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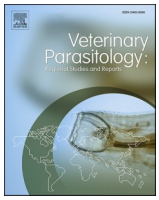

Original Article

The potential role of roaming dogs in establishing a geographically novel life cycle of taeniids (Echinococcus spp. and Taenia spp.) in a non-endemic area

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ABSTRACT

Introduction: Cystic Echinococcosis (CE) is endemic in humans and livestock in many pastoral communities in Kenya. The distribution of the disease is enhanced by several factors, including livestock trade, which has allowed for the spread of CE to non-endemic areas such as western Kenya. Dogs' roaming behaviour, with consequent contamination of the environment with intestinal parasites, could then lead to parasite establishment. This study examined dogs' infection levels with taeniid eggs and their potential role in contaminating the environment with intestinal parasites.

Methodology: We selected sixteen ruminant slaughterhouses in Busia and Bungoma Counties, and around each slaughterhouse we identified ten homesteads owning free-roaming dogs. We administered a questionnaire on dog management practices to the homestead owner and collected a faecal sample from the dog's rectum. In homesteads around 8 of the 16 slaughterhouses, we collared dogs with a GPS tracker to assess their movement patterns. The faecal samples were examined microscopically following zinc-chloride sieving-floatation technique for the presence of taeniid eggs and other canine intestinal parasites. Polymerase Chain Reaction – Restriction Fragment Length Polymorphism of NADH dehydrogenase subunit 1 gene and sequencing were used to confirm taeniid eggs identified during microscopy. Additionally, the Coproantigen-ELISA was used to detect the presence of taeniid antigen in a sub-set of the faecal samples.

Results: Helminths detected in the 155 dogs sampled included hookworms ($n = 92$; 59.4%), ascarids ($n = 15$; 9.7%), and taeniids (n = 1; 0.6%). Through Copro-PCR, 13 eggs extracted from the sample of the only taeniid infected dog were sequenced and identified as *E. canadensis* (G6/7) [n = 1], *Taenia multiceps* [n = 1], and *Taenia serialis* [$n = 6$]; the remaining were indeterminate. Of the 77 faecal samples tested for *E. granulosus* sensu *lato* (*s. l.*) with the Copro-ELISA, 64 (83.1%) were negative, 12 (15.6%) were positive, while 1 (1.3%) was suspicious. The dogs travelled a median of 13.5 km daily, and 28 dogs visited the slaughterhouses during the 5-day recording period.

Conclusion: The results indicate a relatively high carriage of zoonotic parasites by free-roaming domestic dogs in western Kenya, which poses a risk to human and livestock populations. We report for the first time a domestic lifecycle of *Echinococcus canadensis* and *Taenia multiceps* in western Kenya, as well as a presumptive sylvatic cycle of coenurosis by *T. serialis*. We recommend an extensive and ongoing Copro-antigen survey of dog faeces, broader assessment of dog parasites with zoonotic potential, adherence to slaughterhouse management practices, and dog-ownership programmes to highlight the importance of deworming and restricted dog movements.

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1. Introduction

The relationship between dogs, humans, and domestic livestock dates to prehistoric times (Morey, 1994; Shipman, 2009). Besides the many benefits that humans get from owning dogs, there are also associated health risks due to possible passage of disease agents such as echinococcosis, toxocariasis, rabies, leishmaniasis, plague, Chagas disease, and some bacterial infections (Chomel and Sun, 2011; Deplazes et al., 2011). The presence of dog faeces in public areas allows for the transmission of both dog to dog, and dog to human infections, with the latter leading to public health concerns (Macpherson, 2012). Taeniid tapeworms are important parasites that infect people and animals globally. Over thirteen Taeniid related genera are known but only four are taxonomically recognized, namely *Hydatigera* spp., *Taenia* spp., *Versteria* spp., and *Echinococcus* spp. (Nakao et al., 2013).

Echinococcus granulosus sensu lato (s. l.). consists of at least five genetically distinct species, namely, *E. granulosus* sensu stricto (G1, G3), *E. equinus* (G4), *E. ortleppi* (G5), *E. canadensis* (G6/G7 and G8/G10), and *E. felidis* (Nakao et al., 2013). The parasite *Echinococcus granulosus* s. l. causes the intestinal type of echinococcosis among carnivores. The domestic dog *Canis lupus familiaris* is the dominant definitive host, though in some regions wild carnivores may accidentally contribute to the maintenance of the *Echinococcus granulosus* s. l. life cycle (Thompson, 2017). *E. granulosus* s. l. has a cosmopolitan distribution, with a domestic life cycle that alternates between dogs as definitive hosts, and a wide range of ungulates as intermediate hosts (Moro and Schantz, 2009). An increase in the dog population, and their capacity to roam unrestricted, especially around slaughterhouses, could contribute to *E. granulosus* s. l. transmission (Otero-Abad and Torgerson, 2013). This is further exacerbated by the common practice of feeding offal to dogs, particularly after unregulated livestock slaughter (Torgerson, 2013; Alishani et al., 2017). Canines get infected when they consume hydatid cysts with viable protoscoleces contained in the offal (McManus et al., 2003). *Echinococcus* spp. eggs may be passed through the faeces of an infested dog and be taken up by grazing sheep or other ungulates where they cause Cystic Echinococcosis (CE). Humans may also get infected by inadvertently ingesting tapeworm eggs excreted by infected carnivores (Torgerson and Heath, 2003).

