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Sympatric occurrence of *Taenia saginata* and *Sarcocystis* spp. in cattle from Narok County, Kenya: meat inspection findings with molecular validation

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Abstract

The epidemiological picture of *Taenia saginata* infections in Kenya is fragmented with limited available data. Although *Sarcocystis* species are significant meat-borne parasites, few studies have explored their occurrence in Kenya. This study aimed to estimate the occurrence of bovine cysticercosis and screen for the presence of *Sarcocystis* spp. A meat inspection-based survey was conducted in ten abattoirs in Narok County, Kenya, and inspection for *T. saginata* cysticerci was limited to the *Triceps brachii* muscle. The apparent occurrence of the parasite was 5.4% (95% CI, 3.8, 7.6, *n*=573). Molecular confirmation of *T. saginata* was done via nested polymerase chain reaction targeting the mitochondrial 12S ribosomal RNA gene and restricted fragment length polymorphism. *Sarcocystis* species were identified using a multiplex polymerase chain reaction method targeting the 18S ribosomal RNA gene sequences and the mitochondrial cytochrome c oxidase subunit I gene. Of the 31 cystic lesions tested, 26/31 (83.9%) were confirmed to be *T. saginata. Sarcocystis cruzi* and *S. hominis* were detected in 8/31 (25.8%) and 1/31 (3.2%) of the cystic lesions, respectively. Co-infections of *S. cruzi and T. saginata* were found in 6/31 lesions (19.4%). The confirmation of bovine cysticercosis and *S. hominis* is suggestive of the presence of risky culinary and sanitation practices that facilitate transmission. This is the first report and molecular confirmation of *Sarcocystis* spp. in cattle in the country. The presence of both zoonotic *S. hominis* and pathogenic *S. cruzi* highlights an underexplored concern of veterinary and human health significance, warranting further epidemiological investigation.

Introduction

Bovine cysticercosis negatively impacts vulnerable communities and the beef industry, and it poses a public health burden. The economic losses are attributed to carcass condemnations, treatment of lightly infected carcasses, their subsequent drop in value post-treatment, and, indirectly, restriction on exports (Jansen *et al.* 2018). The adult parasite is the cause of *Taenia saginata* taeniosis, a cosmopolitan neglected cyclozoonoses estimated to affect 50 million people worldwide (OIE 2005; WHO 2016). The adult helminth resides in the small intestines of man, and the metacestode stage parasitizes the muscles of cattle and occasionally other bovids (Grove 1990). Tapeworm carriers rarely report clinical symptoms; however, they may present with nausea, abdominal discomfort, emesis, diarrhoea, weight loss, peri-anal symptoms, and, rarely, cholecystitis (Hakeem *et al.* 2012; Uyguer-Bayramicli *et al.* 2012).

Bovine cysticercosis is asymptomatic. Post-mortem examination (meat inspection) of slaughtered carcasses through incision, palpation, and visual inspection remains the main method for screening infected carcasses. It is, however, insensitive due to its reliance on predilection sites and the intensity of infestation (Minozzo *et al.* 2002; Wanzala *et al.* 2003; Lopes *et al.* 2011; Jansen *et al.* 2017; Jansen *et al.* 2018). Sources of false positives may stem from morphologically similar pathological lesions such as small abscesses, neoplasms, fat tissue, and other tissue parasites (Ogunremi *et al.* 2004; Abuseir *et al.* 2006). Serological tests for *T. saginata* detection in cattle have been reported to have inconsistent sensitivities, have rather poor performances in detecting light infections, and cross-react with other taeniid infections (Onyango-Abuje *et al.* 1996a; Wanzala *et al.* 2002; Jansen *et al.* 2017). Still, serological tests offer a higher potential performance than meat inspection. Nevertheless, their limited commercial availability and restricted accessibility to researchers have led to a reliance on meat inspection in many studies. Additionally, serological tests are yet to be adapted for operational use in slaughterhouses.

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The prevalence of bovine cysticercosis in Kenya has shown varying trends over time and across regions. Surveillance data are limited and have huge chronological gaps. Notably, however, Kenya is among the African countries referred to as having 'hyperendemic pastoral cysticercosis', characterized by a simultaneous high burden of *T. saginata* infection in humans and cattle (OIE 2005). Early reports from the 1960s revealed high prevalence rates, with 31.7% reported at Kenya Meat Commission (receives slaughter cattle countrywide) and an estimated 53% in the Narok region (Froyd 1960; Froyd 1965). Between 1974 and 1991, the prevalence ranged from 8% to 1.1% in different regions (Kangethe 1995). In the late 1990s, the reported prevalence at Kenya Meat Commission was 7.6%. An aggregated prevalence estimate of 16% was observed in several pastoralist-occupied areas in the same duration, with the Narok region reporting the highest occurrence rate at 31% (Onyango-Abuje *et al.* 1996b). Findings from a study conducted in 2009 indicated the prevalence to be 3.2% in the Northern Turkana area (Asaava *et al.* 2009).

Another important meat parasite in cattle is *Sarcocystis.* The genus *Sarcocystis* represents over 200 heteroxenous cosmopolitan coccidian parasites of vertebrates. Only 26 have known life cycles, which commonly adopt a predator–prey pattern (Dubey 2015; Castro-Forero *et al.* 2022). Carnivores and omnivores act as the definitive hosts and get infected upon ingesting infected muscles from intermediate hosts. In their gut, oocysts are excreted to contaminate the environment, leading to the acquisition of infection by intermediate hosts. The parasite adopts asexual forms in the intermediate host, leading to the formation of muscular sarcocysts. Pigs and cattle are the only recognized livestock intermediate hosts of zoonotic *Sarcocystis* spp. Cattle act as the intermediate host for seven known *Sarcocystis* spp. Two of these are zoonotic (i.e., *S. hominis* and *S. heydorni*). Intestinal sarcocystosis is, however, frequently asymptomatic, although nausea, inappetence, vomiting, diarrhoea, bloat, and stomachache have been reported (Fayer 2004; Dubey 2015; Castro-Forero *et al.* 2022). *Sarcocystis cruzi* is the most significant *Sarcocystis* spp. in cattle, it is the most prevalent globally, and it is responsible for high economic losses. Canids act as the definitive host for *S. cruzi.* Infection with *S. cruzi* in cattle rarely manifests clinically; however, it is linked with the pathogenesis of bovine eosinophilic myositis (BEM). BEM leads to high economic losses as it necessitates carcass condemnation (Dubey 2015; Castro-Forero *et al.* 2022; Dini *et al.* 2023; Dubey and Rosenthal 2023).

