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Original Article

Genetic characterization of *Echinococcus* species in eastern Ethiopia

Terefe Y.[a], Addy F.[b], Alemu S.[a], Mackenstedt U.[c], Romig T.[c], Wassermann M.[c,*]

[a] College of Veterinary Medicine, Haramaya University, Dira Dawa, Ethiopia
[b] Department of Biotechnology, University for Development Studies, Faculty of Agriculture, Tamale, Ghana
[c] Department of Parasitology, University of Hohenheim, Stuttgart, Germany

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ABSTRACT

Cystic echinococcosis (CE) is a neglected zoonotic disease with considerable economic and public health burden worldwide, particularly affecting developing countries like Ethiopia. To initiate effective prevention and control of CE, comprehensive data on the local lifecycles of the various species/genotypes of *Echinococcus* are needed. In the present study, conducted in eastern Ethiopia, a total of 1106 livestock animals were examined at three slaughterhouses, which resulted in combined prevalence of morphologically and molecularly confirmed CE of 8.4% (75/891) in cattle, 1.1% (1/95) in sheep, 0.0% (0/95) in goats and 12.0% (3/25) in camels. All cystic lesions recovered during post mortem examination were assessed for cyst condition and underwent molecular characterization by PCR and sequencing of a 1081 bp fragment of the mitochondrial *cox1* gene. A total of 175 cysts belonged to *E. granulosus* s.s. ($n = 165$), *E. ortleppi* ($n = 6$) and *E. canadensis* G6/7 ($n = 4$). Of all examined cysts, only 14 were fertile and contained protoscoleces, all from the lungs of cattle: 5 were *E. granulosus* s.s., 6 *E. ortleppi* and 3 *E. canadensis* G6/7. In sheep, only one sterile liver cyst of *E. granulosus* s.s. was found, while in camels seven sterile or caseated/calcified cysts of *E. granulosus* s.s. and *E. canadensis* G6/7 were found in liver and lungs. In conclusion, the prevalence of CE was rather low compared to other regions of Ethiopia, and, based on the number of fertile cysts, three *Echinococcus* spp. contributed almost equally to transmission. Cattle seem to be, epidemiologically, the most important livestock species. Our data provide a substantial basis for more detailed investigations of the transmission dynamics of CE in the study area.

1. Introduction

Cystic echinococcosis (CE) is a zoonotic parasitic disease of mammals caused by the larval stage (metacestodes) of the taeniid cestode *Echinococcus granulosus* sensu lato (s. l.). Dogs and some wild carnivores are definitive hosts harbouring adult worms in their small intestines and shedding the eggs with the faeces. The herbivorous or omnivorous intermediate hosts become infected via ingestion of eggs with contaminated food or water. The development of cysts, which are localized mainly in liver and lungs, can cause severe pathological effects in the intermediate hosts. Also humans, as dead-end hosts, can acquire this disease when ingesting eggs (Kern et al., 2017; Romig et al., 2017; Thompson, 2017).

CE is a major economic and public health challenge, particularly in developing regions with limited economic resources for management of zoonotic diseases (Cardona and Carmena, 2013; Budke et al., 2006). Annually, about USD 2 billion financial loss is imposed by CE on the livestock industry globally (Cardona and Carmena, 2013). Estimated annual global impact of CE in humans in terms of disability-adjusted life years (DALYs) is 1,009,662 in addition to a USD 1,918,318,955 monetary loss, whereas the financial impact in Sub-Saharan Africa was estimated to be about USD 5,176,229 (Budke et al., 2006).

E. granulosus s. l. is globally distributed, causing high prevalence of animal and human CE mainly in regions with extensive and traditional livestock husbandry. This applies to many regions in Sub-Saharan Africa including Ethiopia, Kenya and Sudan (Romig et al., 2011). There are several transmission foci in Africa, that are usually geographically restricted and do not cover entire countries. Prevalence levels and presence of different *Echinococcus* species are very heterogeneously distributed, as is the resulting impact on animal and human health (Addy et al., 2012; Kagendo et al., 2014; Mbaya et al., 2014; Mutwiri et al., 2013; Omer et al., 2010). It follows, that the epidemiological situation in one part of a country cannot be extrapolated to others. In Ethiopia, numerous studies were performed on CE prevalence in livestock, highlighting the central and western part of the country as a hotspot of animal CE in Africa (lit. in Deplazes et al., 2017). In contrast, only limited information is available from the eastern part of Ethiopia, which borders to Somalia and is characterized by very different cultural

* Corresponding author at: Department of Parasitology, University of Hohenheim, Emil-Wolff-Str. 34, 70599 Stuttgart, Germany.
E-mail address: Marion.Wassermann@uni-hohenheim.de (M. Wassermann).

