

Taenia hydatigena cysticercosis in slaughtered pigs, goats, and sheep in Tanzania

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Abstract Few studies have been carried out in Africa to estimate the prevalence of *Taenia hydatigena*. With the aim to determine the prevalence of *T. hydatigena* in slaughtered pigs and small ruminants (goats and sheep) in Mbeya, Tanzania, two cross-sectional surveys were carried out investigating pigs in April to May 2014 and small ruminants in September 2012. In total, 243 pigs were examined post-mortem for *T. hydatigena* cysts which were found in 16 (6.6 %) pigs. The majority (80 %) of cysts were found on the omentum and the rest on the liver (20 %), all on the visceral surface. Two pigs were also found infected with *Taenia solium* but showed no signs of other infections. A total of 392 goats and 27 sheep were examined post-mortem, and the prevalence of *T. hydatigena* was similar in goats and sheep with 45.7 and 51.9 %, respectively. DNA sequencing of the mitochondrial cytochrome *c* oxidase subunit 1 gene (*cox1*) from a subsample of metacestodes from goats and sheep confirmed the *T. hydatigena* infection. The prevalence found in small

ruminants was comparable to other studies conducted in Africa, but for pigs, it is one of the highest recorded to date. The present study also confirms the occurrence of *T. hydatigena* and *T. solium* in pigs from Mbeya. Further studies are needed to determine the impact of *T. hydatigena* on production under sub-Saharan conditions and the financial consequences for smallholder farmers.

Keywords *Taenia hydatigena* · Cysticercosis · Pigs · Small ruminants · Tanzania · Prevalence

Introduction

The tapeworm *Taenia hydatigena* has canines, primarily dogs, as definitive hosts. The larval stage of the parasite infects a wide range of intermediate hosts, but most often domestic hosts are goats, sheep, and pigs (Pathak and Gaur 1982; Sweatman and Plummer 1957). Few prevalence studies have been carried out in Africa on *T. hydatigena* in small ruminants and even fewer in pigs (Table 1). All reported studies have used post-mortem examination of slaughtered animals to determine the prevalence, and most of the porcine studies are based on small sample sizes and non-random sampling strategies resulting in uncertainties regarding reported prevalences. Overall, *T. hydatigena* is widely distributed in the countries studied, and the prevalence in goats and sheep is higher compared to that of pigs. The only study conducted in Africa with a relative large sample size involving pigs was in Nigeria where the prevalence was 1.7 % based on 360 slaughtered pigs (Ajayi et al. 1988). To date, the highest prevalence in pigs from Africa was reported in Ghana at 6.7 % by Permin et al. (1999), but based on a relative small sample size ($n=60$).

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Table 1 Prevalence of *Taenia hydatigena* cysticercosis in sheep, goats and pigs from Africa based on post-mortem examinations

Country	Prevalence						Reference
	Sheep (%)	<i>N</i>	Goats (%)	<i>n</i>	Pigs (%)	<i>n</i>	
Benin	58.2	366	53.1	390	–	–	Attindehou and Salifou (2012)
Burkina Faso	43.3	60	–	–	–	–	Belem et al. (2005)
Egypt	29.8	369	33.3	240	–	–	El-Azazy and Fayek (1990)
Egypt	16.9	189	–	–	–	–	Sultan et al. (2010)
Ethiopia	37.1	560	–	–	–	–	Bekele et al. (1988)
Ethiopia	32.9	92	34.0	91	–	–	Abede and Esayas (2001)
Ethiopia	79	655	53	632	–	–	Sissay et al. (2008)
Ethiopia	40.0	630	46.6	768	–	–	Samuel and Zewde (2010)
Ethiopia	56.8	576	63.9	576	–	–	Wondimu et al. (2011)
Ghana	–	–	–	–	6.7	60	Permin et al. (1999)
Nigeria	21.4	1800	34.2	1260	–	–	Dada and Belino (1978)
Nigeria	–	–	33.3	120	–	–	Nwosu et al. (1996)
Nigeria	13.0	261	–	–	–	–	Saulawa et al. (2011)
Nigeria ^a	30.2 ^a	116	–	–	–	–	Fakae (1990)
Nigeria	–	–	–	–	1.7	360	Ajayi et al. (1988)
Sudan	32.4	3478	29	960	–	–	El Badawi et al. (1978)
Zambia	–	–	–	–	6.2	65	Dorny et al. (2004)
Tanzania	–	–	–	–	1.4	70	Ngowi et al. (2004)