The status of *Sarcocystis* infection in cattle in Kenya is largely unknown. A clear and comprehensive epidemiological picture of *T. saginata* infections is also lacking in the country; data are limited and confounded by the use of poorly performing diagnostic tools. This study seeks to contribute to the growing body of knowledge in this area by estimating the occurrence of bovine cysticercosis in cattle from at-risk communities based on meat inspection, combined with molecular confirmation of the suspected *T. saginata* lesions. Moreover, lesions were also tested for *Sarcocystis* spp. as a potential differential diagnosis and to contribute to preliminary investigations into the occurrence of *Sarcocystis* spp. in Kenyan cattle.

Materials and methods

Study area

The study area was Narok County located in southwestern Kenya at coordinates $1^0$15'S, $35^0$37'E. It has a total area spanning 17,921.2 km^2, which is fragmented into 6 sub-counties and 30 wards. The sub-counties are Kilgoris, Narok North, Narok South, Narok East, Narok West, and Emurua Dikirr (see Figure 1). The ambient temperature ranges between 12 and 28°C, and annual rainfall averages between 500 and 1800 mm. Economic activities include mining, crop farming, tourism, and livestock farming. Its cattle density is estimated at 1.5 million. Pastoralism production system is widely practiced. The human population is approximately 1,157,873 of which only 35% use improved sanitation and 20% use improved drinking water sources (KNBS 2019).

Narok County has 5 main slaughterhouses and 14 active slaughter slabs with an average daily throughput of approximately 200 cattle carcasses (unpublished Narok County veterinary records). The animals that are slaughtered are primarily sourced locally. However, the Maasai community of pastoralists, who are the primary cattle keepers in the region, extend their presence to neighbouring Tanzania.

Sampling and sample size determination

A cross-sectional study design was employed. The sample size (n) for bovine carcasses was computed as per the proportions survey formulae by Thrusfield (2005), where expected prevalence (Pexp) was set at 50% to maximize the sample size, the desired absolute precision (d) set at 0.05, and the confidence level set at 95%, to give a sample size of 384. However, additional sampling was conducted due to its positive effect on precision. Individual carcasses were selected by systematic random sampling. Sampling targeted 5/5 of the slaughterhouses and 5/14 of the slaughter slabs, with the selection of slaughter slabs being random.

Carcass inspection

The sampling was conducted from April to July 2021. Bovine carcasses were inspected for *T. saginata* cysticerci by a meat inspector accompanied by the researcher. This was done by examining the *Triceps brachii* muscles predilection site whereby three cuts of approximately 2-cm thickness were made using a sharp knife with subsequent visual identification of cysticerci if present (Meat Control Act 2012). Suspected tissue lesion sections of approximately 0.5 cm^3 containing the cysticercus were excised and stored in labelled 2-ml vials containing 70% ethanol until analyses. The number of carcasses positive on visual inspection was noted. The number of cysticercus lesions detected, and their stage, was noted; they were classified as either viable or degenerated (Abuseir *et al.* 2006).

DNA extraction

One suspected cysticercus per carcass was utilized for DNA extraction. Where both viable and degenerated cysticerci were obtained from a single carcass, tissue from the viable cysticercus was used. DNA extraction was conducted as per the protocol from the manufacturer, DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany). Extracted DNA was stored at -20°C pending further tests.

Polymerase chain reaction-restricted fragment length polymorphism

A semi-nested polymerase chain reaction (PCR) technique was utilized to target the mitochondrial 12S rDNA gene of *Taenia* spp. The specific primers used in this PCR method and protocol

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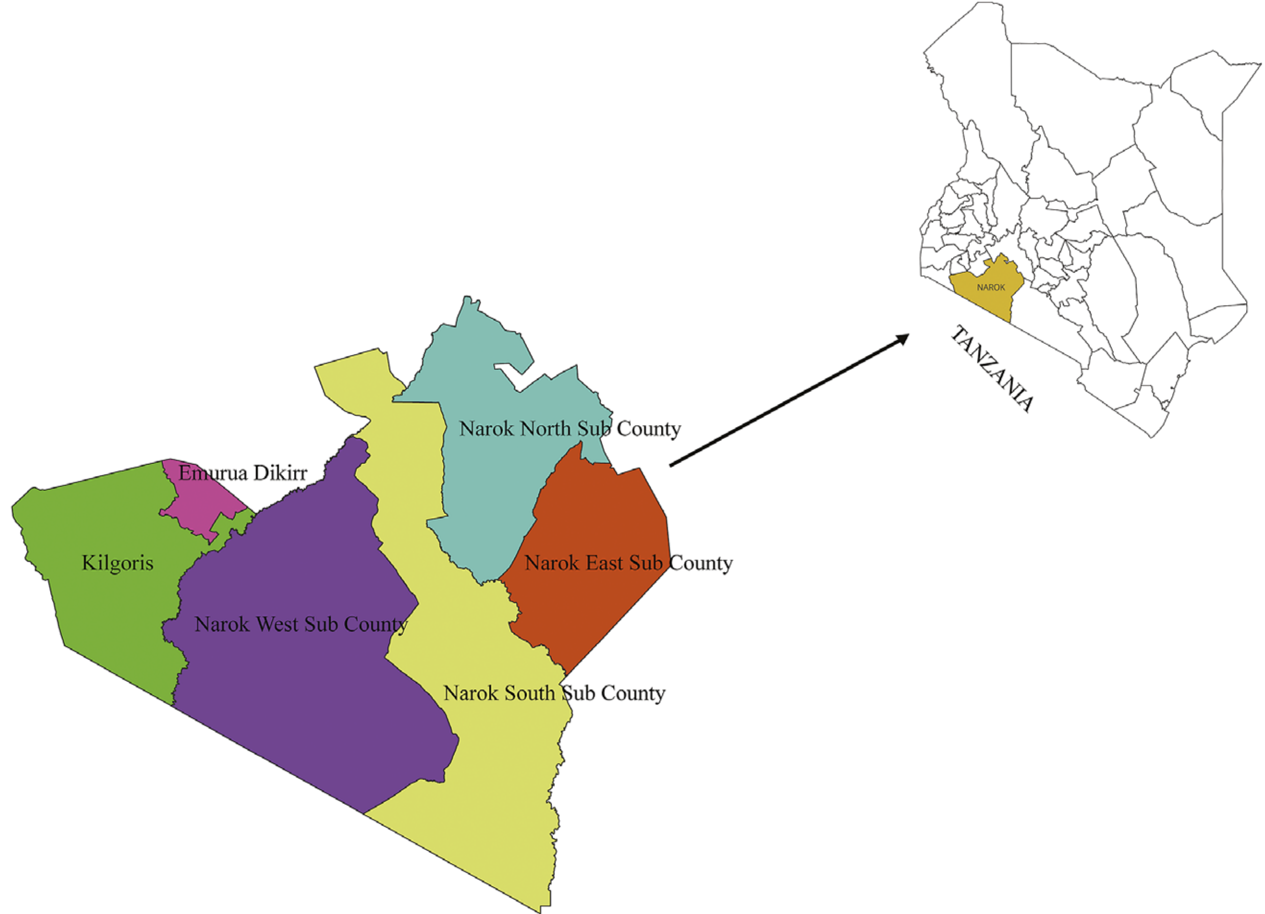

