Molecular Characterization of Sudanese strains of Echinococcus granulosus

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A molecular survey of cystic echinococcosis in Sudan

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1. Introduction

Cystic echinococcosis (CE) is a zoonotic disease affecting mainly various species of livestock and humans. It is caused by metacestodes of dog tapeworms of the \textit{Echinococcus granulosus} complex. The metacestodes usually form fluid-filled cysts ('hydatids') located in liver, lungs and other organs. CE is distributed worldwide, acquiring public health or economic significance in areas where extensive livestock production provides suitable conditions for the cyclic transmission between dogs and livestock animals. CE is considered an emerging disease in many parts of the world, in some regions re-emerging after initially successful control (Eckert et al., 2000; Jenkins et al., 2005). The global burden of CE is estimated at >1,000,000 DALYs (disability adjusted life years) lost, which gives CE a greater impact than onchocercosis, Dengue fever and Chagas disease, and approaches the burden caused by African trypanosomosis and schistosomiasis (Budke et al., 2006).

CE has been reported from the majority of countries in sub-Saharan Africa (Macpherson and Wachira, 1997). However, as it is typically a disease affecting pastoral communities which often live in remote areas, reliable data on prevalence of CE in humans or animals are only known from few regions. CE in livestock seems to be widespread and frequent especially in eastern and southern Africa. In contrast, high-prevalence regions of human CE are focally distributed in Kenya, northern...
Tanzania and southern Sudan, where prevalence levels can approach 6% in nomadic populations (Macpherson et al., 1989). In Sudan, human CE is frequent in the extreme southeast of the country at the border to Kenya, where cystic echinococcosis was found in 2–3.5% of the human population examined by ultrasound techniques (Magambo et al., 1996, 1998). Elsewhere, human disease seems to be sporadic throughout the country. For 2001, 40 cases of CE were reported by the National Health Institute of Sudan (personal communication). In a recent ultrasound survey, one of 300 villagers in central Sudan was identified as a CE patient (Elmahdi et al., 2004). The most extensive recent survey of CE in livestock was confined to central Sudan, where 6.9% of sheep (n = 5595), 3.0% of cattle (n = 2386) and 44.6% of camels (n = 242) were found infected (Elmahdi et al., 2004), confirming older reports on the frequency of CE in this region (Elkhawad et al., 1979a; Saad and Magzoub, 1989a, b). Few data are available from other parts of Sudan, but in an older study infection rates of 25%, 12% and 10% were found in cattle, sheep and goats, respectively, in western Sudan (Elkhawad et al., 1979b).

CE is caused by metacestodes of the *E. granulosus* complex, which is composed of highly diverse taxa in terms of genetic distances, morphology of the adult worms, host preferences and other biological traits. This has recently led to a splitting of ‘*E. granulosus*’ into *E. granulosus* sensu stricto (G1–G3), *Echinococcus ortleppi* (G4), *Echinococcus ovostrati* (G5), *Echinococcus canadensis* (G6–G10), and *Echinococcus felidis* (Thompson and McManus, 2002; Nakao et al., 2007; Hüttner et al., 2008). The internal taxonomy of the cluster G6–G10 is still under study, which may in future lead to a split of G6/7 from *E. canadensis* under a separate name—for discussion on this, see Thompson (2008) and Saarma et al. (2009). All of these taxa are known or suspected to occur in subsaharan Africa including the Sudan. However, as no morphological distinction of the metacestodes is possible, genetic identification of isolates is necessary to study the distribution and impact of these parasites. Several studies have identified *E. granulosus* s.s. (G1 and G2) and *E. canadensis* (G6) in northern Africa (Bardonnet et al., 2002; Tashani et al., 2002; Azab et al., 2004; Bart et al., 2004; Maillard et al., 2007), but from subsaharan Africa only few data are available: *E. granulosus* s.s. (G1) was recorded from Ethiopia (cattle and sheep) and Kenya (human, cattle, camel, sheep, goat and pig), *E. canadensis* (G6) was recorded from Kenya (human, cattle, camel, goat and pig) and Sudan (cattle, camel and sheep), and *E. ortleppi* was recorded from Kenya (pig) and Sudan (cattle) (Wachira et al., 1993; Dinkel et al., 2004; Maillard et al., 2007). All previously genotyped samples from Sudan originated from the central part of the country (Dinkel et al., 2004).