^a Prevalence for goats and sheep combined

Cysticercosis in small ruminants caused by *T. hydatigena* may cause production loss through clinical disease or condemnation of meat, organs and offal (Kusiluka and Kambarage 1996; Kusiluka et al. 1998; Perry and Sones 2007). The financial loss from condemnation is considered to be high, but very few studies have estimated the loss due to *T. hydatigena* in ruminants (Christodouloupoulos et al. 2008; Oryan et al. 1994; Pathak and Gaur 1982; Saulawa et al. 2011), and to our knowledge, no such studies have been conducted for pigs. However, early reports of experimental infections with *T. hydatigena* in pigs revealed severe losses among the experimental animals (Herbert and Oberg 1975), but it is unclear whether this also applies under natural transmission settings.

Taenia hydatigena can only be diagnosed with a specificity of 100 % by post-mortem examination. Diagnosis can also be made by serology using an antigen(Ag)-ELISA (Brandt et al. 1992), but the assay is only genus specific and cross-reacts with other *Taenia* spp. (Dorny et al. 2003). The use of the Ag-ELISA assay in *Taenia* spp. co-endemic areas is therefore problematic, especially as a diagnostic tool for porcine cysticercosis. Uncertainties regarding prevalence, economic losses for farmers and complications with cross-reactions in sero-diagnostics highlight the importance of obtaining information on the distribution and prevalence of the parasite, especially in

Taenia solium co-endemic areas. The presence of *T. hydatigena* has been documented in sheep, goats, and pigs in Tanzania (Kusiluka et al. 1995; Mellau et al. 2011; Ngowi et al. 2004), but no studies have so far been conducted in Mbeya Region which is highly endemic for *T. solium* (Braae et al. 2014; Komba et al. 2013; Mwanjali et al. 2013). Therefore, this study aimed to determine the prevalence of *T. hydatigena* in slaughtered small ruminants (goats and sheep) and pigs in Mbeya, Tanzania.

Materials and methods

Study area

The study was carried out in Mbeya district, Tanzania, at two government slaughter slabs as small ruminants and pigs were slaughtered at different slabs. Both slaughter slabs were located in Mbalizi. The small ruminant and pig production is almost exclusively conducted as small-scale farming in Mbeya Region. In 2008, the small ruminant and the pig population in Mbeya Region were 642, 695 and 346,466, respectively (URT 2012). Goats and sheep made up 85 and 15 % of the small ruminant population, respectively.

Study design

The study was carried out as two cross-sectional surveys investigating pigs in April to May 2014 and small ruminants in September 2012. All animals slaughtered during the visits to the slaughter slab were included in the study.

Data collection and analyses

Post-mortem examination for T. hydatigena in pigs

The sex of pigs was noted before slaughter. The exact age was not determined but expected to be around 1 year based on size and appearance. The origin of the pig was established by questioning the trader. Pigs were cut open at the slaughter slab and the surface of the lungs, liver and all other abdominal viscera, along with the entire peritoneal cavity examined for *T. hydatigena* cysts with emphasis on the omentum and the mesentery, which were examined ex situ (OIE 2008). Cysts were considered to be *T. hydatigena* metacestodes if they were singular, transparent and filled with clear fluid, with a discrete white spot denoting a scolex (Samuel and Zewde 2010; Sweatman and Plummer 1957). The anatomic location, number and size of the cysts were noted. Since the liver was only inspected on the surface, metacestodes embedded in the liver parenchyma could not be identified.