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and climatic conditions.

Unlike in neighbouring Kenya and Sudan, few studies with molecular identification of the parasites have been undertaken in Ethiopia. Previous data show that *E. granulosus* s.s., *E. canadensis* G6/7 and *E. ortleppi* are present (Hailemariam et al., 2012; Maillard et al., 2006; Tigre et al., 2016). Very recently Wassermann et al. (2016) identified a novel taxon of *Echinococcus* from a human patient in Ethiopia, which shows that the knowledge on the diversity in the region is incomplete. Sub-Saharan Africa remains the region with the highest diversity of *Echinococcus* taxa and more studies reveal previously unknown aspects of *Echinococcus* within the region (Romig et al., 2017). For a comprehensive understanding of the echinococcosis situation in the region, a differential diagnosis of prevailing *Echinococcus* spp. in all endemic localities is required. The present study presents data on the CE situation in livestock and the causative *Echinococcus* spp. circulating in eastern Ethiopia.

2. Material and methods

2.1. Study area and setting

The study was conducted between February 2015 and September 2016 in the municipal abattoirs in Harar and Dire Dawa towns and the slaughterhouse at the Haramaya University (main campus), Eastern Ethiopia. (Fig. 1). The abattoirs were managed by the respective city administrations which aim to provide officially inspected meat (beef, mutton, goat and camel meat) for the consumers. The beef from the slaughterhouse in Haramaya University is provided to students and the staff.

2.2. Study animals and their management

The animals included in this study were adult indigenous cattle, sheep, goat and camels that originated from a catchment area of approximately 100 km radius in the surrounding areas of the East Hararghe Zone of Oromia Regional State and the Ethiopian Somali National Regional State (Fig. 1). Livestock in these areas are raised in extensive pastoral management systems where animals are allowed to graze freely on available pastures. It was difficult to identify the exact community origin of the animals, but the abattoirs are supplied with animals from Haramaya, Kombolcha, Chelenko, Kersa, Awash, Meso, Jijiga, Babile, Weter, Kulubi and Shinele zones. In total 1106 animals were examined: 891 cattle, 25 camels, 95 sheep and 95 goats.

2.3. Cyst isolation and morphological characterization

Post-mortem examination of lung, liver, heart, spleen and kidney was done by visual inspection and palpation. The infected organs from each positive animal were collected and suspected hydatid cysts were resected; the status of the cyst was identified by macroscopic and microscopic examinations and recorded as fertile (containing protoscoleces), sterile (without protoscoleces but with viable appearance), and caseous / calcified (non-viable, degenerated lesions). The total number of cysts was counted per infected organ. The viability of the protoscoleces was not assessed. Protoscoleces (from fertile cysts) and germinal layers (from sterile/caseous/calcified cysts) were rinsed in physiological saline and subsequently preserved in 70% ethanol until further analysis.

Final diagnosis of non-fertile cysts was only done after molecular confirmation.

2.4. Molecular characterization and species identification

2.4.1. DNA extraction

Single protoscoleces were transferred into PCR tubes containing 10 µl of 0.02 M NaOH and lysed at 95 °C for 10 min in thermocycler (Nakao et al., 2003). The tissue of sterile cyst samples was thoroughly washed in ddH$_2$O to remove ethanol. For each sample a tissue pieces of approximately the size of 0.5 cm^2 were transferred into a PCR tube containing 30 µl of 0.02 M NaOH and lysed for 10 min at 95 °C. The lysates were used directly as DNA templates in the following PCR.

2.4.2. DNA amplification and sequencing

A partial fragment of the mitochondrial cytochrome *c* oxidase subunit 1 (*cox1*) gene was amplified in a nested PCR. The first PCR was performed with a final volume of 25 µl and the second (nested) PCR with a 50 µl final volume. The reaction mixtures contained 10 mM Tris-HCl (pH 8.3); 50 mM KCl, 2 mM MgCl$_2$, 200 µM of each deoxynucleotide triphosphate (dNTP), whereas the primer and Taq Polymerase concentrations and DNA in the 1st/nested PCR were 10/20 pmol, 0.625/1.25 U and 1 µl of lysate/first PCR product, respectively. The primers used to obtain the 1082 bp long fragment were forward 5'-GTCTAGTATTTTTAGTTCTATCA-3′ and reverse 5'-GCATGATGCAAAA GGCAAATAAAC-3′ in the 1st PCR and forward 5'-CTGTTTTGTTATTG GTTACGTTGC-3′ and reverse 5'-GACTAATAATCAACTTAGACTTAC-3′ in the nested PCR (Ebi, unpublished; Hüttner et al., 2008). The conditions during both PCRs were as follows: initial denaturation 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, elongation at 72 °C for 1 min and a final elongation at 72 °C for 5 min.