Here we present the results of a recent prevalence survey in various parts of Sudan with emphasis on the western state of Darfur. In contrast to previous surveys, a substantial proportion of the cysts (11.3%) underwent genetic analysis, and the study was supplemented by the genetic characterization of cyst isolates from human patients from various parts of Sudan.

2. Materials and methods

2.1. Survey and cyst collection

2.1.1. Abattoir surveys

Multiple visits were made to slaughterhouses in different regions of Sudan (Khartoum, Tamboul and Wad Medani in central Sudan, Nyala in Darfur State (western Sudan), Juba and Malakal in southern Sudan (Fig. 1) during the period of May 2001 to July 2003. A total number of 21,659 animals (779 camels, 4893 cattle, 10,422 sheep and 5565 goats) were examined. From this survey, 494 cysts samples from camel (207), cattle (107), sheep (115), and goats (65) were preserved in 70% ethanol for genetic characterization (Table 1). Additional 38 cyst samples from camel, cattle and sheep where obtained from slaughterhouses in eastern (Kassala and Gedaref), northern (Halfa, Atbara and Eldamir) and central Sudan (Khartoum). As these samples were not obtained in the course of the prevalence survey, the results are treated separately (Fig. 1, Table 2).

2.1.2. Isolates from humans

Cyst isolates were obtained after surgery from five human patients in Khartoum (3) and Juba (2) medical teaching hospitals. The patients operated in Khartoum were one female (49 years) and two males (46, 53 years) with lung hydatid cysts. The female patient originated from the Nuba Mountains and had lived in Khartoum for 7 years before she presented with chest problems. One male
2.2 Parasitological examination

Lungs, liver, heart, spleen, kidneys and peritoneal cavity of the surveyed slaughter animals were thoroughly inspected for cysts, and their number and location were recorded. Additional data for cysts and clinical presentation could not be obtained.

2.3 Genetic characterization

In this survey, we used a previously described PCR system (Dinkel et al., 2004) for species discrimination. As this system does not allow to discriminate between subspecific genotypes (G1/G2/G3, or G6/G7), we also sequenced the partial cox1 and nad1 genes of a subset of these samples (Bowles et al., 1992; Bowles and McManus, 1993).

2.3.1 DNA extraction

DNA extraction was done according to Dinkel et al. (1998). Briefly, 500 μl of hydatid fluid containing protoscolices was centrifuged and the resulting pellet was digested with 500 μl digestion buffer containing 10 μl 1 M dithiothreitol and 60 μl proteinase K. DNA was then extracted using phenol–chloroform–isoamylalcohol (25:24:1) and precipitated with absolute ethanol. The DNA concentration was photometrically measured and 200 ng DNA of each sample was used for PCR.

Table 1
Results of the parasitological and molecular survey.

