Antwerp, Belgium (ITM) for molecular analysis. For reference purposes, genomic DNA from G1 (from Armenia), G5 (from Vietnam) and G6 (from Kenya) genotypes were obtained from the Department of Parasitology, University of Hohenheim, Emil-Wolff, Germany.

## 2.3. DNA extraction

Genomic DNA was extracted from the protoscolices and germinal layers by means of a modified Boom extraction method (Boom et al., 1990; Geysen et al., 2007).

## 2.4. Primers and enzyme selection

To select the primers and the restriction enzyme used in this study, we first obtained complete genomic sequences of mitochondrial DNA for G1 (AF297617), G5 (AB235846), G6 (AB208063) and G7 (AB235847) strains from GenBank at the National center for Biotechnology Information (NCBI). The ITM TnR/TaenF primer pair that was previously developed for the characterization of *Taenia* species (Geysen et al., 2007) was now used to amplify a  $\pm 900$  bp band of *Echinococcus* DNA. The resulting DNA bands were then digested with the *AluI* restriction enzyme to distinguish between different *Echinococcus* species. PCR reactions were simulated with AmplifX (version 1.5.4; <http://crn2m.univ-mrs.fr/pub/amplifx-dist>), while RFLP assays were simulated with EnzymX 3 (<http://nucleobytes.com/index.php/enzymex>). Finally, the theoretical results (Table 1) were compared with actual digests of the reference samples.

## 2.5. Strain characterization

### 2.5.1. PCR-RFLP

A total volume of 25  $\mu$ l, containing Yellow Sub (GeneoBioTech-Products, Hamburg, Germany), 5  $\mu$ l of sample (0.5  $\mu$ l template DNA and 4.5  $\mu$ l water), 200  $\mu$ M of each dNTP, Promega GoTAQ FlexiBuffer (5X), 1.5 mM MgCl<sub>2</sub>, 20 pmol of each primer, and 0.5 U of DNA polymerase (Promega) were used for PCR amplifications. The DNA amplification was performed with a Thermocycler (Biometra, Goettingen, Germany) for 40 cycles as follows: denaturation for 45 s

at 94 °C, annealing for 45 s at 57 °C and elongation for 1 min at 72 °C. 5  $\mu$ l of each PCR product was then analyzed by electrophoresis on 2% agarose gels (100 V for 20 min; Eurogentec, Liège, Belgium) followed by ethidium bromide staining. When a  $\pm 900$  bp band was observed, 6  $\mu$ l of the amplification product was digested with *AluI* (New England Biolabs, Ipswich, MA, USA) according to the manufacturer's recommendations. The digested products were analyzed by electrophoresis on a 10% polyacrylamide gel (100 V for 2.5 h) followed by SYBR green staining. A 100 bp DNA size marker was included for both the PCR and the RFLP electrophoresis.

### 2.5.2. Genomic sequencing

Samples for which strain characterization by PCR-RFLP was doubtful were sent to the VIB Genetic Service Facility (University of Antwerp, Belgium) for sequencing. The obtained sequences were edited and aligned with BioEdit (Hall, 1999) and BLAST was performed with BLASTN (version 2.2.26+) using the NCBI non-redundant database (nr) and the NCBI Reference Sequence Database (refseq\_genomic).

## 3. Results

The fertility rate of hydatid cysts was 34%, 0%, 50% and 0% in cattle, goats, camels and pigs, respectively.

From the 139 samples, 119 could be unequivocally characterized by PCR-RFLP; 20 were sent for sequencing. From the 20 samples sent for genomic sequencing, we obtained readable sequences of  $\pm 900$  nucleotides for 18 isolates. Two samples, both from goats, gave a negative sequencing result. The 18 samples were identified by BLASTN. Identical results were obtained regardless of the NCBI database that was used. From these 18 isolates, 16 showed  $\geq 99\%$  homology with the G6 genotype (GenBank accession no. AB208063) and two showed  $\geq 98\%$  homology with the G5 genotype (GenBank accession no. AB235846).

From the 137 samples characterized by PCR-RFLP and sequencing, 115 (83.9%) were identified as *E. granulosus* s.s. (G1, common sheep strain), 6 (4.4%) as *E. ortleppi* (G5, cattle strain) and 16 (11.7%) as *E. intermedicus* (G6/7, camel strain). A summary of the different genotypes of *E. granulosus* according to the different regions of Ethiopia and animal species is presented in Table 2. In cattle both G1 and G5 genotypes were found. Four of the five G5 cysts were fertile while fertility was only 31% for G1 cysts ( $N=28$ ) in cattle. Both G1 and G6/7 cysts were found in goats and camels; while all hydatid cysts were sterile in goats, in camels 52% of G1 ( $N=11$ ) and 45% of G6/7 ( $N=21$ ) were fertile. Finally, 2 cysts were found in pigs, which were sterile, one G1 and one G5 (which was confirmed by sequencing).

## 4. Discussion

Many studies reported a high prevalence of CE in Ethiopian livestock. Compiled data for the period 1990–2011 from 23 slaughterhouses from different parts of the country showed a prevalence of CE of 27.6% (2369/8590) in cattle, 11.5% (450/3900) in sheep, 6.8% (131/1937) in goats, 23.8% (292/1226) in camels and 0% (0/150) in pigs (Van Pelt, Devleeschauwer, Tigre, Dorny, unpub-

**Table 2**  
Species of *Echinococcus granulosus* s.l. identified from different animals species from different parts of Ethiopia.