After successful amplification, the nested PCR products were purified using the High Pure PCR product purification kit (Roche Diagnostics GmbH, Germany) following the manufacturer's instruction and sent to GATC Biotech AG (Konstanz, Germany) for sequencing. Obtained DNA sequences were analysed and edited with GENtle v. 1.9 (Manske M. 2003, University of Cologne, Germany) and compared with existing sequences in the GenBank databases by using BLAST (www.blast.ncbi.nlm.nih.gov/blast.cgi).

3. Results

A total of 1106 livestock animals were examined post-mortem with 79 animals harbouring at least one molecularly confirmed *Echinococcus* cyst. Prevalence was 8.4% (75/891) in cattle, 1.1% (1/95) in sheep, 0.0% (0/95) in goats and 12.0% (3/25) in camels. Out of 409 cysts or suspected lesions that were initially collected, 175 cysts out of 79 animals (at least one from each positive animal) were molecularly confirmed. They belonged to *E. granulosus* s.s. (n = 165), *E. ortleppi* (n = 6) and *E. canadensis* G6/7 (n = 4). Of all examined cysts, only 14 were fertile and contained protoscoleces, all from the lungs of cattle: 5 of these were *E. granulosus* s.s., 6 were *E. ortleppi* and 3 were *E. canadensis* G6/7. In sheep, only one sterile liver cyst of *E. granulosus* s.s. was found, while in camels seven sterile or caseated/calcified cysts of *E. granulosus* s.s. and *E. canadensis* G6/7 were found in liver and lungs (Table 1).

4. Discussion

Cystic echinococcosis (CE) is a neglected zoonotic disease, warranting increased attention particularly in resource-poor countries. Reliable estimates of infection levels in humans and livestock as well as correct species diagnosis of the causative parasites is mandatory to recognize transmission patterns in any given area (Romig et al., 2011; Ziaei et al., 2011), and to be able to respond effectively using appropriate control methods (Craig et al., 2017) and limit the transmission to humans (Ito et al., 2017; Otero-Abad and Torgerson, 2013).

Prevalence levels found in our survey were lower compared to most studies in other parts of Ethiopia (Deplazes et al., 2017). This emphasizes the fact that the situation of CE in Ethiopian livestock varies considerably in the different regions of the country. In previous studies, prevalence figures ranged from 6 to 60% in cattle, from 7 to 68% in sheep, from 0 to 65% in goats and from 14 to 24% in camels (Debela et al., 2015; Fromsa and Jobre, 2011; Getaw et al., 2010; Koskei, 1998;

Fig. 1. Map of Ethiopia indicating the abattoir locations (dots) and the approximate catchment area of slaughtered livestock (circle) (relief map: CIA, University of Texas Libraries, Perry-Castanedas – public domain).

Sissay et al., 2007). One possible reason for this variation is the geographic origin of the examined animals in the various studies. It is known that environmental factors such as humidity and temperature influence the viability of eggs and therefore the infection pressure on the intermediate host (Veit et al., 1995). Fromsa and Jobre (2011) have demonstrated a correlation between altitude and CE prevalence in Ethiopia. There is a significant decline in the prevalence of CE in livestock from the more humid highland toward the more arid lowland zones of Ethiopia. Further factors could act on transmission, such as the level of community awareness about the disease, education and economic status of the population and the farming community. It is still common practice in some areas to slaughter sheep and goats at home and leave the offal accessible to dogs. Moreover, also abattoirs may discard their offal on dumpsites where carnivores have free access (YT, personal observations). Such practices enhance the transmission of the parasite in these areas and increase the risk for human infection.

The majority of cysts (94%) found in our study belonged to *Echinococcus granulosus* s.s. and were found in cattle, camel and sheep. Further identified species were *E. canadensis* G6/7 in cattle and camel and *E. ortleppi* in cattle. These findings correlate with the few studies from Ethiopia where *Echinococcus* species were determined, however, none of them giving information about the prevalence. *Echinococcus granulosus* s.s. was previously recorded from sheep, cattle, camel, goat and pig, *E. canadensis* G6/7 from cattle, camel and goat and *E. ortleppi* from cattle and pig. The presence of *E. granulosus* s.s., *E. ortleppi* and *E. canadensis* G6/7 was confirmed in Central and North Ethiopia, whereas in South and East Ethiopia only *E. granulosus* s.s. and *E. canadensis* G6/7 had been detected before (Hailemariam et al., 2012; Maillard et al., 2006; Romig et al., 2011; Tigre et al., 2016). In the present study, *E. ortleppi* was found for the first time in the East of Ethiopia, suggesting that the distribution of *Echinococcus* spp. across the country is rather uniform. *Echinococcus granulosus* s.s. appears to be the most common

Table 1
Occurrence of CE infection in animal slaughtered in the study areas.