Post-mortem examination for T. hydatigena in small ruminants

Age was determined by evaluating dentition. If two pairs of permanent incisors had erupted, the animal was categorised as adult (>1.5 years), whereas if none or only the first pair of permanent incisors had erupted, it was categorised as young (0.5–1.5 years) (Samuel and Zewde 2010). The origin of the animal was determined based on brands and ear clippings. The abdominal, pelvic and thoracic cavities were inspected for cysticerci after the removal of intestines and fore stomachs. The lungs and liver were inspected in situ, whereas the omentum and the rumen were inspected ex situ. The intestines were inspected on each side of the spiral disc and the corresponding side of the mesentery. One specimen was collected from each of 12 randomly selected *T. hydatigena*-positive small ruminants (six goats and six sheep). The host tissue surrounding the cysts was removed manually and the cysts washed thoroughly in physiologic saline before being transferred to a 30-ml collection tube containing 70 % ethanol. The ethanol was discarded after 24 h and replaced with fresh ethanol for preservation. The samples were stored at room temperature until analysis.

DNA analysis of metacestodes from small ruminants

From each metacestode collected, DNA was extracted from half of the scolex and a portion of the metacestode membrane using the DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions, except that metacestode tissue was homogenised using a pestle, and digested using 10 µl proteinase K (50 µg/µl) in 290 µl Buffer ATL for 3 h. DNA concentration and purity was evaluated using NanoDrop ND-1000 (Thermo Scientific) and the concentration adjusted to ~20 µg/µl.

The mitochondrial cytochrome *c* oxidase subunit 1 gene (*cox1*) was chosen for amplification due to high conservation of this locus, making it a good target for species identification (Blouin 2002). Sequences from GenBank (accession numbers FJ518620, JN831298, JN831307 and JN831305) and Primer3 (<http://primer3.wi.mit.edu/>) were used to identify the following primers: THCOIF: 5'-TTGATCCATTAGGTGGTGGAG-3' and THCOIR: 5'-TCCAGTAATTAAGGTCACCATC-3' targeting 551 bp of the *cox1* gene. PCR amplification was performed using standard PCR conditions in a final volume of 40 µl: 2 µl template DNA (20 ng/µl), 4 µl 10× PCR buffer (15 mM MgCl₂), 2 µl dNTPs (4 mM), 1.6 µl forward and reverse primer (10 µM), 0.4 µl TAMPase Hot Start DNA-polymerase (Amplicon) (5 units/µl), and 28.4 µl MilliQ water. The PCR reaction was carried out in a Techne TC-512 thermo cycler with the following cycling conditions: initial denaturation (95 °C, 15 min), followed by 35 cycles of denaturation (95 °C, 30 s), annealing (52 °C, 40 s) and extension (72 °C, 60 s), and a final extension step (72 °C, 10 min). The amplification of a single fragment of the expected size was verified by gel electrophoresis on a 1.5 % agarose gel (TAE, 0.5 %) stained with ethidium bromide.

PCR products were purified using the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions, except that 40 µl elution buffer was used. Sequencing was performed by Macrogen (Seoul, Korea) using the same primers as for the PCR reaction. All PCR products were sequenced in both directions, checked and edited manually using Vector NTI (Life Technologies). Sequences were aligned and trimmed using ClustalW multiple alignments as implemented in Bioedit (www.mbio.ncsu.edu/bioedit/bioedit.html) using default settings. All sequences were analysed using BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) to compare the obtained sequences with sequences from GenBank. MEGA, version 5 (www.megasoftware.net) was used to construct a Neighbour-Joining tree with the genetic distances calculated using Kimura 2-parameter. The robustness of the tree topology was examined using bootstrap analysis (1000 replications). For comparison, sequences of *T. hydatigena*, *Taenia saginata*, *Taenia taeniaeformis*, *T. solium* and *Taenia multiceps* from GenBank were included in the analysis with accession numbers, host and country origins as

well as references given in Table 2. Lastly, the p distance between all sequences was calculated using MEGA.

Statistics

All data were entered into an Excel spread sheet (Microsoft Office Excel 2010®) and imported into the statistical programme R (www.r-project.org) or SAS 9.0 for prevalence and 95 % confidence interval estimation.

Ethical considerations

Permission to conduct the study was obtained through Sokoine University of Agriculture in Morogoro, Tanzania, in addition to regional and district authorities. All animals included in the study were slaughtered as part of a daily slaughter routine, and no sampling was done until the animals were dead. Employees at the slaughter slabs were informed of the objective of the study and verbally agreed to allow the sampling.

