Prevalence and diversity of cystic echinococcosis in livestock in Maasailand, Kenya

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Abstract Cystic echinococcosis (CE) is a zoonotic disease caused by several members of the *Echinococcus granulosus* species complex. In East Africa, several species/strains are known to occur in livestock and humans, but host preferences, relative frequencies and spatial distribution of these taxa are poorly known. Here, we contribute livestock data for Maasailand of southern Kenya. Total CE prevalence was 25.8 % in cattle (151/587), 16.5 % in sheep (71/430) and 10.8 % in goats (21/194), which is a significant increase compared to surveys done about three decades ago. The majority of cysts occurred in the liver (56 % in cattle, 70 % in sheep and 65 % in goats). Molecular characterization by PCR–RFLP and sequencing of parts of the mitochondrial *nad*-1 gene was done for a subsample of 285 cysts. *E. granulosus* G1 was dominant in all host species (200 of 201 cysts from cattle, 68 of 69 from sheep and 11 of 15 from goats); the remaining taxa were *Echinococcus canadensis* G6 (one cyst from sheep, four from goats) and *Echinococcus ortleppi* (one cyst from cattle). Considering cyst fertility, sheep appear to be the most important hosts for *E. granulosus* G1, while goats were found to be suitable hosts for *E. canadensis* G6 (three of four cysts were fertile). For the first time, *E. ortleppi* was found in cattle from southern Kenya. Our data show an intense and possibly increasing level of CE transmission in southern Kenya, and the predominance of *E. granulosus* G1, which appears to be particularly pathogenic to humans, calls for urgent control measures.

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Introduction

Cystic echinococcosis (CE) is a zoonotic disease caused by the larval stage of *Echinococcus granulosus* sensu lato. The various species in this cluster were previously considered as strains (Nakao et al. 2007). They use canids and/or felids as definitive hosts and a wide range of ungulates as intermediate hosts, where cysts develop in the liver, lungs and other organs. The disease occurs worldwide and has a particular economic and medical impact on rural pastoral societies (Eckert et al. 2001). It is widespread in Africa, posing a public health concern in most countries with extensive livestock economy (Wachira et al. 1993; Dinkel et al. 2004; Magambo et al. 2006; Maillard et al. 2007, 2009; Hüttner et al. 2008, 2009; Omer et al. 2010; Romig et al. 2011). With exception of western and central Africa, from where only sporadic cases are known, CE is endemic among humans and livestock populations in all countries of the continent (Magambo et al. 2006; Romig et al. 2011). According to the currently available information on species, strains and genotypes of CE agents, Africa is arguably the continent with the highest diversity of these parasites (Romig et al. 2011).

To date, five species of *Echinococcus granulosus* s. l. have been identified in Africa: *E. granulosus* sensu stricto

(common sheep strain G1), *Echinococcus equinus*, *Echinococcus ortleppi*, *Echinococcus canadensis* (camel/pig strain G6/G7) and *Echinococcus felidis* (Wachira et al. 1993; Thompson and McManus 2001; Hüttner et al. 2008; Maillard et al. 2009; Casulli et al. 2010; Omer et al. 2010). In East Africa, the parasite presents a complex pattern of infectivity and prevalence, with several species or strains occurring sympatrically in different or the same livestock species. Based on the limited number of studies so far, there seem to be considerable differences in species/strain composition among different regions (Romig et al. 2011). This scenario depicts a complex epidemiology of the disease which is not fully understood. Regions populated by pastoral communities (e.g. Maasai and Turkana) in Kenya and others in some neighbouring countries seem to be transmission foci of these parasites.

The main life cycles involve domestic dogs and various livestock species (cattle, camels, sheep and goats) (Wachira et al. 1993; Dinkel et al. 2004). *Echinococcus* taxa from wildlife may also contribute to the infection of livestock and humans (Hüttner et al. 2009). Surveys for CE done in Maasailand about 30 years ago established that the region was a hyperendemic focus with high prevalence levels in livestock and frequent occurrence of human cases (Macpherson 1985; Macpherson et al. 1989). In the meantime, several cyst isolates from southern Kenya have been genotyped (Wachira et al. 1993; Dinkel et al. 2004). However, the relative contribution of the identified species to the total CE burden is not known because the collection of samples had not been done in the context of systematic surveys. Here, we provide an update on the CE prevalence in all livestock species of economic relevance in southern Kenya and quantify the relative impact of different *Echinococcus* species on their hosts. In addition, we attempt to estimate the relative importance of different livestock species for the transmission of *Echinococcus* spp. in Maasailand.

