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SHORT COMMUNICATION

First detection of *Echinococcus granulosus* sensu stricto *(G1)* in dogs in central Sudan

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Abstract

Eighty-four stray dogs shot as a part of a governmental rabies control program in two neighboring towns of central Sudan were examined for the presence of *Echinococcus* spp. and other intestinal helminths. *Echinococcus* worms were identified to species level by PCR and gene sequencing. For comparative reasons, rectal content of the necropsied dogs was examined for helminth eggs and subjected to copro-PCR for *Echinococcus*. At necropsy, 51.2% (43/84) of the dogs harbored *Echinococcus canadensis* (G6/7) worms with worm burdens ranging from 22,000 to 80,000. *Dipylidiun caninum* was found in 53.6% of the dogs. At coproscopy, taeniid eggs were found in 37 of the 43 dogs which were positive for *Echinococcus* at necropsy, but none in the 41 necropsy-negative dogs. In addition, 58% of the rectal samples contained eggs of *Toxocara* spp., 34.5% eggs of *Trichuris* spp. (34.5%), and 26% eggs of *Ancylostoma caninum*. Copro-PCR gave positive results for *E. canadensis* with 97.5% (39/40) of nonhibiting samples from the necropsy positive dogs; the one remaining dog tested positive for *E. granulosus* sensu stricto *(G1)*, whose partial cox1 and nad1 sequences showed a 100% identity with various reference sequences of the G1 genotype. 100% of 38 non-inhibited samples taken from the necropsy-negative dogs were also negative in copro-PCR. This is the first study which combines prevalence and genetic identification of *Echinococcus* spp. in dogs of Sudan. Together with a recent report from cattle, it confirms the autochthonous presence, at low level, of *E. granulosus* sensu stricto in Central Sudan.

Keywords Echinococcosis · *Echinococcus granulosus* · *Echinococcus canadensis* · Dogs · Sudan · Genetic identification

Introduction

Cystic echinococcosis (CE) is a zoonotic disease caused by various species of the cestode genus *Echinococcus*. Adult tapeworms are intestinal parasites of dogs and some other carnivores which acquire the worms by feeding on metacestodes that develop in various species of livestock as intermediate hosts, as well as in some dead-end hosts including humans. These become infected after accidental ingestion of *Echinococcus* eggs from the environment. The epidemiology of CE in sub-Saharan Africa is still incompletely understood (Romig et al. 2017). An exceptionally high incidence of human disease is restricted to parts of northern and eastern Africa, where *E. granulosus* sensu stricto *(s.s.)* is present in dogs and livestock. Human infections are far less frequent or even absent in other regions where other species of *Echinococcus* occur (Deplazes et al. 2017). This is in line with the observation that, worldwide, 88.4% of human CE is caused by *Echinococcus granulosus s.s.* (Alvarez Rojas et al. 2014).

E. granulosus s.s. is present in the southernmost region of South Sudan, but in the Republic of the Sudan, this parasite has only been observed sporadically in human patients where the origin of infection was not completely clear (Omer, unpublished) and, most recently, as a rare parasite of cattle from Khartoum area (Ahmed et al. 2018). In contrast, *E. canadensis*

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(G6/7, the 'camel strain') is extremely frequent in central Sudan, causing high prevalence of CE particularly in camels, but also in other livestock.

Whether or not the human-pathogenic *E. granulosus* is an autochthonous parasite in central Sudan would be important information in order to provide countermeasures against its transmission. Since the data on its life cycle in Sudan are inconclusive, any records of dog infection are urgently needed. We therefore conducted a study in two rural areas of central Sudan, where all epidemiological conditions for transmission of *E. granulosus* are given: sheep as the preferred hosts of *E. granulosus s.s.* are frequent, there are large numbers of dogs (owned, semi-stray or stray), traditional methods of animal husbandry, unsupervised home slaughtering of livestock, and frequent absence of appropriate anthelmintic care which favors the transmission of *Echinococcus*. Nevertheless, human infections seems to occur rather sporadically in such areas except for some high-risk foci (Ahmed et al. 2010; Elmahdi et al. 2004). The present study represents a trial to investigate the prevalence of *Echinococcus* spp. in stray dogs in Tamboul and Rofaa cities in central Sudan using necropsy, microscopic egg detection of rectal samples and genetic identification.