Variable	Species (%)			Total
	<i>E. granulosus</i> s.s. (G1)	<i>E. intermedius</i> (G6/7)	<i>E. ortleppi</i> (G5)	
Study area				
Jimma area	91	1	5	97
Shinele	1	2	–	3
Borena	18	9	–	27
Fentale	4	1	–	5
Central Ethiopia	1	3	1	5
Animal species				
Cattle	90	–	5	95
Camel	21	11	–	32
Goat	3	5	–	8
Pig	1	–	1	2
Total	115 (83.9%)	16 (11.7%)	6 (4.4%)	137

lished results). The same authors also collected information from 9 different areas in the country on the prevalence of infection with *E. granulosus* s.l. in dogs. In 32.4% (81/250) of euthanized dogs *E. granulosus* s.l. tapeworms were found at autopsy. These data clearly show the high endemicity of *E. granulosus* s.l. in the country. However, very few data are available on the impact on the human population of this zoonotic parasite. Characterizing the strains of *E. granulosus* infecting different livestock species, dogs and humans adds valuable information on the epidemiology of this cestode.

In this study, *E. granulosus* s.s. (G1), *E. ortleppi* (G5) and *E. intermedius* (G6/7) were found in different animal species from different parts of Ethiopia. These findings are in agreement with previous studies on smaller numbers of isolates in the country (Maillard et al., 2007; Romig et al., 2011; Hailemariam et al., 2012) and in neighboring Sudan and Kenya (Wachira et al., 1993; Dinkel et al., 2004; Maillard et al., 2007; Casulli et al., 2010; Omer et al., 2010). The predominance of *E. granulosus* s.s. (G1) in this study agrees with several studies conducted in different regions of the world including Eastern African countries. The G1 strain was identified in 52 of 53 hydatid cysts obtained from cattle in Kenya and was also the predominant strain in sheep, goats, camels and dogs in that country (Wachira et al., 1993). In our study, 28 of the 90 *E. granulosus* s.s. (G1), (31%) cysts in cattle were fertile, showing the importance of cattle in the transmission of the “sheep strain”. Similar to reports from other East African countries, this study showed the predominance of the G6/7 strain (camel strain; *E. intermedius*) next to *E. granulosus* s.s. (G1). In this study, 11/32 of the hydatid cysts collected from camels and 5/8 from goats were identified as G6/7. These animals originated from the drier Central, Eastern and Southern parts of the country. In Sudan, the G6/7 strain was characterized from all of the 215 and 65 cysts from camels and goats, respectively (Omer et al., 2010). Similarly, Casulli et al. (2010) reported the predominance of the G6 strain next to the G1 strain in the Turkana district of Kenya. These findings indicate the importance of the camel strain (G6) and the significant role of camels and goats as intermediate hosts for this strain in arid parts of East African countries, including Ethiopia. The existence of *E. ortleppi* (G5) in Ethiopia was reported in the Northern part of the country by Romig et al. (2011). In this study, six isolates (five from cattle and one from pig) were identified as *E. ortleppi*. The G5 has been reported from Europe, Africa, parts of Asia and South America (CFSPH, 2011). Dinkel et al. (2004) reported two isolates of *E. ortleppi* out of 70 cattle cysts from Sudan and one out of the five cysts from pigs in Kenya. *E. ortleppi* was also characterized in one of 107 cattle cysts from Sudan (Omer et al., 2010). Though the proportion of the G5 was found to be low compared to the G1 and G6 strains, its area coverage in Ethiopia seems wide. The G5 strain was characterized from five samples from cattle in southwest Ethiopia and from one sample from a pig

in central Ethiopia. Our study is the first to report CE in pigs in the country.

Ethiopia has one of the largest populations of livestock in Africa with more than 54, 26, 25 and 0.9 million of cattle, sheep, goats and camels, respectively (FAOSTAT, 2014). Extensive management systems prevail, and different livestock species graze on communal land. In most parts of the country, there are large numbers of free roaming domestic and stray dogs. In rural areas, slaughtering of almost all small ruminants and most of the cattle in the backyard and feeding of dogs with non-treated infective offal is common. Large dog populations are usually seen in and around slaughterhouses and these have access to condemned carcasses and organs because proper destruction facilities are inexistent, even in newly constructed export slaughterhouses. These conditions are very conducive for the transmission of *E. granulosus* s.l. Because domestic dogs are left to roam during the day and watch the house at night, exposure to the parasite combined with close contact of the dogs with the owners poses a serious risk for human infection. However, the lack of reports on human CE in the country is striking. The few hospital-based studies show few cases (Kebede et al., 2009; Van Pelt, Devleeschauwer, Tigre, Dorny, unpublished results). One (outdated) study reports on a hotspot of human CE in two tribes in the extreme southwest of the country, close to the Kenyan Turkana hyperendemic area (Fuller and Fuller, 1981). There are few data on the clinical characteristics and severity of these cases, and also on the strain(s) causing these human infections. *E. granulosus* s.s. (G1) is the primary cause of human CE worldwide (Craig and Larrieu, 2006; Nakao et al., 2010) followed by the G7/6 strain, especially in camel producing communities (Casulli et al., 2010). From the 59 hydatid cysts characterized from humans in the Turkana district of Kenya, 49 (83%) and 10 (17%) were G1 and G6, respectively (Casulli et al., 2010). *E. ortleppi* (G5) rarely causes human CE (Nakao et al., 2010).

In conclusion, our study reports the predominance of *E. granulosus* s.s. (G1) and *E. intermedius* (G6/7) in Ethiopian livestock, and shows the existence of *E. ortleppi* (G5) in the southwest and center of the country. For the first time, we show the presence of CE in pigs in Ethiopia. The presence of these strains and especially the fact that the zoonotic G1 and G6 genotypes are dominant, make *E. granulosus* an important public health concern in Ethiopia. To this effect, epidemiological studies on human CE and the strains of the parasite infecting humans are highly recommended.

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