Materials and methods

Study area

The current study was done in three abattoirs located in Kitengela town (approximately 30 km south of Nairobi) and Suswa (approximately 50 km west of Nairobi), which are key slaughter facilities for Maasai livestock, supplying the meat markets of Nairobi and environs. Maasailand occupies the southern part of the East African Rift valley in southern Kenya and northern Tanzania. The communities known as Maasai continue to practise traditional semi-nomadic pastoralism. People live in semi-permanent settlements of several families pasturing their stock together, 10–20 huts surrounded by a thorn fence into which the livestock is driven at night. The landscape is dominated by grazing

land and large-scale cereal production; some sections of Maasailand have been converted into National Parks and game reserves. The area receives low to moderate rainfall (average 500–1,800 mm/a) and is known for recurrent years of drought when a considerable number of livestock perishes (Evangelou 1984 and Kaplan et al. 1976).

Isolation of cysts

During the month of October 2011, a total of 1,211 carcasses of cattle (587), sheep (430) and goats (194) were inspected for cysts in all organs of the pleural and abdominal cavities. The age of each slaughtered animal was estimated. Eight hundred twenty-nine cysts were obtained. Cyst contents were microscopically inspected for the presence of protoscolices. Cysts with viable protoscolices were considered fertile; cysts without protoscolices and calcified cysts were considered non-fertile. Protoscolices and pieces of cyst wall (germinal layer) intended for molecular characterization were fixed and stored in 70 % ethanol. Characterised isolates of *E. granulosus* G1, *E. ortleppi* and *E. canadensis* G6 from Kenya, Vietnam and Sudan, respectively, were used as positive controls during species differentiation.

DNA extraction

DNA was extracted from protoscolices or tissue pieces by lysing in 0.02 M NaOH at 95 °C for 10 min as previously described by Nakao et al. (2003). In a few instances where the above process failed to yield adequate DNA, genomic DNA was extracted as described elsewhere (Dinkel et al. 2004). About 0.5-g cyst wall (germinal layer) was cut into small pieces and digested in the presence of 2 mg/ml proteinase K in 500 μl of 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.

Polymerase chain reaction

A nested PCR assay was conducted to amplify the NADH dehydrogenase subunit 1 (*nad*-1) gene using the following primer pair: *for.*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: *for.*CAG TTC GGT GTG CTT TTG GGT CTG/*rev.*GAG TAC GAT TAG TCT CAC ACA GCA, for the nested reaction (Hüttner et al. 2008). In both reactions, a 50-μl reaction mixture was made up of DNase/RNase-free water, 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 2 mM MgCl$_2$, 200 μM of each dNTPs, 12.5 pmol of each primer and 1.25 U Taq polymerase.

Amplification conditions were as follows: start denaturation 95 °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 final elongation 72 °C for 5 min post cycling. Amplification results were detected on 1.5 % (w/v) agarose gel stained with Gel Red® (Biotium, Inc.).

Restriction fragment length polymorphism of nad-1

The nad-1 amplicons were digested as described previously (Hüttner et al. 2009) with the restriction enzyme Hph I (Fermentas GmbH, Germany). A total reaction mixture of 30.5 μl constituted of 15 μl PCR amplicons, 2 μl buffer B (supplied with enzyme), DNase/RNase-free water and 0.5 μl enzyme. Reaction mixture was incubated at 37 °C for 3 h, followed by deactivation of the enzyme at 65 °C for 20 min. Banding patterns were detected on 3 % (w/v) agarose stained with Gel Red® (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 analysed 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).