Figure 1. Map of Kenya depicting Narok County and its sub-counties.

were adapted from Geysen *et al.* (2007). The forward primer was 5'-CTCAATAATAATCGAGGGTGACGG-3', (ITM TnR, Primer 1), and the reverse primer was 5'-GTTTGCCACCTCGATGT TGACT-3'(TaenF, Primer 2) and 5'-CGTGAGCCAGGTCGGTTC TTAT-3' (nTAE, Primer 3). The nested PCR (nPCR) was genus-specific, producing *Taenia* spp. amplicons of 789–798 bp with ITM TnR/nTAE primers. Extracted DNA was prepared in pairs of 1/1 dilution and 1/10 dilution for the first round of PCR (i.e., 5 µl DNA and 0.5 µl DNA templates). For the second round of PCR, a 0.5µl DNA template was used. The quantities of the constituents of the mastermix were 2 × Promega GoTaq Mastermix (Promega, Madison, USA) (12.5 µl both PCR rounds), nuclease-free water (6.7 µl round 1 PCR, and 11.2 µl round 2 PCR), Primer 1 (0.4 µl for both PCR rounds), Primer 2 and 3 (0.4 µl). Primers were constituted to a concentration of 25 pmol/ µl. A total volume of 25 µl PCR reaction mix was used for both rounds of PCR. Primers 1 and 2 were used for the first round of PCR, and Primers 1 and 3 for the second round of PCR. Amplification was conducted using Applied Biosystems Veriti Thermal Cycler®, with the following settings used for the 1st round PCR: initial denaturation at 95°C for 2 min, followed by 40 cycles of amplification of 92°C for 45 s, 57°C for 45 s, 72°C for 60 s, and a final elongation at 72°C for 10 min. The second round PCR utilized the same parameters, but the cycle number was reduced to 25. The amplicons were analyzed by electrophoresis in 2% (w/v) agarose gels for 60 min at 120 V, followed by ethidium bromide staining for 20–30 min and photography under UV illumination. Amplicons corresponding with *Taenia* spp. Positive wells from round 2 of PCR were further subjected to restriction endonuclease digestion using enzymes *DdeI, HinfI*, and *HpaI* (New England Biolabs, Ipswich, USA). Post PCR, restricted fragment length polymorphism (RFLP) using the endonucleases achieves diagnostic DNA fragments, enabling the identification of *Taenia* species. Amplicons of 471 bp, 165 bp, and 128 bp are diagnostic for *T. saginata.* Where both the 1/1 dilution and 1/10 dilution samples gave positive amplification bands, the 1/10 nPCR product was preferred. The enzymes were used at their initial concentration of 10–20 U/µl and concentrated restriction enzyme buffers at 10×. The RFLP mastermix was constituted following the manufacturer's instructions. The final mixture per sample comprised 6 µl of PCR products from round 2 of PCR and 9 µl of RFLP mastermix. The vials were then incubated at 37°C for 4 hrs. The digested products were analyzed by electrophoresis for 60 min at 120 V, on a 2% (w/v) agarose gel. A DNA size marker of 100 bp was included for reference. This was followed by ethidium bromide staining for 40 min and photography under UV illumination. Known positive controls for *T. saginata* were included in the RFLP analysis.

Echinococcus spp.-Taenia spp. multiplex polymerase chain reaction

All DNA isolates were subjected to an *Echinococcus* spp.-*Taenia* spp. multiplex PCR. This test targets sequences of part of the mitochondrial genes for NADH dehydrogenase subunit 1 (*nad1*) and the small subunit of ribosomal RNA (*rrnS*). The protocol utilized was adapted from Trachsel *et al.* (2007). The assay is deemed more sensitive than