In contrast to livestock, prevalence data from dogs are difficult to obtain using the gold standard detection method (necropsy), as killing of dogs in relevant numbers for the purpose of disease monitoring is ethically not accepted. To explore alternative options using faecal samples, this opportunity was used to obtain data on the sensitivity and specificity of two different copro-diagnostic methods.

Materials and methods

Study area

The study was conducted in and around the neighbouring towns of Tamboul and Rofaa, situated close to the Blue Nile approximately 120 km east of Khartoum in central Sudan, with a population of some 50,000 residents. People practice animal husbandry and seasonal farming. A traditional slaughterhouse is located near the market where a large number of stray and semi-stray dogs are frequently seen feeding on the condemned offal.

Dogs

Eighty-four dogs were shot during May 2004 in the context of a rabies control program by the Epidemics Control Unit of the Ministry of Animal Resources, and permission was granted to examine these dogs for intestinal parasites. The dogs originated from the towns of Tamboul ($n = 66$) and Rofaa ($n = 18$). The intestine of each dog was removed and opened longitudinally after securing its content by making double ligatures at both ends of the intestine. The mucosa was scraped with a scalpel, the content was washed with 0.9% saline through an 80-mesh-per-inch brass sieve and the resulting material examined for the presence of *Echinococcus* worms. The number of worms recovered from each individual animal was estimated using worm counts of aliquots. Other intestinal cestodes were also reported. Samples of rectal content were obtained from all dogs.

Preservation of the faecal samples and harvested worms

Fifteen *Echinococcus* worms from each individual dog were preserved in 70% ethanol for molecular characterization. Samples of rectal content were kept refrigerated for 6 weeks, then placed at − 80 °C for at least 5 days (to inactivate eggs) and subsequently stored at − 20 °C until use.

Coproscopic examination

Rectal samples were analyzed as described before (Mathis et al. 1996). Briefly, 2–3 g of the sample were diluted 1:4 with PBS Tween 20 and centrifuged. The sediment was suspended with ZnCl2 (1.45 g/ml) and centrifuged. The supernatant was then passed through a filter of 31-μm mesh size in a glass container and passed again through a filter of 20-μm mesh size. The solution was then examined microscopically at a magnification power of 100–200×.

DNA extraction

DNA was extracted from suspensions of 15 worms collected from each individual dog as described before using proteinase K digestion and phenol-chloroform-extraction (Dinkel et al. 1998). The DNA concentration was measured photometrically. 200 ng DNA of each sample was used for PCR. DNA was extracted from faecal samples as described earlier using alkaline hydrolysis and phenol-chloroform-extraction (Dinkel et al. 1998).

Polymerase chain reactions

Characterization of genotypes and species of *Echinococcus* was done using a previously described nested PCR system and published primers. As a first step, the primer pairs P60 and P375 were used (Dinkel et al. 1998). This was followed for all samples by G5/6/7 PCR as described before (Dinkel et al. 2004): all G5/6/7 positive samples underwent semi-nested PCRs specific for G6/7 and for *E. ortleppi*. All G5/6/7 negative samples were tested with a G1 PCR. To control for PCR inhibition, 10 μl of *E. canadensis* G6 DNA was added to each negative sample and the P60/P375 PCR was repeated. If

no signal was obtained, the result was considered inconclusive.