Results

Prevalence and distribution

Results of the prevalence survey are shown in Table 1. The highest prevalence of CE was found in cattle (25.8 %) followed by sheep (16.5 %) and goats (10.8 %). The average number of cysts per surveyed animal was 1.0, 0.4 and 0.3 for cattle, sheep and goats, respectively. The number of cysts varied widely from one to over 16 cysts per infected animal. About half of the infected livestock harboured more than one cyst (Table 2). In some extreme cases, one sheep and goat each harboured 34 and 19 cysts, respectively. Prevalence was correlated with age in cattle (Fig. 1a), and infection intensity showed a trend towards an increase with age in cattle and sheep (Fig. 1b).

Table 2 Load of *Echinococcus* spp. in infected livestock

Infected livestock species	Number of cysts				
	1	2–5	6–10	11–15	16 or more
Cattle (n=151)	75	47	21	5	3
Sheep (n=71)	34	28	5	2	2
Goat (n=21)	10	10	0	0	1

Despite the high prevalence and infection intensity of *Echinococcus* spp. among cattle, cyst fertility in cattle was low (6.5 %) compared to sheep (25.6 %) and goats (17.6 %) (Table 3). The liver was found to be the most frequently involved organ (Table 3), but liver cysts were less frequently fertile compared to lung cysts in all species: in cattle (2.9 against 11.3 %), in sheep (22.6 against 34.8 %) and in goats (0 against 50 %). Multiple organ involvement was common in all three livestock species (Tables 1 and 3). Other organs aside the liver and lungs were the heart, kidney and spleen, but these organs only harboured non-fertile cysts in our survey.

Genetic characterisation

A total of 285 cyst isolates from cattle (201), sheep (69) and goats (15) recovered during the survey were characterised to the species/genotype level by PCR/RFLP and confirmatory partial mtDNA sequencing of the selected samples (Table 3). Three species of *Echinococcus* were identified: *E. granulosus* G1, *E. ortleppi* and *E. canadensis* G6. *E. granulosus* G1 was, by far, the dominant species in our survey (279 of 285 isolates) which often reached fertility in sheep (approx. 25 %) and goats (45.5 %) but rarely in cattle (approx. 6 %) (Table 3). In addition, one fertile lung cyst from cattle belonged to *E. ortleppi*, and the lungs of one sheep and four goats were infected with cysts of *E. canadensis* G6; three of the four goat cysts were fertile.

Discussion

Our results demonstrate the persistence of cystic echinococcosis in Maasailand, Kenya. The prevalence levels of

Table 1 Prevalence of cystic echinococcosis and cyst location in livestock

Livestock	Prevalence (95 % CI)	Liver (n)	Lungs (n)	Heart (n)	Li/Lu (n)	Li/Sp (n)	Li/Lu/Ki (n)	Li/Lu/Ki/Sp (n)
Cattle (n=587)	25.8 (12.3–29.5)	59	43	2	45	1	1	0
Sheep (n=430)	16.5 (13.2–20.4)	42	14	1	13	0	0	1
Goat (n=194)	10.8 (6.8–16.1)	9	10	0	2	0	0	0

Prevalence p<0.001

CI confidence interval, Li liver, Lu lungs, Ki kidney, Sp spleen, n number of animals