<table>
<thead>
<tr>
<th>Animal species</th>
<th>n</th>
<th>Prevalence in % (Cl. 95%) (no. of infected/no. of examined)</th>
<th>Mean no. cysts per infected animal</th>
<th>Fertility (no. of fertile/no. of examined)</th>
<th>Predilection site of cysts</th>
<th>No. of cysts examined by PCR (no fertile–non-fertile)</th>
<th>Species/genotype (PCR &amp; Seq.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Central Sudan</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Camel</td>
<td>214</td>
<td>55.6 (48.7–62.4) (119/214)</td>
<td>3.0</td>
<td>75% (270/360)</td>
<td>Lungs (304/360)</td>
<td>87 (57–30)</td>
<td>EC (87) G6 (2)</td>
</tr>
<tr>
<td>Cattle</td>
<td>250</td>
<td>20.0 (15.2–25.3) (50/250)</td>
<td>1.3</td>
<td>100% (63/63)</td>
<td>Liver (49/63)</td>
<td>27 (27–0)</td>
<td>EC (27) G6 (2)</td>
</tr>
<tr>
<td>Sheep</td>
<td>400</td>
<td>2.5 (1.2–4.5) (10/400)</td>
<td>1.2</td>
<td>0% (0/12)</td>
<td>Liver (6/12)</td>
<td>12 (0–12)</td>
<td>EC (12) NR (2)</td>
</tr>
<tr>
<td>(b) Western Sudan</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Camel</td>
<td>565</td>
<td>61.4 (57.3–65.4) (347/565)</td>
<td>5.8</td>
<td>74% (1490/198)</td>
<td>Lungs (1323/198)</td>
<td>120 (100–20)</td>
<td>EC (120) G6 (2)</td>
</tr>
<tr>
<td>Cattle</td>
<td>4318</td>
<td>20.0 (15.2–25.3) (226/4318)</td>
<td>1.1</td>
<td>75% (186/247)</td>
<td>Liver (138/247)</td>
<td>60 (30–30)</td>
<td>EC (60) G6 (2)</td>
</tr>
<tr>
<td>Sheep</td>
<td>9727</td>
<td>11.9 (10.9–12.2) (1162/9727)</td>
<td>1.3</td>
<td>0% (0/32)</td>
<td>Liver (52/32)</td>
<td>20 (5–10)</td>
<td>EC (20) NR (1)</td>
</tr>
<tr>
<td>Goats</td>
<td>5552</td>
<td>1.9 (1.5–2.3) (103/5552)</td>
<td>1.0</td>
<td>33% (34/103)</td>
<td>Liver (34/103)</td>
<td>3 (0–3)</td>
<td>EC (3) G6 (1)</td>
</tr>
<tr>
<td>(c) Southern Sudan</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cattle</td>
<td>325</td>
<td>7.1 (4.5–10.4) (23/325)</td>
<td>1.0</td>
<td>35% (8/23)</td>
<td>Liver (19/23)</td>
<td>20 (5–15)</td>
<td>EC (19) G5 (1)</td>
</tr>
<tr>
<td>Sheep</td>
<td>295</td>
<td>2.7 (1.2–5.3) (8/295)</td>
<td>1.0</td>
<td>0% (0/8)</td>
<td>Liver (8/8)</td>
<td>8 (0–8)</td>
<td>EC (8) G6 (1)</td>
</tr>
<tr>
<td>Goat</td>
<td>42</td>
<td>7.1 (1.5–19.5) (3/42)</td>
<td>1.0</td>
<td>0% (0/3)</td>
<td>Liver (3/3)</td>
<td>3 (0–3)</td>
<td>EC (3)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Camel</td>
<td>779</td>
<td>59.9 (466/779)</td>
<td>5.1</td>
<td>74% (1760/2378)</td>
<td>Lungs (1627/2378)</td>
<td>207 (157–50)</td>
<td>EC (207) G6 (4)</td>
</tr>
<tr>
<td>Cattle</td>
<td>4893</td>
<td>6.1 (299/4893)</td>
<td>1.1</td>
<td>77% (256/333)</td>
<td>Liver (206/333)</td>
<td>107 (62–45)</td>
<td>EC (106) G6 (4)</td>
</tr>
<tr>
<td>Sheep</td>
<td>10,422</td>
<td>11.3 (1180/10,422)</td>
<td>1.3</td>
<td>19% (289/1514)</td>
<td>Periton cavity (1242/1514)</td>
<td>115 (55–60)</td>
<td>EC (111) G5 (1)</td>
</tr>
<tr>
<td>Goats</td>
<td>5565</td>
<td>1.9 (106/5565)</td>
<td>1.0</td>
<td>32% (34/106)</td>
<td>Periton cavity (51/106)</td>
<td>65 (10–55)</td>
<td>EC (65) G6 (2)</td>
</tr>
</tbody>
</table>

EC = E. canadensis G6/7, EO = E. ortleppi. NR = No result, PCR: results of species-specific PCR system, Seq.: mt gene sequencing (cox1 and nad1) for determination of the genotype.

In Table 2, identity of additional isolates of Echinococcus spp. from livestock, obtained by opportunistic sampling in various parts of Sudan.