Mitochondrial gene sequencing

A total of 12 samples was sequenced to confirm the species identification. Sequencing was done for the partial mitochondrial cox1 gene with primer pair 2575 and 3021 (Bowles et al. 1992) and nad1 gene using primer pair JB11 and JB12 (Bowles and McManus 1993). PCR products were purified over QIAquickTM columns and cycle sequencing was done as described in Dinkel et al. (2004) on the Gene Amp 2400 (Perkin Elmer) using the ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) for 25 cycles (denaturation for 10 s at 94 °C and annealing for 4 min at 60 °C). Electrophoresis was performed on the ABI Prism 310 Genetic Analyzer (Applied Biosystems) and nucleotide sequence analysis was done using the BLAST programs and databases of the National Centre for Biotechnology Information. Reference sequences (KY766891.1 KY766890.1 KY766889.1, KY766888.1, KX039965.1, KX039962.1, KX039960.1, KU925433.1, KU925428.1 and KU925390.1) of the cox1 genes of *E. granulosus s.s.* used in this study were obtained from GenBank® (https://www.ncbi.nlm.nih.gov/genbank/) using the blast algorithm (https://blast.ncbi.nlm.nih.gov). DNA sequence alignments were performed by MUSCLE v.3.8.31 on the European Bioinformatics Institute (EBI) homepage (http://www.ebi.ac.uk/Tools/msa/muscle). All positions containing gaps and missing data were eliminated.

Results

Necropsy

A total of 51.2% (43/84) of the necropsied dogs contained *Echinococcus* spp. with little difference between the two study sites: 53.0% (35/66) in Tamboul and 44.4% (8/18) in Rofaa). Worm burden ranged from 22,000 to 80,000. As the only other cestode, *Dipylidium caninum* was found in 53.6% (45/84) of the examined dogs. Fifteen dogs had both *Echinococcus* and *Dipylidium* worms.

Microscopic examination of the rectal samples

Compared to necropsy results, in 86% (37/43) of the *Echinococcus*-positive dogs and 0.0% (0/41) of the *Echinococcus*-negative dogs, taeniid eggs were found in the rectal samples. Eggs of other internal parasites could also be seen, namely *Toxocara spp*. (58%), *Trichuris spp.* (34.5%) and *Ancylostoma caninum* (26%).

Genetic characterization

All collected worm suspensions gave PCR signals for *E. canadensis* (G6/7). Concerning copro-PCR, in Tamboul, 33 of the 35 dogs which harboured *Echinococcus* worms at necropsy were found positive for *E. canadensis* (G6/7), the two remaining samples gave inconclusive result due to PCR inhibition. In Rofaa, six of the eight necropsy-positive dogs tested positive for *E. canadensis*, one sample was inhibiting, and the remaining dog sample gave a PCR signal for *E. granulosus s.s.* (G1). Of the 41 samples obtained from necropsy-negative dogs, 38 were also PCR negative, PCR of the remaining three was inhibited (Table 1). Sequencing of the partial cox1 and nad1 genes of 11 samples that were PCR positive for *E. canadensis* showed 100% identity with the camel strain G6 when compared with the data on GenBank® (Accession No. AB271912). The single sample that was determined as genotype G1 of *E. granulosus s.s.* showed 100% identity with reference sequences of *E. granulosus s.s.* (G1) from GenBank® sequences. This sequence was deposited under the Accession No. HQ 012553.

Discussion

Cystic echinococcosis is highly prevalent in all species of livestock of Sudan and neighbouring countries (Deplazes et al. 2017). Prevalence is particularly high in camels, where reported prevalence in Sudan ranges from 30% in the Blue

Table 1 Necropsy and copro-PCR results for *Echinococcus* spp. in two study areas

Area	*Echinococcus* positive at necropsy ($n = 43$)			*Echinococcus* negative at necropsy ($n = 41$)			Species of *Echinococcus* (copro-PCR)
	PCR +	PCR −	inhibited	PCR +	PCR −	inhibited	
Tamboul	33	0	2	0	31	0	*E. canadensis* G6 $n = 33$
Rofaa	7	0	1	0	7	3	*E. canadensis* G6 (n = 6) *E. granulosus* s. s. (G1) ($n = 1$)
Total	40	0	3	0	38	3	