Results

Post-mortem examination of pigs

In total, 243 slaughtered pigs from Mbeya district of which the majority were female (70 %) were examined post-mortem for *T. hydatigena* cysts. *Taenia hydatigena* cysts were found in 16 (6.6 %, CI 4.1–10.4) pigs. Intensity of infection was low, with the majority of pigs harbouring one cyst and a few pigs harbouring two cysts. The majority (80 %) of cysts were found on the omentum, and the rest (20 %) were found on the liver visceral surface. The *T. hydatigena* cysts were all translucent in appearances, between 2 and 4 cm in diameter, and had one visible scolex with a long neck characteristic of *T. hydatigena*. In addition, two pigs (1.2 %) were found infected with *T. solium* of which one had several calcified cysts in the heart, tongue and muscle tissue and the other had several viable cysts in muscle tissue and the heart, but no cysts could be seen on the tongue. These two pigs showed no other signs of infection at inspection. However, several pigs showed clinical signs of *Ascaris suum* infection on the liver.

Post-mortem examination of small ruminants

A total of 392 goats and 27 sheep were included in the study. The origin of the goats represented three areas in Mbeya Region (Chunya district, Momba district, and Mbeya district) as well as a small group (five animals) from neighbouring Rukwa Region. The prevalence of *T. hydatigena* was similar in goats and sheep with 45.7 % (CI 40.7–50.7) and 51.9 % (CI 32.4–70.8), respectively. The highest prevalence was

observed in goats from Momba district (51.3 %, CI 35.0–67.3) and Chunya district (50.2 %, CI 43.7–56.7), and the lowest prevalence in goats from Mbeya district (34.9 %, CI 26.1–44.8). *Taenia hydatigena* cysts were not found in the five animals from Rukwa Region. In goats, the intensity of infection was low, with the majority of infected animals harbouring one to three cysts (84.9 %), 11.2 % four to six cysts and 3.9 % harbouring seven or more cysts. Similarly, 57.1 % of infected sheep harboured one to three cysts, 14.3 % four to six cysts, and 28.6 % had seven or more cysts. Most cysts in goats were found in the omentum (76.0 %), mesentery (22.9 %), liver (19.6 %) and peritoneum (13.4 %). In two cases (1.1 %), cysts were evident on the lungs, and in another two cases, the location of the cysts was undetermined as they fell out of the carcass upon evisceration. The cysts in the peritoneum were found primarily adjacent to the descending colon or bladder. On the liver, all cysts were located on the visceral surface. In the mesentery, the cysts were mainly found in the ruminal serosa, and few were located in the centre of the spiral colon disc. Migratory tracts on the surface of the liver were not observed.

DNA analysis of metacestodes

A total of 12 metacestodes were collected for DNA analysis, six from goats and six from sheep. For all 12 metacestodes, DNA extraction and PCR amplification were successful, with only a single band of the expected size (~550 bp) visible after gel electrophoresis. After trimming the DNA sequences for comparison with sequences in GenBank, only 421 bp remained for analysis. The BLAST analysis revealed that in all cases except one there were 100 % coverage with at least 99 % homology to *cox1* sequences from *T. hydatigena* deposited in GenBank. The 12 samples in this study consisted of four haplotypes, and differences between these were one to three substitutions, corresponding to 0.23–0.71 % of the nucleotides. In the phylogenetic analysis, all *T. hydatigena* sequences clustered together with distinction from *T. saginata*, *T. taeniaeformis*, *T. solium* and *T. multiceps* (Fig. 1). The low bootstrap values indicate that the topology within the *T. hydatigena* clade was not robust, reflecting low genetic variability among *T. hydatigena* samples. The p distance between all *T. hydatigena* sequences ranged from 0.000 to 0.028, and the p distance between *T. hydatigena*, and *T. solium*, *T. saginata*, *T. multiceps*, and *T. taeniaeformis* ranged from 0.118 to 0.163.