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the nPCR described by Geysen *et al.* (2007) (pers. comm) and therefore a useful test for detecting *Taenia* spp, especially in populations where *Taenia* spp. is co-endemic with *Echinococcus* spp. A positive *Taenia* spp. amplicon is identified by a band weight of 267 bp. The oligonucleotide sequences for the primers used were as follows for *E. multilocularis*: 5'- TGCTGATTTGTTAAAGT-TAGTGATC-3'(Primer-Cest1), 5'-CATAAATCAATGGAAACAA CAACAAG-3' (Cest2), *E. granulosus,* 5'-GTTTTTG TGTGTTA-CATTAATAAGGGTG-3' (Cest4), 5'-GCGGTGTGTACMTGA GCTAAAC-3' (Cest5) and *Taenia* spp. detection, 5'-YGAYTCTTT TTAGGGGAAGGTGTG-3' (Cest3), and 5'-GCGGTGTGTACMT GAGCTAAAC-3'(Cest5). The amplification mixture volume per reaction was constituted to a total of 25 μl, and a DNA template of 0.5 μl was mixed with a cumulative mastermix of 24.5 μl. The Qiagen multiplex kit mastermix (Qiagen, Hilden, Germany) was used at 12.5 μl, primer mix at 2.5 μl (Primer concentrations were 2 mM of Cest1-4 and 16 mM of Cest5 in Tris-EDTA or water) and nuclease-free water at 9.5 μl constituted as per manufacturer's instruction. The PCR assay settings were as follows: an initial denaturation step at 94°C for 15 min, followed by 40 cycles at 94°C for 30 s, 58°C for 90 s, 72°C for 10 s, and a final extension at 72°C for 7 min. To visualize the amplicons, PCR products were analyzed by electrophoresis in 2% (w/v) agarose gels for 60 min at 120 V, followed by ethidium bromide staining for 20–30 min and photography under UV illumination. This process was repeated for all loaded gels. Digital photos of the results were saved for later interpretation.

Sarcocystis spp. multiplex polymerase chain reaction

All previously isolated DNA was subjected to a *Sarcocystis* spp. multiplex PCR targeting the 18S ribosomal RNA (18S rRNA) gene sequences and the mitochondrial cytochrome c oxidase subunit I (COI) gene. The protocol utilized was adapted from Rubiola *et al.* (2020). The multiplex PCR was capable of detecting *S. hominis*, *S. cruzi*, *S. hirsuta*, *S. bovifelis*, and *Sarcocystis* spp. The anticipated fragment sizes for different organisms in the DNA analysis are as follows: 108 bp for *S. hirsuta*, 200–250 bp for *Sarcocystis* spp (these were further analyzed via sequencing), 300 bp for *S. cruzi*, 420 bp for *S. hominis*, and 700 bp for *S. bovifelis*. The primers used were 5'-AACCCTAATTCCCCGTTA-3'(Sarco_Rev), 5'-TGGCTA ATACATG CGCAAATA-3' (SarF), 5'-CATTTCGGTGATT ATTGG -3'(Hirsuta), 5'-ATCAGATGAAAATCTACTACATGG-3'(Cruzi), 5'-AATGTGGTGCGGTATGAACT-3'(COI_HB), 5'- -GGCACCAACGAACATGGTA-3'(COI_H), and 5'-TCAAAA ACCTGCTTTGCTG-3'(COI_B). The amplification mixture volume per reaction was constituted to a total of 25 μl. A DNA template of 2.5 μl was mixed with a cumulative mastermix of 22.5 μl (12.5 μl Taq DNA polymerase, 6 μl nuclease free water, Sarco-Rev 1 μl, all other primers at 0.5 μl (Primer conc. 0.5 μM in all, except Sarco-Rev at 1 μM)). The PCR assay involved a denaturation step at 95°C for 3 min, followed by 35 cycles at 95°C for 60 s, 58°C for 60 s, and 72°C for 30 s and a final extension at 72°C for 3 min. PCR products were analyzed by electrophoresis in 2% (w/v) agarose gels for 70 min at 120 V, followed by ethidium bromide staining for 20–30 min and photography under UV illumination.

Sequencing

PCR products were sent for sequencing at Eurofins Genomics in Ebersberg, Germany. Primer Cest5seq was used for sequencing PCR products from the *Echinococcus-Taenia* spp. multiplex PCR (Trachsel *et al.* 2007) and primer Sarco-Rev was used for sequencing PCR products from *Sarcocystis* spp. multiplex PCR (Rubiola *et al.* 2020). DNA sequences were first viewed and manually edited using GENtle v. 1.9.4 (http://gentle.magnusmanske.de). The sequences were identified by comparing with those available in the National Centre for Biotechnology Information database using the basic local alignment search tool (http://www.ncbi.nlm.nih.gov/BLAST/) (Altschul *et al.* 1997).

Data handling and analysis

The data were entered in a Microsoft Excel spreadsheet, cleaned, and validated to check for any errors and omissions. Data were exported to the computer package R version 4.3.1 for analysis. Data on the meat inspection and PCR findings were summarized as proportions and presented in tables.

Results

Bovine cysticercosis detection in abattoirs

During the abattoir survey, 573 randomly sampled bovine carcasses from 10 slaughter facilities were partially inspected for *T. saginata* cysticerci. The estimated overall prevalence in the study area was 5.4 % (95% CI, 3.8, 7.6). All positive carcasses came from Narok Town Ward, which hosts most (40%) of the surveyed slaughter facilities and carcasses inspected (59%). The aggregate prevalence for Narok Town Ward was 9.1% (95% CI, 6.5–12.7, $n = 339$). Narok Town Ward had a significantly higher prevalence than Mulot Ward, which had a prevalence of 0% (95% CI, 0–2, $n = 187$) denoting non-overlapping confidence intervals of the point prevalence for bovine cysticercosis reported in the respective Wards.

The final sampling frame and proportion of positive carcasses per facility are shown in Table 1.

Intensity of infection

Of the 31 infected carcasses, 10 had viable cysts, and 21 had degenerated cysts. One (1/31) infected carcass had both viable and degenerated cysts. Most (24/31) infected carcasses had a single cyst. A total of 14 viable cysts were recovered from one carcass. The

Table 1. The prevalence of bovine cysticercosis in abattoirs, Narok County, April to July 2021

Facility name	Ward	No. of carcasses inspected	No. of positive carcasses	Prevalence and 95% CI
East African SH	Narok Town	190	15	7.9, (4.8, 12.6)
Narok Town SH	Narok Town	61	10	16.4, (9.2, 27.6)
Maleto SH	Narok Town	48	5	10.4, (4.5, 22.2)
Kerempe SH	Narok Town	40	1	2.5, (0.4, 12.9)
Mulot SH	Mulot	187	0	0, (0, 2)
Kimogoro SS	Sogoo	8	0	0, (0, 32.4)
Ololulunga SS	Ololulunga	20	0	0, (0, 16.1)
Duka moja-Suswa SS	Keekonyokie	3	0	0, (0, 56.2)
Nairragie Enkare SS	Keekonyokie	3	0	0, (0, 56.2)
Ntulele SS	Mosiro	13	0	0, (0, 22.8)
Total		573	31	5.4, (3.8, 7.6)

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Table 2. Parasites identified from cattle tissue cyst material using various molecular tools

Parasite(s) identified	Proportion of positive cysts, *n*= 31
T. saginata spp.	26/31 (83.9%)
Taenia-like spp.	5/31 (16.1%)
S. cruzi	8/31 (25.8%)
S. hominis	1/31 (3.2%)
S. bovifelis	0/31 (0%)
Taenia-like spp + *S. cruzi*	2/31 (6.5%)
T. saginata + *S. cruzi*	6/31 (19.4%)
T. saginata + *S. hominis*	1/31 (3.2%)

mean number of cysts per infected carcass was 1.6 ≈ 2. A total of 51 cysts were recovered from 31 infected carcasses; 25 were viable, and 26 were degenerated.