<table>
<thead>
<tr>
<th>Species and origin</th>
<th>n</th>
<th>PCR species/genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Camel</td>
<td>5</td>
<td>E. canadensis G6/7 (5)</td>
</tr>
<tr>
<td>Eastern Sudan</td>
<td>3</td>
<td>E. canadensis G6/7 (3)</td>
</tr>
<tr>
<td>Northern Sudan</td>
<td>3</td>
<td>E. canadensis G6/7 (2)</td>
</tr>
<tr>
<td>Cattle</td>
<td>4</td>
<td>E. ortleppi (1)</td>
</tr>
<tr>
<td>Eastern Sudan</td>
<td>4</td>
<td>E. canadensis G6/7 (4)</td>
</tr>
<tr>
<td>Northern Sudan</td>
<td>23</td>
<td>E. canadensis G6/7 (23)</td>
</tr>
</tbody>
</table>

* Confirmed by sequencing (cox1 and nad1).
2.3.2. Polymerase chain reaction (PCR)

Characterization of genotypes and species of *Echinococcus* was done using a previously described PCR system and published primers (Dinkel et al., 2004). In short, this method includes a PCR assay specific for *E. granulosus* G1 (g1 PCR) and PCR assays specific for G6/7 and *E. ortleppi* (g5/6/7 PCR, g6/7 PCR and g5 PCR). G5/6/7 PCR was performed with all samples. The 50 μl reaction mixture consisted of 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 2 mM of MgCl2, 200 μM of each dNTP, 25 pmol of each primer and 1.25 units of Ampli-Taq Polymerase (Applied Biosystems) and amplification was done for 40 cycles (denaturation for 30 s at 94 °C, annealing for 1 min at 53 °C and elongation for 40 s at 72 °C). All samples which gave positive results with g5/6/7 PCR underwent semi-nested PCRs specific for G6/7 (g6/7 PCR) (Fig. 2) and for *E. ortleppi* (g5 PCR) (Fig. 3) in a second step. Semi-nested PCRs were performed both in a 50 μl volume containing 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 2 mM of MgCl2, 200 μM of each dNTP, 25 pmol of each primer and 1.25 units of Ampli-Taq Polymerase (Applied Biosystems) for 40 cycles (denaturation for 30 s at 94 °C, annealing for 1 min at 60 °C and elongation for 30 s at 72 °C). With all samples which were negative with g5/6/7 PCR the g1 PCR was done. The 50 μl reaction mixture consisted of 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 2 mM of MgCl2, 200 μM of each dNTP, 25 pmol of each primer and 1.25 units of Ampli-Taq Polymerase (Applied Biosystems) and amplification was done for 40 cycles (denaturation for 30 s at 94 °C, annealing for 1 min at 57 °C and elongation for 40 s at 72 °C). 10 μl of the amplification products were detected on a 1.5% ethidium bromide stained agarose gel.

With DNA samples that were negative in both g5/6/7 PCR and g1 PCR (mainly the calcified samples—Table 1), a cestode-specific PCR (Dinkel et al., 2004) was added as a pre-amplification step for increased sensitivity.

2.3.3. Mitochondrial gene sequencing

A total of 20 samples were sequenced to determine the intraspecific genotype. Sequencing was done of 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 QiAquick™ columns and cycle sequencing was done as described by Dinkel et al. (2004) on the Gene Amp 2400 (PerkinElmer) 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). Cycle sequencing 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 Center for Biotechnology Information.

3. Results

3.1. Parasitological survey

The results of the parasitological slaughterhouse survey are presented in Table 1. In the west (Darfur), the camel was by far the most affected species in terms of prevalence (61.4%), infection intensity (5.8 cysts per infected animal) and – together with cattle – cyst fertility (74%). Cattle, sheep and goats were much less frequently infected, rarely harboured more than one cyst, and cyst fertility was low in sheep and goats. These data were very similar to those obtained in central and southern Sudan, although the prevalence in cattle was higher in central Sudan (20.0%), and the prevalence in sheep was lower in central and southern Sudan (2.5% and 2.7%).

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3.2. Genetic characterization

A total of 490 cysts from the slaughterhouse survey were determined to species level by PCR, which is 11.2% of all cysts encountered in the survey (Table 1). All cysts but one (99.8%) belonged to *E. canadensis* (G6/7). Sequencing of partial cox1 and nad1 genes of 14 of these samples showed 100% identity with the ‘camel strain’ G6 of *E. canadensis* when compared with data on GenBank™ (Accession No. 208063). The one exception was a calcified liver cyst from cattle in southern Sudan, which was determined as *E. ortleppi* by PCR and sequencing (Table 2) with 100% conformity to *E. ortleppi* when compared with data on GenBank™ (Accession No. 235846). (Table 1).