Definitive diagnosis for *Echinococcus* spp. eggs in dogs and other definitive hosts has been challenging due to the indistinguishable nature of *Taenia* spp. and *Echinococcus* spp. eggs. Purgation of dogs using arecoline hydrobromide has been used to help activate bowel evacuation and collection of faecal content for examination. However, this method has the risk of environmental contamination (Craig et al., 2015). Another method is the microscopic examination of dogs' small intestines during necropsy, but the parasite can be missed when there is a low worm burden. The Coproantigen-ELISA method allows for the detection of specific antigens of *Echinococcus* spp. in faecal samples (Allan et al., 1992; Deplazes et al., 1992; Allan and Craig, 2006; Van Kesteren et al., 2013), and studies have shown the sensitivity and specificity of Copro-ELISA to be above 80% (Christofi et al., 2002). This diagnostic method is advantageous as numerous samples can be examined by one person in a day and can therefore be used to screen individual dogs in populations. Lastly, *E. granulosus* s. l. infection can be confirmed or excluded by the PCR test which is a highly sensitive and specific secondary test (Eckert and Deplazes, 2004). Copro-PCR techniques have been used to identify DNA, either from faecal material or eggs, from definitive hosts (Mathis et al., 1996; Deplazes et al., 2003). This method is superior in that it enables the detection of specific species using DNA isolated from individual eggs (Hüttner et al., 2008; Mulinge et al., 2018).

Western Kenya was previously considered as non-endemic for *Echinococcus* spp. However, documentation of a net inward trade in livestock destined for the local and growing beef market, numerous cases of open disposal of offal within slaughterhouse compounds, continuous scavenging opportunities by roaming dogs, as well as frequent interactions between humans, livestock, and dogs (Falzon et al., 2021; Muinde et al., 2021), indicate the possible establishment of the parasite's life cycle in this study area, with consequent public health implications. Understanding the dog population size, basic dog ecology and behaviour, and infection rates with *Echinococcus* spp. are therefore needed to guide the implementation of efficient CE control programmes (Van Kesteren et al., 2013).

The aim of this study was to determine whether *Echinococcus* spp. infection is present in dogs in the study area and, more specifically, to: (i) describe the frequency of management practices conducive to CE transmission and establishment, (ii) screen dogs for *Echinococcus* spp. infection and other intestinal parasites using microscopy, Copro-PCR, and Copro-ELISA, and (iii) determine whether dog movement parameters are associated with *Echinococcus* spp. infection exposure.

2. Methods

2.1. Study area

This study was conducted in the Counties of Busia and Bungoma in western Kenya. The annual average rainfall levels are 1742 mm in Busia and 1102 mm in Bungoma (Climate data, 2022). Busia County has a population of 893,681 and sits in an area of 1696 km^2, while Bungoma County has a population of 1,670,570, and sits in an area of 2069 km^2 (Brinkhoff, 2020).

Sixteen sites, eight in each County, were selected for inclusion in this study. In Busia County the sites were: Amerikwai, Amukura, Bumala, Busia town, Funyula, Malaba, Mudembi and Nambale; in Bungoma County these were: Chwele, Kamukuywa, Kimilili, Kimwanga, Mayanja, Misikhu, Naitiri and Wanaichi. The sites were selected so as to capture the demographic diversity within each County and included rural, peri-urban, and urban centers. Furthermore, the selection of each site was based on the presence of a ruminant slaughterhouse, while the selection of homesteads was based on the presence of roaming owned dogs. Data were collected in May–June 2017 (Busia County) and May–June 2018 (Bungoma County).

2.2. Homestead and dog selection

At each site, the ruminant slaughterhouse was our departure point. From there, and with the help of a village elder or local chief, we identified ten homesteads within a maximum of 1.5 km radius that owned a free-roaming dog (i.e., a dog that was allowed to roam freely for any amount of time during the day and/or night). Dogs that were younger than 6 months or that appeared in ill health were not considered eligible. At each homestead that met our inclusion criteria, we explained to the homestead head or another adult present the purpose of the study and what participation would entail. If they agreed to participate, a consent form was signed, and an electronic questionnaire was administered. The structured questionnaire, which was administered by one of the investigators in Kiswahili, contained close-ended multiple-choice questions on homestead and dog demographics, and dog and livestock management practices.