Fig. 1 Prevalence of cystic echinococcosis (**a**) and mean infection intensity of *Echinococcus* species (**b**) in cattle and sheep at different ages. *Error bar* with 5 % probability; *n* at age 2: 76 cattle, 204 sheep; at age 3: 157 cattle, 207 sheep; at age 4: 240 cattle, 19 sheep; at age 5:90 cattle; and at age 6: 24 cattle. No sheep were recorded at ages 5 and 6. Goats were not included due to the smaller number of infected animals (21/194)
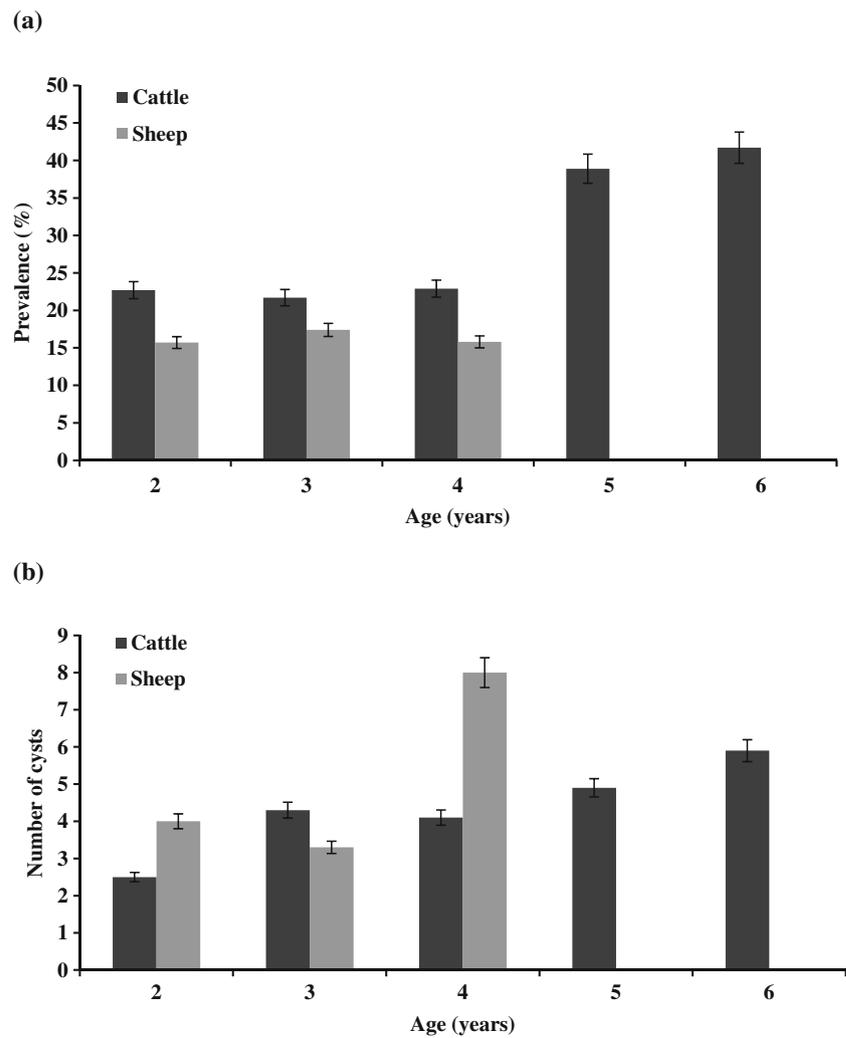
Echinococcus spp. among livestock in the present study are significantly higher than those reported from the last comprehensive survey done in Maasailand three decades ago, at least in cattle and sheep (25.8 vs. 8.9 % and 16.5 vs. 8.1 %, respectively) (Macpherson 1985). They are also higher than those reported from other parts of Kenya (Njoroge et al. 2002) and neighbouring Sudan (Omer et al. 2010; Ibrahim et al. 2011), which confirm this region in southern Kenya as one of the hot spots of CE in Africa. Whether our data reflect a true increase of prevalence is difficult to conclude, as various factors which could influence the outcome of surveys (pre-selection of livestock to be slaughtered, requirements of abattoirs) may have changed between the two survey periods. Importantly, no figures on animal age were given in

Table 3 Conditions and species/genotypes of *Echinococcus* cyst isolates

Livestock species	Cyst condition	Liver		Lungs		Heart		Kidney		Spleen	
		n	*E.* spp.	*n*	*E.* spp.	*n*	*E.* spp.	*n*	*E.* spp.	*n*	*E.* spp.
Cattle	Fertile (*n*=40)	10	8 G1	30	14 G1/1 G5	0	0	0	0	0	0
	Non-fertile (*n*=574)	335	78 G1	235	98 G1	2	1 G1	1	1 G1	1	0
Sheep	Fertile (*n*=42)	26	14 G1	16	10 G1	0	0	0	0	0	0
	Non-fertile (*n*=122)	89	22 G1	30	20 G1/1 G6	1	1 G1	1	0	1	1 G1
Goat	Fertile (*n*=9)	0	0	9	5 G1/3 G6	0	0	0	0	0	0
	Non-fertile (*n*=42)	33	1 G1	9	5 G1/1 G6	0	0	0	0	0	0

G1 E. granulosus G1; *G5 E. ortleppi*; *G6 E. canadensis* G6

the previous survey, and prevalence is known to be strongly correlated with the age of the host animal. Both surveys agree on the fact that cattle, although showing the highest prevalence, play a minor role in transmission due to the low cyst fertility in this host. Moreover, cattle are clearly over-represented in our survey as they are sold to abattoirs more often than small stock which is usually slaughtered at home.