Molecular detection

All samples were analyzed with PCR-RFLP, whereby 26/31 samples presented a clear *T. saginata* profile on nPCR. Five samples gave an unclear profile and further tested negative for *T. saginata* on RFLP. Additional testing gave positive *Taenia* spp. results on these five samples (*Echinococcus* spp.-*Taenia* spp. multiplex PCR). *Taenia saginata* was detected in 9/10 viable cysts and 17/21 degenerated cysts. *Sarcocystis* spp. was detected in 9/31 samples. A summary of the findings is in Table 2.

Sequencing

Two PCR products were sequenced and identified as *T. saginata* and *S. hominis.* The *T. saginata* isolate sequence was deposited in the GenBank under accession number OR594291 and was 100% identical to several sequences including that of accession number NC_009938 (Jeon *et al.* 2007). The *S. hominis* sequence was short (161 bp) and therefore not deposited into the GenBank; it was 100% identical to sequence accession number OQ184854 (Dini *et al.* 2023).

Discussion

The prevalence of bovine cysticercosis in Kenya has shown varying trends over time and across regions. The documented bovine cysticercosis prevalence estimate in the Narok area prior to this study was 31% (Onyango-Abuje *et al.* 1996b). A report by Asaava *et al.* (2009) demonstrated a lower estimate (3.2%), although this was in a different area – Northern Turkana. An occurrence of 5.4 % determined by incision of the *Triceps brachii* only was reported. The findings indicate a lower value than the previous estimates for Narok area; however, the study employed detection via the incision of a sole predilection site. Multiple studies have highlighted that meat inspection significantly underestimates the true prevalence, and those studies are based on a much more in-depth dissection of predilection sites (Onyango-Abuje *et al.* 1996b; Minozzo *et al.* 2002; Wanzala *et al.* 2003; Lopes *et al.* 2011; Jansen *et al.* 2017). The true prevalence of bovine cysticercosis in Narok area may therefore be higher.

Inter-ward prevalence comparison was limited in Sogoo, Ololulunga, Keekonyokie, and Mosiro Wards due to the small number of carcasses sampled. Bovine cysticercosis was detected in Narok Town Ward, while no occurrences were reported in Mulot Ward. Narok Town Ward hosts a relatively higher concentration of pastoralists who source cattle locally when compared to Mulot Ward (NCG 2016). Thitu *et al.* (2016) indicated that members of this pastoralist community exercise risky practices such as open defecation. Consumption of raw/partially cooked meat is also common (Chege *et al.* 2015). It is noteworthy that Narok County supplies meat to far-flung markets beyond its borders (Mwangi *et al.* 2020) and is therefore of significant spatial consideration in the control of *T. saginata* taeniasis in other parts of Kenya. Due to its proximity to the neighbouring Bomet County, Mulot SH (Mulot Ward) sources most of its slaughter cattle from Bomet County (pers. comm). Notably, Bomet County has higher improved water, sanitation coverage, and literacy rates when compared to Narok County (74%, 78%, and 83% in Bomet County compared to 35%, 20%, and 53% in Narok County) (KNBS 2019).

An average of 1.6 ≈ 2 cysticerci per infected carcass was found, based on incisions in one muscle. Minozzo *et al.* (2002) estimated that for each cysticercus found on routinely inspected tissues, there are at least 6.1 that remain undetected. Meat inspection positivity is therefore only an indicative underestimate. One carcass harboured 14 viable cysts (27% of the total cysts retrieved). The high intensity of infection insinuates a compromised immunity (OIE 2021) or possibly a case of recent exposure to a high number of viable *T. saginata* eggs. Notably, however, a high count of viable cysts is a common finding in carcasses from young animals (Froyd 1960; Wanzala *et al.* 2003; OIE 2021). Such carcasses are potential 'super-spreaders' and pose a relatively higher public health risk where meat inspection is not conducted. Most of the infected carcasses had degenerated cysts (22/31, 71%). This is consistent with other reports (Jansen *et al.* 2017; OIE 2021).

The meat inspection regulation in Kenya outlines a few predilection sites to detect *T. saginata* cysticerci (Cap 356 of the Meat Control Act). It directs the meat inspector to examine the tongue, masseter muscles, *Triceps brachii*, heart, and diaphragm, but it mentions that the inspector has the liberty to proceed with further incisions at predilection sites if there is suspicion of bovine cysticercosis. It also gives guidance on carcass treatment and handling relative to the intensity of infection (Meat Control Act 2012). An observation made in the context of routine surveillance for bovine cysticercosis was that the major predilection sites inspected were the triceps muscles in both forelimbs and the heart. In the event of positive findings on the two sites, seldom were additional incisions made. Information on infection intensity was not commonly captured possibly due to the absence of infrastructure to support carcass treatment. The observed practices were that of excision of affected tissue and therefore a risk of releasing unsafe meat to the public.