Next, the dog was manually restrained, and a faecal sample was collected from the dog's rectum. Samples were collected by a qualified veterinarian using a lubricated glove and stored in a bar-coded 20 ml faecal pot (Greiner Bio-one). If the participants owned more than one eligible dog, the selection of the dog to be included in the study was based on the owner's recommendations and ease of handling.

In Busia County, and as part of a complementary study on dog roaming behaviour (Muinde et al., 2021), the movements of the sampled dogs were tracked for five days. A Mobile Action i-gotU GT-600 GPS logger placed in a customized casing was strapped around each dog's neck. The GPS loggers were set to record the dogs' locations every minute for a period of five days whenever the dog was in motion. Afterwards, the collars were retrieved. The time and date when the collar was put on the neck of the dog, and when it was later retrieved, were

recorded. The GPS coordinates of the site slaughterhouse and the participating homesteads were also recorded.

2.3. Laboratory analysis

The faecal samples were maintained at room temperature during transport to the International Livestock Research Institute Zoonoses laboratory in Busia. Samples collected from sites in Busia County were separated into two aliquots. One aliquot was preserved in 70% ethanol for microscopic examination of taeniid eggs through a floatation technique employing differential sieving, while the second aliquot was placed in 0.3% PBS Tween buffer with 10% formalin for Copro-ELISA taeniid antigen detection. Samples collected from Bungoma sites were not aliquoted as only microscopy and Copro-PCR was conducted.

2.3.1. Isolation of taeniid eggs from dog stool using zinc chloride flotation-sieving method

Ethanol was drained off and 2 g or 2 ml of the faecal content was transferred into 15 ml Falcon tubes. The faecal content was concentrated by slight modification of the zinc chloride flotation-sieving technique, as previously described by Mathis et al. (1996), to help recover any taeniid eggs present. The protocol involved washing the sample with 8 ml of distilled water. The rinsing water was then drained off to obtain faecal pellets. The faecal pellets were rinsed with 1 X PBS 0.3% Tween 20 and one part of the obtained pellets was mixed with four parts of the zinc chloride solution (specific density 1.45). After flotation, the top layer, where any taenid eggs are expected, was collected and filtered using first a 50 μm sieve, followed by a 22 μm sieve (Franz Eckert GmbH, Germany). The eggs were then washed off from the 22 μm sieve into a 15 ml Falcon tube using distilled water. The samples were centrifuged, and the eggs were collected using a Pasteur pipette and stored in 2 ml micro centrifuge tubes at 4 °C. The sample was then examined under the microscope at ×10 magnification for the presence of taeniid eggs and other canine intestinal parasites. In the case of a taeniid positive sample, as many eggs as possible were isolated and transferred into individual 0.2 ml PCR tubes containing 10 μl of 0.02 M NaOH solution. The eggs were lysed at 99 °C for 10 min in a thermocycler, and the lysate was used as a template for nested PCR.

2.3.2. Nested polymerase chain reaction (PCR) for taeniid eggs

A nested PCR based on the NADH dehydrogenase subunit 1 (NAD 1 gene) was used to detect taeniid eggs. This tool amplifies a region of approximately 545–552 base pairs (bp). In a 25 μl reaction: 2 μl of the taeniid lysate was used, 1 × DreamTaq Green Buffer (containing 20 mM Tris-HCl at pH of 8.0), 1 mM DTT, 0.1 mM EDTA, 100 mM KCl, 0.5% (*v*/v) Nonidet P40, 0.5% (v/v), Tween 20 (ThermoScientific), 0.2 mM dNTPs, 0.25 μM of forward and reverse primers, 2 mM $MgCl_2$ and 0.625 units of DreamTaq Green DNA Polymerase (ThermoScientific). The PCR cycling conditions were: 5 min of initial denaturation at 94 °C, 40 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min, and a final extension at 72 °C for 5 min. The same conditions were used for secondary PCR except that 2 μl of primary PCR product were used as a template in the secondary PCR. The primers used in the primary PCR were: Nadnest A (TGT TTT TGA GAT CAG TTC GGT GTG) and NAD C (CATAAT CAA ACG GAG TAC GAT TAG), while the primers used for the secondary PCR were Nadnest B (CAG TTC GGT GTG CTT TTG GGT CTG) and NAD D (GAG TAC GAT TAG TCT CAC ACA GCA) (Hüttner et al., 2008). PCR products were resolved on 2% agarose gel and visualized under UV following ethidium bromide staining. Restriction digestion was performed on the PCR amplicons in a 20 μl volume reaction containing of 10 μl of the secondary PCR product, 0.5 μl (5 U) *Hph*I endonuclease (Thermo Scientific), 1 × Buffer and 7.5 μl of nuclease free water. The digestion reactions were performed overnight at 37 °C in an incubator and resolved on 3% agarose gel stained with ethidium bromide. To identify *Echinococcus* spp. known positive controls of *E. granulosus* sensu stricto, *E. ortleppi*, *E. canadensis* (G6/7) and *E. felidis* were resolved alongside the test samples as explained by Mulinge et al. (2018).