Nile region (Kamal et al. 2011) to 61% in western Sudan (Omer et al. 2010). This high prevalence in camels is likely to be a function of old age at slaughter and the predominant presence of *E. canadensis* (the 'camel strain') as CE agent in the country, which is well adapted to camels as intermediate hosts. The predominating presence of *E. canadensis* G6/7 in Sudan was confirmed in our study. *E. canadensis* G6/7 is also well known as an agent of human CE, although the proportional contribution of this species to human CE, at a global scale, is far smaller (11%) compared with *E. granulosus* (88%) (Alvarez Rojas et al. 2014). Typically, countries with a predominance of *E. canadensis* in livestock show comparatively few human CE cases, while global foci of human CE (e.g. in East Africa of central Asia) are associated with the frequent presence of *E. granulosus s.s.* (Deplazes et al. 2017). The situation of human CE in Sudan is data deficient, but the number of cases appears to be moderate despite the identification of hot spots (Elmahdi et al. 2004; Ahmed et al. 2010). In this context it is of importance that *E. granulosus s.s.* seems to be of only sporadic presence in central Sudan, e.g. in cattle (Ahmed et al. 2018) and human patients (Omer, unpublished).

Given the fact that *E. granulosus s.s.* is highly prevalent in neighbouring South Sudan and Ethiopia (Deplazes et al. 2017), possible translocation or emergence of this human-pathogenic species in Sudan is a public health concern. Any data that help to elucidate the reasons for absence or presence of this parasite are therefore highly warranted, and our finding of an infected dog provides baseline information for further research in this region and possible adaptations of control strategies. The study area is located in central Sudan and serves as the largest market for camel meat in the country. Villagers own dogs for the purpose of guarding, but often these dogs are semi-stray and feed on the offal from slaughter slabs. Also, truely stray dogs are obviously increasing in that area.

In the current study, we compared two approaches of copro-diagnosis with necropsy results, both giving good correlations to necropsy as a gold standard. Detection of parasite eggs in the faeces showed satisfactory 86% sensitivity and 100% specificity. This was, however, aided by the absence of any *Taenia* worms in our dogs, whose eggs cannot be distinguished morphologically from those of *Echinococcus* spp.. Nevertheless, the high sensitivity of the egg detection method opens the possibility to combine this with genetic identification of individual eggs (Hüttner et al. 2009) as an alternative diagnostic method for living dogs. The second copro-diagnostic approach was copro-PCR, which gave 100% sensitivity (40/40) and 100% specificity (38/38) for *Echinococcus* with the noninhibiting samples (6/84 samples showed PCR inhibition and thus gave inconclusive results). This excellent correlation was probably aided by the fact that rectal content was used rather than faecal samples from the environment, where DNA degrading factors would act on the material. Concerning species identification, PCR results from necropsy (worms) and copro-PCR agreed in all but one case. In that dog, worm PCR detected *E. canadensis*, whereas coproPCR was negative for *E. canadensis* and positive for *E. granulosus s.s.*. We explain this discrepancy by the presence of a mixed infection, where *E. granulosus* worms were missed to be included in the PCR samples, while the *E. canadensis* worms may not have shed eggs into the rectal content.

Apart from *Echinococcus*, our study showed the presence of other zoonotic helminths in stray dogs of central Sudan at high prevalence. This is true for *Dipylidium*, *Toxocara* and *Ancylostoma*, which may have considerable health impact particularly in children. With respect also to other zoonotic agents known to be present in Sudanese dogs (e.g. leishmaniasis—Dereure et al. 2003), it is recommended that a combination of dog population control and public education be implemented.

Conclusion

The study has confirmed a high prevalence of *Echinococcus canadensis* G6/7 in Sudan and, for the first time, the occurrence of *Echinococcus granulosus* sensu stricto in Sudanese dogs. Other parasites of zoonotic nature were also frequent. This highlights the need for improved control of zoonotic diseases and calls for attention to stray and semi-stray dogs as sources of zoonoses.

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