Molecular tools were able to amplify and detect parasite DNA from all 31 cyst tissues despite 21/31 of the cysts being classified as degenerated. PCR-RFLP (Geysen *et al.* 2007) was able to confirm *T. saginata* larvae in 26/31 (84%) of the cysts, and 5 samples were noted as 'positive but doubtful' from the assessment of results from the genus-specific nested PCR. This was due to the presence of a slightly lighter band than *Taenia* spp. positive control samples and other positives (i.e., 750–775 bp instead of 798 bp). These samples were negative for *T. saginata* during post PCR- RFLP with *Ddel, Hinfl*, and *Hpal* enzymes. The 5 samples were, however, positive for *Taenia* spp. in the *Echinococcus* spp.-*Taenia* spp. multiplex PCR

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(Trachsel *et al.* 2007), which is genus-specific for *Taenia* spp. These findings suggest the presence of other *Taenia* spp. as a cause of bovine cysticercosis in Kenya. Sequencing analysis will, however, be required to confirm the identity of the taeniid. Hailemariam *et al.* (2014)) reported non-*T. saginata* cysticerci parasitizing cattle in Ethiopia and hypothesized that the parasite could be *T. hyaenae.* The sampled carcasses are from cattle owned by pastoral communities that have close interactions with wildlife, exposing them to taeniids from the wild.

This is the first report and molecular confirmation of *S. cruzi* and *S. hominis* in Kenya. Concurrent infection by *S. cruzi* and *T. saginata* (6/31) and *T. saginata* and *S. hominis* (1/31) was also confirmed. *Sarcocystis cruzi* and *S. hominis* are microscopic intracellular parasites (Dubey 2015). Infected muscle tissue adjacent to the cyst wall was likely included during the DNA extraction procedure. Most of the *Sarcocystis* spp. positive DNA samples (8/9) were also derived from degenerated cyst lesions. The chronic inflammatory response around the cysticerci wall may have increased the likelihood of inadvertently including infected muscle tissue due to its adhesion to the surrounding structures, attributed to collagen infiltration (Dini *et al.* 2023).

Previous reports on bovine sarcocystosis in the country are limited. The scarcity of published literature in Kenya on sarcocystosis implies a low research focus consequently leading to potential latent economic losses and health implications. This is corroborated by the confirmation of infection in various livestock including cattle in other African countries such as Egypt (Ahmed *et al.* 2016; El-Kady *et al.* 2018; Gareh *et al.* 2020; El-Morsey *et al.* 2021), Algeria (Taibi *et al.* 2020), Ethiopia (Mekibib *et al.* 2019), Tunisia (Amairia *et al.* 2016), and Nigeria (Obijiaku *et al.* 2013). This assertion considers the parasite's relatively high occurrence in the screened samples (29%) despite the survey design not being optimized to detect *Sarcocystis* spp. *Sarcocystis cruzi* in cattle seldom manifests with a severe syndrome but is nonetheless associated with BEM, a cause of economic losses due to carcasses condemnation (Dubey 2015; Castro-Forero *et al.* 2022; Dini *et al.* 2023; Dubey and Rosenthal 2023). *Sarcocystis* infection due to *S. hirsuta* can confound *T. saginata* cysticerci, leading to false positives during routine meat inspection (Dubey *et al.* 1990; Ogunremi *et al.* 2004). Cattle infections with zoonotic *S. hominis* have been reported in Algeria (Nedjari 2003; Taibi *et al.* 2020), Tunisia (Amairia *et al.* 2016), and Nigeria (Obijiaku *et al.* 2013). *Sarcocystis hominis* infections in cattle are associated with environmental contamination due to open defaecation, similar to bovine cysticercosis (Castro-Forero *et al.* 2022). Conducting further investigations into the epidemiology of sarcocystosis in Kenya is crucial to preventing human infections and minimizing economic losses within the beef industry.

Conclusion

Study findings point towards the persistence of bovine cysticercosis in cattle within the study area with hot spots in Narok Town Ward. Further investigations are required to reveal source villages/sublocations for targeted control efforts. The cystic lesions were confirmed to be *T. saginata* cysticerci via molecular methods, which also revealed the possibility of other *Taenia* spp. being responsible for bovine cysticercosis in the study area. The presence of bovine cysticercosis suggests suboptimal sanitation practices, which may include open defecation due to the lack of toilets/pit latrines, the persistence of tribal taboos that discourage toilet use, and inadequate meat inspection protocols. Additionally, culinary habits such as consuming raw or partly cooked beef may be practiced in the area, potentially facilitating transmission.

Consequently, it is imperative to invest in initiatives aimed at reducing the transmission of *T. saginata.* Interventions at the slaughterhouse level may involve upgrading facilities to treat infected carcasses and enforcing strict adherence to the inspection regulations. On the community level, interventions include education on the prevention and control of *T. saginata* taeniosis; improving water, sanitation, and hygiene capacity; and ensuring proper cooking of meat. These measures can then be supported by diligently monitoring infection in cattle.

This is the inaugural report on *Sarcocystis* spp. infection in cattle in the country. Disseminating information regarding the presence of sarcocystosis in the Kenyan cattle population to meat inspectors, coupled with capacity building on its detection, is imperative. Other facets of the national veterinary health system could then look into comprehensive surveillance that will inform effective disease management, mitigation of production losses, and integration into disease control initiatives. The zoonotic nature of *S. hominis* and *T. saginata* necessitates a One Health approach in both surveillance and control.

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Competing interest. The authors declare none.

References

Abuseir S, Epe C, Schnieder T, Klein G, and Kühne M (2006) Visual diagnosis of *Taenia saginata* cysticercosis during meat inspection: is it unequivocal? *Parasitology Research* **99**, 405–409.

Ahmed AM, Elshraway NT, and Youssef AI (2016) Survey on *Sarcocystis* in bovine carcasses slaughtered at the municipal abattoir of El-Kharga, Egypt. *Veterinary World* **9**(**12**), 1461–1465. doi:10.14202/vetworld.2016.1461-1465.

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, and Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* **25**(**17**), 3389–3402. doi:10.1093/nar/25.17.3389.

Amairia S, Amdouni Y, Rjeibi MR, Rouatbi M, Awadi S, and Gharbi M (2016) First molecular detection and characterization of Sarcocystis species in slaughtered cattle in North-West Tunisia. *Meat Science* **122**, 55–59. doi: 10.1016/j.meatsci.2016.07.021.

Asaava LL, Kitala PM, Gathura PB, Nanyingi MO, Muchemi G, and Schelling E (2009) A survey of bovine cysticercosis/human taeniosis in Northern Turkana District, Kenya. *Preventive Veterinary Medicine* **89**, 197–204.