An additional 38 isolates from camels, cattle and sheep, obtained by opportunistic sampling in eastern, northern and central provinces also were predominantly allocated to *E. canadensis* G6/7 by PCR (Table 2). Only one isolate, a fertile liver cyst from cattle in eastern Sudan (close to the border of Ethiopia), was identified as *E. ortleppi* by PCR and sequencing (Table 2), showing 100% identity to sequences in GenBank™ (Accession No. 235846).

The isolates from five human patients from hospitals of Khartoum and Juba were all determined as *E. canadensis* G6 by PCR and sequencing (Table 3) with 100% conformity to sequences in GenBank™ (Accession No. 208063).

### Table 3

<table>
<thead>
<tr>
<th>Origin</th>
<th>Sex</th>
<th>Age</th>
<th>Cyst location</th>
<th>Cyst condition</th>
<th>PCR</th>
<th>Sequencing&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Khartoumb&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Female</td>
<td>49</td>
<td>Lung</td>
<td>Viable, fertile</td>
<td><em>E. canadensis</em></td>
<td>G6</td>
</tr>
<tr>
<td>Khartoumb&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Male</td>
<td>46</td>
<td>Lung</td>
<td>Viable, fertile</td>
<td><em>E. canadensis</em></td>
<td>G6</td>
</tr>
<tr>
<td>Khartoum&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Male</td>
<td>53</td>
<td>Lung</td>
<td>Viable, fertile</td>
<td><em>E. canadensis</em></td>
<td>G6</td>
</tr>
<tr>
<td>Juba Female&lt;sup&gt;c&lt;/sup&gt;</td>
<td>27</td>
<td>Liver</td>
<td>Viable, fertile</td>
<td><em>E. canadensis</em></td>
<td>G6</td>
<td></td>
</tr>
<tr>
<td>Juba Female&lt;sup&gt;c&lt;/sup&gt;</td>
<td>38</td>
<td>Liver</td>
<td>Viable, fertile</td>
<td><em>E. canadensis</em></td>
<td>G6</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> cox1 and nad1.

<sup>b</sup> Migrant from Nuba mountains.

<sup>c</sup> Migrant from Nyala.

### 4. Discussion

The prevalence estimates of CE found in animals during our slaughterhouse survey are closely similar to those reported in previous studies from Sudan. Due to the large proportion of genotyped samples and the overwhelming presence of *E. canadensis* (G6) we propose that the figures from this (and previous) surveys reflect closely the epidemiological situation of that species. There is an unspecified margin of error in this, as, for practical reasons, the samples used for genotyping could not be collected at random, and the proportion of genetically characterized samples is lower for western Sudan than for the other regions. However, examination of cyst samples from all regions, host species and conditions resulted in the diagnosis of *E. canadensis* with two exceptions only, so we conclude that this species is predominant at high prevalence levels in all regions of the country.

We confirm the importance of camels for the transmission of this taxon, because prevalance estimates both in central (55.6%) and western Sudan (61.4%) far exceed those in other host animals, and the fertility rate was high (74%). Cattle, although more rarely infected, also seem to be well suited for the development of fertile cysts. Comparisons between host species have to be done with caution, however, as the development of prevalence and cyst fertility of *Echinococcus* spp. strongly depends on the age of the host which was not possible to determine with reliability in this survey (Lahmar et al., 1999; Dueger & Gilman, 2001; Torgerson et al., 2003). In contrast to goats, sheep are considered to be poor hosts for *E. canadensis*. This has been shown both for the ‘camel strain’ G6 in Kenya (Dinkel et al., 2004) and for the closely related ‘pig strain’ (G7) in Greece (Varcasia et al., 2007). We can confirm this observation only for our samples from central and southern Sudan, but found a surprisingly high prevalence (12%) and proportion of fertile cysts (19%) in sheep from Darfur province. There are two possible explanations. Our sampling might have been biased, and we only managed to examine 98 out of almost 2000 cysts from sheep in that region and might have overlooked a different *Echinococcus* taxon occurring there. We consider this explanation unlikely, as the genotyping concentrated on fertile cysts, and all 55 of these were identified as *E. canadensis*. The second possibility is an influence of the breed or husbandry conditions of sheep – which differ within Sudan – on the development of *E. canadensis* cysts, a hypothesis that needs exploration.