When considering the high fertility rate of cysts in sheep, it becomes clear that this species, together with goats, possibly is the most important intermediate host that maintains transmission of the disease in the Maasai population. Sheep slaughtered at home without inspection will easily pass on the parasites to dogs and consequently perpetuate the disease in the population (Macpherson 1985). High infection intensity in older animals may be caused by a continuous acquisition of new infections over time or may reflect the time needed for small, inconspicuous cysts to reach a detectable size. In either case, older animals, e.g. breeding stock, are the most affected animals in a population. This is important especially in the case of sheep and goats because such animals have low market value and are more likely to be slaughtered at home without supervision.

Echinococcus spp. indeed has wide organ infection range (Varcasia et al. 2006, 2007; Berhe 2009; Omer et al. 2010; Abdul et al. 2010) which has implications for meat inspection practises. Also, multiple organ infection translates into greater economic loss due to condemnation of affected organs as well as reduction in market value of entire carcasses.

The identification of *E. granulosus* G1, *E. ortleppi* and *E. canadensis* G6 is in support of earlier accounts of these species/genotypes isolated from African livestock (Wachira et al. 1993; Dinkel et al. 2004; Maillard et al. 2009; Omer et al. 2010; Ibrahim et al. 2011). However, the finding of G1 as the dominant taxon among cattle, sheep and goats (Wachira et al. 1993; Dinkel et al. 2004) is in contrast to the situation in neighbouring Sudan where the camel strain of *E. canadensis* is the dominant taxon (Dinkel et al. 2004; Omer et al. 2010; Ibrahim et al. 2011). A frequent presence of G1 was also reported from the North and Northeast of Africa (Maillard et al. 2007). Considering the predominance of *E. granulosus* G1 in the survey area, the above-discussed characteristics of CE in Maasailand (prevalence, fertility in hosts, organ involvement) are representative for this taxon. The dominance of G1 could also explain the high prevalence of human CE among the Maasai people, as this taxon has been shown to be the most pathogenic form of CE in humans (Wachira et al. 1993; Dinkel et al. 2004).

The account of *E. canadensis* G6 (camel strain) in sheep and goat in Maasailand where camels are mostly absent supports the previous findings (Wachira et al. 1993), where G6 was isolated from a cow and goat in Maasailand. In recent studies, the taxon was found in various livestock species including sheep and goats (Dinkel et al. 2004). The high

fertility rate of G6 cysts in goats (three fertile cysts out of four) shows that this parasite is well adapted to goats which seem to be able to maintain the lifecycle in places where camels are absent. This is in agreement with the suitability of goats as hosts of the closely related 'pig strain' *E. canadensis* G7, as was shown in southern Europe (Varcasia et al. 2007).

Prior to our finding of *E. ortleppi* in its typical host, cattle, this taxon in Kenya had only been reported from pigs in the South (Dinkel et al. 2004) and from cattle in the north-central part of the country (Mulinge et al. 2011). The data available so far suggest that *E. ortleppi* is widespread but rare in eastern Africa, as in most other parts of the world. This appears difficult to explain, given the ubiquity of cattle in the area. However, cattle (in contrast to sheep) are rarely slaughtered at home, so local dogs rarely have access to cysts from cattle and may therefore not be able to pass the parasite to the local cattle population.

None of the examined isolates belonged to *E. felidis*, which was recently recorded from lions and warthogs in the Queen Elizabeth National Park, Uganda (Hüttner et al. 2009). No information on its range of intermediate hosts is available, and it may be present in livestock from Maasailand where animals graze around or in the vicinity of game reserves such as Maasai Mara or Amboseli.

In a pending further investigation of the diversity of CE in Maasailand, the disease was confirmed to be highly endemic with sympatric taxa coexisting in the same livestock population. Further evaluation of the current situation, especially in dogs, humans and wildlife will improve our understanding on the epidemiology of the disease in Maasailand and provide the background for the design of cost-efficient control strategies.

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