Castro-Forero SP, Bulla-Castañeda DM, López Buitrago HA, Díaz Anaya AM, Madeira de Carvalho LM, and Pulido-Medellín MO (2022) *Sarcocystis* spp., a parasite with zoonotic potential. *Bulgarian Journal of Veterinary Medicine* **25**(**2**), 175–186. doi:10.15547/bjvm.2019-0129.

Chege PM, Kimiywe JO, and Ndungu ZW (2015) Influence of culture on dietary practices of children under five years among Maasai pastoralists in Kajiado, Kenya. *International Journal of Behavioral Nutrition and Physical Activity* **12**, 131. doi: 10.1186/s12966-015-0284-3.

Dini FM, Caffara M, Jacinto JGP, Benazzi C, Gentile A, and Galuppi R (2023) A case of bovine eosinophilic myositis (BEM) associated with co-infection

by *Sarcocystis hominis* and *Toxoplasma gondii*. *Animals* **13**(**2**), 311. doi: 10.3390/ani13020311.

Dubey JP, **Udtujan RM**, **Cannon L, and Lindsay DS** (1990) Condemnation of beef because of *Sarcocystis hirsuta* infection. *Journal of the American Veterinary Medical Association* **196**(**7**), 1095–1096.

Dubey JP (2015) Foodborne and waterborne zoonotic sarcocystosis. *Food Waterborne and Parasitology* **1**, 2–11. doi:10.1016/j.fawpar.2015.09.001.

Dubey JP and Rosenthal BM (2023) Bovine sarcocystosis: *Sarcocystis* species, diagnosis, prevalence, economic and public health considerations, and association of *Sarcocystis* species with eosinophilic myositis in cattle. *International Journal for Parasitology* **53**(**9**), 463–475. doi:10.1016/j.ijpara.2022.09.009.

El-Kady AM, **Hussein NM, and Hassan AA** (2018) First molecular characterization of *Sarcocystis* spp. in cattle in Qena Governorate, Upper Egypt. *Journal of Parasitic Diseases: Official Organ of the Indian Society for Parasitology* **42**(**1**), 114–121. doi:10.1007/s12639-017-0974-7.

El-Morsey A, **Abdo W**, **Zaid AAA, and Sorour SSG** (2021) Morphologic and molecular identification of three macroscopic *Sarcocystis* species infecting domestic sheep (*Ovis aries*) and cattle (*Bos taurus*) in Egypt. *Parasitology Research* **120**(**2**), 637–654. doi:10.1007/s00436-020-07002-w.

Fayer R (2004) *Sarcocystis* spp. in human infections. *Clinical Microbiology Reviews* **17**(**4**), 894–902. doi:10.1128/CMR.17.4.894-902.2004.

Froyd G (1960) Cysticercosis and hydatid disease of cattle in Kenya. *Journal of Parasitology* **46**, 491–496.

Froyd G (1965) Bovine cysticercosis and human taeniasis in Kenya. *Annals of Tropical Medicine & Parasitology* **59**(**2**), 169–180. doi:10.1080/00034983.1965.11686296.

Gareh A, **Soliman M**, **Saleh AA**, **El-Gohary FA**, **El-Sherbiny HMM**, **Mohamed RH, and Elmahallawy EK** (2020) Epidemiological and histopathological investigation of *Sarcocystis* spp. in slaughtered dromedary camels (*Camelus dromedarius*) in Egypt. *Veterinary Sciences* **7**(**4**), 162. doi:10.3390/vetsci7040162.

Geysen D, **Kanobana H**, **Victor B**, **Rodriguez-Hidalgo R**, **De Borchgrave J**, **Brandt J, and Dorny P** (2007) Validation of meat inspection results for *Taenia saginata* cysticercosis by PCR-restriction fragment length polymorphism. *Journal of Food Protection* **70**(**1**), 236–240. doi:10.4315/0362-028X-70.1.236.

Grove DI (1990) *A history of human helminthology*. Wallingford, CAB International. p. 14.

Hailemariam Z, **Nakao M**, **Menkir S**, **Lavikainen A**, **Iwaki T**, **Yanagida T**, **Okamoto M, and Ito A** (2014) Molecular identification of species of Taenia causing bovine cysticercosis in Ethiopia. *Journal of Helminthology* **88**(**3**), 376–380. doi:10.1017/S0022149X13000138.

Hakeem SY, **Rashid A**, **Khuroo S and Bali RS** (2012) *Taenia saginata*: a rare cause of gall bladder perforation. *Case Reports in Surgery* **2012**, 1–3.

Jansen F, **Dorny P**, **Berkvens D**, **Van Hul A**, **Van den Broeck N**, **Makay C**, **Praet N**, **Eichenberger RM**, **Deplazes P, and Gabriël S** (2017) High prevalence of bovine cysticercosis found during evaluation of different post-mortem detection techniques in Belgian slaughterhouses. *Veterinary Parasitology* **244**, 1–6. doi:10.1016/j.vetpar.2017.07.009.

Jansen F, **Dorny P**, **Berkvens D, and Gabriël S** (2018) Bovine cysticercosis and taeniosis: the effect of an alternative post-mortem detection method on prevalence and economic impact. *Preventive Veterinary Medicine* **161**, 1–8. doi:10.1016/j.prevetmed.2018.10.006.

Jeon HK, **Kim KH, and Eom KS** (2007) Complete sequence of the mitochondrial genome of *Taenia saginata*: comparison with *T.* solium *and* T. asiatica. Parasitology International **56**(**3**), 243–246. doi:10.1016/j.parint.2007.04.001.

Kang'ethe EK (1995) The impact of meat inspection on the control of bovine hydatidosis in Kenya. *Bulletin of Animal Health and Production in Africa* **63**, 261–268.

KNBS (2019) *Narok County*. https://shorturl.at/emwAX (accessed 2 August 2023).

Lopes WD, **Santos TR**, **Soares VE**, **Nunes JL**, **Mendonça RP**, **de Lima RC**, **Sakamoto CA**, **Costa GH**, **Thomaz-Soccol V**, **Oliveira GP, and Costa AJ** (2011) Preferential infection sites of *Cysticercus bovis* in cattle experimentally infected with *Taenia saginata* eggs. *Research in Veterinary Science* **90**(**1**), 84–88. doi:10.1016/j.rvsc.2010.04.014.