Discussion

This study found a high prevalence of *T. hydatigena* in all three species of animals investigated in Mbeya district, Tanzania, and the first to document presence of this infection in

Table 2 Sequences from GenBank used for genetic comparison

Species	Country	Host species	GenBank accession no.	Reference
<i>T. hydatigena</i>	China	Goat	JN831304	Unpublished
<i>T. hydatigena</i>	China	Pig	JN831313	Unpublished
<i>T. hydatigena</i>	China	Goat	JN831298	Unpublished
<i>T. hydatigena</i>	China	Dog	FJ518620	Liu et al. (2011)
<i>T. hydatigena</i>	China	Sheep	GQ228819	Jia et al. (2010)
<i>T. hydatigena</i>	Turkey	Goat	JN827307*	Utuk and Piskin (2012)
<i>T. saginata</i>	Thailand	Human	JN986718	Unpublished
<i>T. taeniaeformis</i>	China	Cat	FJ597547	Liu et al. (2011)
<i>T. solium</i>	China	Pig	AB086256	Nakao et al. (2003)
<i>T. multiceps</i>	China	Sheep	FJ495086	Liu et al. (2011)

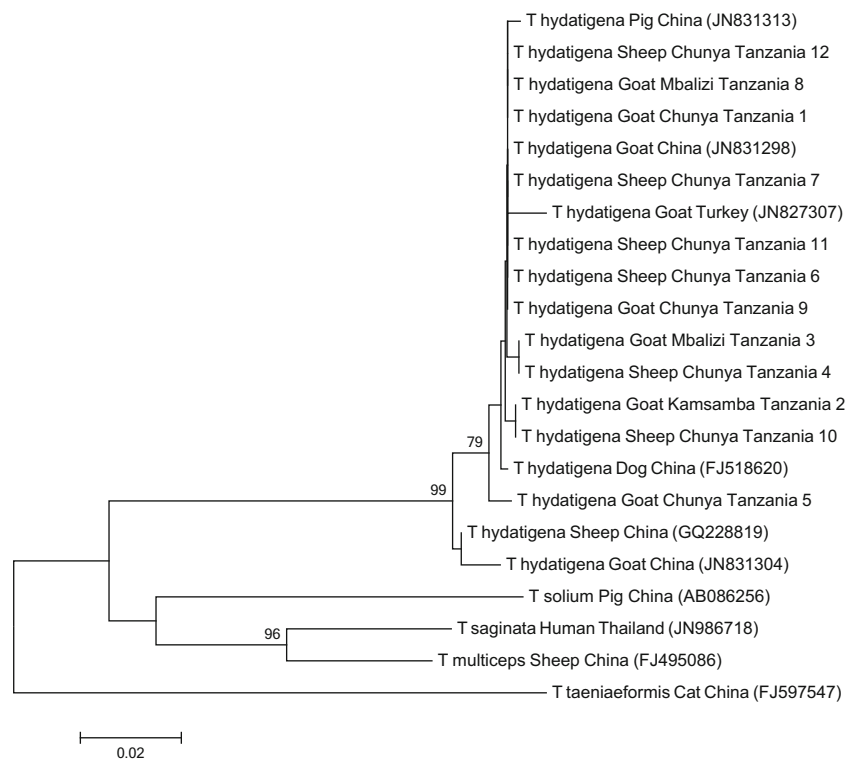
*=This sequence had 94 % coverage of the fragment used in this study

goats, sheep, and pigs in the region. The high prevalence in small ruminants is comparable to previous studies, but for pigs high, compared to studies performed in Africa (Ajayi et al. 1988; Dorny et al. 2004; Ngowi et al. 2004), and the highest so far recorded in Tanzania. The present study also confirms the co-occurrence of both *T. hydatigena* and *T. solium* in pigs from Mbeya district.