When estimating the importance of each host species for transmission, the relative frequency of fertile cysts from each of these species available after slaughter has to be considered rather than prevalence and cyst fertility. Looking at western Sudan, fertile cysts from sheep (289) come second to those from camels (1490) and range in number before those from cattle (186) and goats (34). We cannot exclude a certain bias in our figures as the proportion of slaughtered livestock species may not accurately reflect the real situation, but our figures come close to the official statistics on livestock in Sudan (Ministry of Finance and National Economy) which estimate the populations at 37 million cattle, 46 million sheep, 38 million goats and 3 million camels for the entire country, with some differences in geographical distribution (e.g., cattle are more frequent in the southern, camels in the western, central and northern parts). An additional bias is caused by the fact, that some proportion of livestock (especially sheep) is slaughtered at home and does not reach slaughterhouses. However, those sheep are usually young animals which are not yet likely to have developed fertile cysts, and might therefore not contribute considerably to the transmission.

Only one sample from this survey (derived from a southern Sudanese bovine) was identified as *E. ortleppi*.
additional one of three isolates obtained from cattle of eastern Sudan, close to the border to Ethiopia, was identified as that species. Together with two previous records from cattle of central Sudan (Dinkel et al., 2004) this raises the total number of Sudanese records to four. Clearly, this parasite is widespread in Sudan, but is generally rare and/or of local occurrence, which has also been shown for other countries like Kenya (Dinkel et al., 2004) or Italy (Casulli et al., 2008).

For the first time, cyst isolates from humans in Sudan could be genetically examined. All of five cysts samples were *E. canadensis* G6. As the origin of the patients is widely distributed over central, western and southern Sudan, the disease seems to occur sporadically in a large part of the country. Generally, CE is a rather rare disease in Sudan. For example, only 40 cases of human hydatidosis were recorded in the National Health Institute, Khartoum, Sudan for 2001 (personal communication). However, there might be foci of human CE in high-risk areas, as was demonstrated by a recent ultrasound survey of villagers in central Sudan where one out of 300 persons was found infected (Elmahdi et al., 2004). The camel strain (G6) of *E. canadensis* has been previously identified from human patients all over the world, e.g. in Argentina (Kamenetzky et al., 1997), Nepal (Zhang et al., 2000), Mauritania (Bardonnet et al., 2002), Iran (Harandi et al., 2002), Egypt (Azab et al., 2004) and Kenya (Dinkel et al., 2004). It was suggested that this strain may have a lower pathogenicity to humans due to its sporadic occurrence. In a focus of human CE in northern Kenya, only one human case with G6 infection was found among 189 CE patients, the others being infected with the ‘sheep strain’ (G1) of *E. granulosus*, despite the fact that both forms of *Echinococcus* were frequent in livestock in the same area (Dinkel et al., 2004).

For Sudan, we can come to a similar conclusion if we compare the high prevalence of that parasite in animals and the scarcity of the disease in humans. This is especially so, as all epidemiological conditions for autochthonous transmission of CE are given: in rural areas there are large numbers of dogs in and around villages, and infection can occur with offal from slaughterhouses or during unsupervised home slaughtering. Nevertheless, even if the parasite may have lower infectivity to humans, the infection can occasionally get established and progress to clinical CE, and all samples from the patients in our study were viable and contained protoscolices.

From our study we conclude that the epidemiological situation in the largest part of Sudan is characterized by intense transmission of *E. canadensis* G6 in domestic animals – predominantly between camels and dogs – and the sporadic occurrence of human cases caused by that species. *E. granulosus* sensu stricto (G1–G3), the taxon which is usually frequent in areas of high human CE incidence, is conspicuously absent in our sample and is most likely absent or very rare in most of Sudan. However, there is one exceptional focus of human CE in the extreme southeast of Equatoria province close to the borders with Kenya and Ethiopia. Prevalence rates based on ultrasound surveys can reach 3.5%, e.g. with the Toposa people, but the focus does not seem to extend into the western parts of southern Sudan (Magambo et al., 1996, 1998). It is highly suggestive that this situation is caused by the presence of the ‘sheep’ strain (G1) or related genotypes of *E. granulosus*, as is the case in the immediately neighbouring Turkana region of Kenya. If the absence or rarity of this malignant form of *Echinococcus* is the reason for the rather low number of human patients in the largest part of Sudan, the accidental introduction of this pathogen e.g. via the livestock trade has to be prevented as a matter of urgency.

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