Host species	Animals examined n	Positive animals n (%)	Cyst status, location and molecular identification of *Echinococcus* spp.						
			Cyst status and location				*E. granulosus* s.s.	*E. canadensis* G6/7	*E. ortleppi*
			Status	n	Location	n			
Cattle	891	75 (8.4%)	Fertile	14	Lung	14	5	3	6
			Sterile	71	Liver	13	13		
					Lung	53	53		
					Spleen	5	5		
			cas./calc.	82	Liver	50	50		
					Lung	32	32		
			Total	167			158	3	6
Sheep	95	1 (1.1%)	Sterile	1	Liver	1	1		
			Total	1			1		
Goats	95	0 (0.0%)	–	–	–	–			
			Total	0					
Camels	25	3 (12.0%)	Sterile	5	Liver	1	1		
					Lung	4	4		
			cas./calc.	2	Liver	2	1	1	
			Total	7			6	1	

Echinococcus species across Ethiopia. Worldwide, the transmission of this parasite is mainly based on sheep as intermediate hosts, where it reaches high fertility rates (Romig et al., 2017). The low prevalence and lack of fertile *E. granulosus* s.s. cysts in sheep in our survey was therefore surprising, possibly an artefact due to low numbers of examined sheep. Cattle are known to be frequently infected with this species, but reported fertility rates are generally very low, which means that cattle play a limited role in transmission (Romig et al., 2017). Our result (3% fertility) is therefore in agreement with most studies showing similarly low fertility rates as reported for neighbouring Kenya, where only 6.9% of the bovine hydatid cysts were found fertile (Addy et al., 2012). However, the fertility rate of this parasite in cattle vary considerably, possibly due to local variants of parasite and cattle breed. In Ethiopia, Hailemariam et al. (2012) found no fertile cysts in cattle, while Tigre et al. (2016) detected protoscoleces in 31% of the samples from bovines. Studies on the transmission cycle of *E. granulosus* s.s. are of particular importance, as this species is known to be the principal cause of human CE. A worldwide analysis of 1661 human cases showed that *E. granulosus* s.s. was responsible for 88.44% of all infection (Alvarez Rojas et al., 2014).

E. ortleppi and *E. canadensis* G6/7 are only reported for the third time Ethiopia, which reflects the unsatisfactory research coverage of this aspect in the country. The number of cysts found positive for *E. ortleppi* (6) and *E. canadensis* G6/7 (3) was very low, but in contrast to *E. granulosus* s.s., fertility in cattle was 100% in both *Echinococcus* species. Therefore, and despite the very different prevalence of the three *Echinococcus* species, cattle are an almost equally important infection source to dogs for each of the three parasites. Particular attention may be warranted concerning the disposal of cattle lungs, as all fertile cysts found in this study were from that organ. The role of camels and goats remains unclear in our study area. Both species are known to support the lifecycle of *E. canadensis* G6/7 (the 'camel strain'), in neighbouring regions of Kenya and Sudan (Omer et al., 2010; Addy et al., 2012; Deplazes et al., 2017). In particular, the extremely high prevalence of *E. canadensis* G6/7 in camels reported from elsewhere could not be confirmed in the present study, where only one non-viable cyst occurred in a camel liver. Whether or not all three species of parasites are more adapted to cattle as intermediate hosts than in other regions, due to the importance of this animal as a source of meat in the area, remains open for investigation. Transmission cycles need further characterization by including additional data like total number of livestock animals of the different species, animal age at slaughter, frequency of *Echinococcus* cysts and their availability to dogs. This should be supplemented by studies on the impact of CE on human health in Ethiopia. Such data are scarce: an ultrasound prevalence of 2.9% was found among the Nilotic agro-pastoralists in southwestern Ethiopia, and a low annual mean incidence rate of 0.18 cases per 100,000 population was estimated for central Ethiopia (2008–2012) (Assefa et al., 2015; Macpherson et al., 1989).

Beside further surveys, the meat inspection and offal disposal in the abattoirs should be standardized so that carnivores have no longer access to contaminated offal. Most importantly, control and educational programs should be implemented to prevent disease transmission and improve the public awareness for this pathogen.

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