The intensity of *T. hydatigena* infection was low, ranging from 1 to 15 cysts in small ruminants and 1 to 2 in pigs, but the high prevalence suggests that the parasite could be a constraint

to small ruminant and pig production within the area. However, knowledge on the actual burden of disease caused by *T. hydatigena* is very scarce. Very little is known about the financial loss incurred due to clinical disease, mortality or condemnation of offal at slaughter in sub-Saharan Africa in terms of small ruminants and less so for pigs, but *T. hydatigena* in small ruminants has been associated with financial losses in Ethiopia due to condemnation of offal (Wondimu et al. 2011). It is important to determine and quantify disease burden, in order to estimate whether control programmes to combat

Fig. 1 Neighbour-Joining tree of *Taenia hydatigena* samples and an out group consisting of four different taeniids based on 551 bp of the *cox1* gene. Accession numbers are given after each species for sequence obtained from GenBank. Bootstrap values at the nodes are given as percentages based on 1000 replications. Scale bar: number of base substitutions per site



T. hydatigena infections are cost-effective and necessary in small ruminants and pigs. Integrating rabies control for dogs with anthelmintic treatment effective against taeniids could prove highly effective against *T. hydatigena*. However, the host spectrum for *T. hydatigena* is exceptionally wide, and wildlife may therefore serve as reservoirs, complicating control.

By sequence analysis of the *cox1* gene, all analysed cysts were all found to be *T. hydatigena*, verifying the macroscopic diagnosis. Both the cluster analysis and the *p* distances between *T. hydatigena* and among other species suggest that *cox1* is a good target gene for identifying *T. hydatigena*, *T. solium*, *T. multiceps*, *T. taeniaeformis*, and *T. saginata* and possibly other taeniids as well. The genetic distance between *T. hydatigena* and other taeniid species in this study (11.8–16.3 %) was also of the same magnitude as seen in other studies that found interspecific differences between taeniids of 12.1–17.6 % (Dai et al. 2012), 2.5–8 % (Gasser et al. 1999) and 12.0–18.2 % (Okamoto et al. 1995) based on the *cox1* gene.

The distribution of *T. solium* is currently not well known in Africa (Braae et al. 2015) and even less so the distribution of *T. hydatigena*. Knowledge on the co-existence within a specific area is essential in order to account for false-positives if an intervention for *T. solium* or *T. hydatigena* is evaluated using Ag-ELISA which is only genus specific (Brandt et al. 1992; Dorny et al. 2003). Co-infections were not observed during this study, supporting the hypothesis that an infection with one *Taenia* sp. could result in protective immunity against another (Conlan et al. 2009). As a result, the increase in prevalence of *T. hydatigena* in pigs could result in a decrease in prevalence of *T. solium* and vice versa. This highlights the importance of establishing co-endemic areas and degrees of co-infections, if any, but comparable data on *T. hydatigena* and *T. solium* co-endemic areas are lacking. The prevalence of *T. solium* reported here was considerably lower than previously reported in the area (Komba et al. 2013). Although pigs are screened by lingual examination prior to slaughter, it is likely that traders from experience refrain from purchasing pigs from households with a porcine cysticercosis history. Pigs found positive for *T. solium* in the screening process are presumed to be slaughtered in clandestine slaughter slabs (Ngowi et al. 2007).

The distribution of *T. hydatigena* and *T. solium*, both in terms of prevalence and intensity, but also the degree of co-infections outside the biased environment of a governmental slaughter slab could be significantly different than the observations presented here. Pigs were brought to the slaughter slabs by pig traders who often trade the pigs several times before slaughter; pinpointing the exact origin of each individual animal is therefore almost impossible. The prevalence of *T. hydatigena* presented here might be an underestimation because early-stage *T. hydatigena* metacestodes are small

and therefore easily missed during post-mortem examinations, as are migratory metacestodes, when livers are not incised. However, considering the presumed age of animals at slaughter, as well as the absence of migratory tracts on liver surfaces, it is unlikely that *T. hydatigena* in the migratory phase was overlooked.

Determining possible variations in prevalence of *T. hydatigena* both spatially and temporally (Oryan et al. 2012; Oryan et al. 1994; Pathak and Gaur 1982) calls for more research on the transmission dynamics of the parasite as well as the interaction with other *Taenia* species, which could be important in the control of *T. solium*. Elucidation of the proportions of *T. hydatigena* and *T. solium* infections in pigs and how these parasites interact in terms of interspecific competition, and the protective properties that might follow, and which effect it has on the transmission dynamics of the parasites could prove vital in the control of both parasites.

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Conflict of interest The authors declare that they have no competing interests.

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