Meat Control Act (2012). *Meat Control Act*. https://bit.ly/2Tyx9qx (accessed 24 June 2023).

Mekibib B, **Abdisa D**, **Denbarga Y, and Abebe R** (2019) Muscular Sarcocystis infection in ruminants slaughtered at municipality abattoir and selected hotels in Hawassa city, southern Ethiopia: prevalence and associated risk factors. *Veterinary Parasitology, Regional Studies and Reports* **18**, 100333. doi: 10.1016/j.vprsr.2019.100333.

Minozzo JC, **Gusso RLF**, **de Castro EA**, **Lago O, and Soccol VT** (2002) Experimental bovine infection with *Taenia saginata* eggs: recovery rates and cysticerci location. *Brazilian Archives of Biology and Technology* **45**(**4**), 451–455. doi:10.1590/S1516-89132002000600008.

Mwangi V, **Owuor S**, **Kiteme B, and Giger M** (2020) Beef production in the rangelands: a comparative assessment between pastoralism and large-scale ranching in Laikipia County, Kenya. *Agriculture* **10**(**9**), 399.

NCG (2016) *Narok County*. https://bit.ly/2tZ9roY (accessed 3 November 2020).

Nedjari M (2003) Untersuchungen zum Sarcocystiose-Vorkommen bei Tieren in Algerien [The occurrence of animal sarcocystiosis in Algeria]. *Berliner und Munchener tierarztliche Wochenschrift* **116**(**3–4**), 139–141.

Obijiaku IN, **Ajogi I**, **Umoh JU**, **Lawal IA, and Atu BO** (2013) Sarcocystis infection in slaughtered cattle in Zango abattoir, Zaria, Nigeria, *Veterinary World* **6**(**6**), 346–349. doi:10.5455/vetworld.2013.346-349.

Ogunremi O, **MacDonald G**, **Geerts S, and Brandt J** (2004) Diagnosis of *Taenia saginata* cysticercosis by immunohistochemical test on formalin-fixed and paraffin-embedded bovine lesions. *Journal of Veterinary Diagnostic Investigation: Official Publication of the American Association of Veterinary Laboratory Diagnosticians, Inc.* **16**(**5**), 438–441. doi:10.1177/104063870401600513.

OIE (2005) *WHO/FAO/OIE Guidelines for the surveillance, prevention and control of taeniosis/cysticercosis*. https://iris.who.int/bitstream/handle/10665/43291/9290446560_eng.pdf?sequence=1 (accessed 31 May 2020).

OIE (2021) *Cysticercosis* (*Including infection with Taenia solium*). https://shorturl.at/cwIR9 (accessed 29 July 2023).

Onyango-Abuje JA, **Hughes G**, **Opicha M**, **Nginyi KM**, **Rugutt MK**, **Wright SH, and Harrison LJ** (1996a) Diagnosis of *Taenia saginata* cysticercosis in Kenyan cattle by antibody and antigen ELISA. *Veterinary Parasitology* **61**(**3–4**), 221–230. doi:10.1016/0304-4017(95)00840-3.

Onyango-Abuje JA, **Nginyi JM**, **Rugutt MK**, **Wright SH**, **Lumumba P**, **Hughes G, and Johnstone P** (1996b) Seroepidemiological survey of *Taenia saginata* cysticercosis in Kenya. *Veterinary Parasitology* **64**, 177–185.

Rubiola S, **Civera T**, **Ferroglio E**, **Zanet S**, **Zaccaria T**, **Brossa S**, **Cipriani R**, **and Chiesa F** (2020) Molecular differentiation of cattle *Sarcocystis* spp. by multiplex PCR targeting 18S and COI genes following identification of *Sarcocystis hominis* in human stool samples. *Food and Waterborne Parasitology* **18**, e00074. doi:10.1016/j.fawpar.2020.e00074.

Taibi M, **Benatallah A**, **Zenia S**, **Aissi M**, **Harhoura K**, **Milla A**, **Guerchaoui A**, **Kaabeche I, and Khodja R** (2020) Prevalence of sarcosporidiosis in carcasses of cattle slaughtered at the Eucalyptus Slaughterhouse-Algeria. *Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca* **77** (**2**), 39–46. doi:10.15835/buasvmcn-vm:2020.0008.

Thitu A, **Kaseje M, and Augustine A** (2016) Factors influencing latrine coverage among the Maasai of Ildamat Location Kajiado District. *Developing Country Studies* **6**(**11**), 22–27.

Trachsel D, **Deplazes P, and Mathis A** (2007) Identification of taeniid eggs in the faeces from carnivores based on multiplex PCR using targets in mitochondrial DNA. *Parasitology* **134**(Pt **6**), 911–920. doi:10.1017/S0031182007002235.

Thrusfield M (2005) *Veterinary epidemiology*. 3rd edn. Oxford, Blackwell Science.

Uygur-Bayramiçli O, **Ak O**, **Dabak R**, **Demirhan G, and Ozer S** (2012) *Taenia saginata* a rare cause of acute cholangitis: a case report. *Acta Clinical Belgica* **67**(**6**), 436–437.

Wanzala W, **Onyango-Abuje JA**, **Kang'ethe EK**, **Ochanda H, and Harrison LJS** (2002) Serodiagnosis of bovine cysticercosis by detecting live *Taenia saginata* cysts using a monoclonal antibody-based antigen-ELISA. *Journal of the South African Veterinary Association* **73**, 201–206.

Wanzala W, **Onyango-Abuje JA**, **Kang'ethe EK**, **Zessin KH**, **Kyule NM**, **Baumann MP**, **Ochanda H, and Harrison LJ** (2003) Control of *Taenia saginata* by post-mortem examination of carcasses. *African Health Sciences* **3**(**2**), 68–76.

WHO (2016) *Neglected zoonotic diseases*. https://bit.ly/2EYpXMc (accessed 10 March 2023).

https://doi.org/10.1017/S0022149X24000129 Published online by Cambridge University Press