2.3.3. Copro-antigen capture ELISA for dog samples from Busia County

The second aliquot of dog faecal samples collected from Busia County and that was placed in PBS Tween extract was shaken vigorously, centrifuged at 2500 ×*g* for 10 min at room temperature, and the supernatant collected and kept at −20 °C. The preparation was shipped to the University of Salford, UK, where it was analysed by the Copro-antigen capture ELISA detection method, as described by Van Kesteren et al. (2013). A cut-off was calculated from 0.14 Optical Densities (ODs), and all samples with ODs between 0.14 and 0.17 were ruled suspicious, while those with ODs above 0.17 were ruled as positive.

2.4. Data cleaning and analysis for Busia County

The questionnaire data were entered into an electronic database using Open Data Kit software and downloaded as a Microsoft Excel file. Laboratory data were first recorded on paper-based records, then entered manually into an Excel spreadsheet by one investigator and cross-checked by a second investigator. Questionnaire and laboratory data were merged, cleaned, and summarized using Stata Statistical Software: Release 14 (College Station, TX: StataCorp LP).

The GPS data collected by the loggers were downloaded using the free *@trip pc* (http://www.atrip.com) software, and then exported as individual .csv files into R statistical software (https://www.R-project.org, version 3.4.0) (R Core Team, 2013) for further cleaning and analysis. The GPS data were cleaned and analysed as described by Dürr and Ward (2014). Individual summaries of GPS fixes were produced for each dog, and these were used to estimate the daily mean distance travelled (km), and the core and extended Home Range (ha); the latter two represent the 50% and 95% area traversed by the dog, respectively, during the recording period.

The straight-line distance between each homestead and the nearest slaughterhouse was estimated. To determine the proportion of time each dog spent within their respective homestead, a standardized area with a 20 m radius around each homestead was defined as the homestead area, and the time spent within this area was then estimated as a proportion of the overall recording period. The proportion of time each dog spent within the slaughterhouse compound was estimated based on the time spent within a defined area around the slaughterhouse. Since the slaughterhouses ranged in size, different areas were specified for each slaughterhouse footprint based on our field observations and measurements. These included: 10 m^2 for Mudembi; 15 m^2 for Amukura; 25 m^2 for Amerikwai; 30 m^2 for Bumala, Funyula and Nambale; 40 m^2 for Malaba; and 45 m^2 for Busia town.

2.4.1. Regression analysis for dogs and exposure to CE in Busia County

A regression analysis was conducted to evaluate the potential association between the dogs' movements and their exposure to *Echinococcus* infection. The outcome of interest was the Copro-antigen capture ELISA test result (dichotomized as Positive/Negative), while the predictors of interest included the dog's daily distance travelled (km), the size of the core and extended Home Range (ha), and the proportion of time spent within the homestead and slaughterhouse compound (%). The dog's age and sex were also assessed for possible confounding effects. All continuous variables were checked for normality and transformed or categorized as needed. The unconditional associations between the predictor variables and the outcome of interest were investigated, with site included as a random effect to account for spatial clustering. Variables that were marginally associated ($p < 0.2$) with the outcome were included in a mixed-effects logistic regression model. Given the limited sample size, statistical significance was considered when the *p*-value was ≤0.1.

3. Results

3.1. Questionnaire data

In total, 159 homesteads were included in this study: 79 and 80 from Busia and Bungoma counties, respectively. The median number of people living in each homestead was 7 (ranging between 2 and 23), while the median number of dogs in each homestead was 2 (ranging between 1 and 10).

Half of the participants ($n = 80$; 50.3%) said their dogs had access to the habitable compartments of the homestead, and many also reported that their dogs accompanied them to work ($n = 97$; 61.0%). Most participants and their family members played with their dogs regularly ($n = 114$; 71.7%), and the observation of other dogs within their homestead compound was a common occurrence ($n = 136$; 85.5%).

Most participants ($n = 108/157$; 68.8%) reported that they did not feed their dogs internal organs; the remaining 49 participants who reported feeding their dogs internal organs obtained these from the slaughterhouse ($n = 42$) or a butchery ($n = 7$). Internal organs were usually cooked prior to feeding them to the dogs ($n = 39/47$; 83.0%), and only 8 participants (17.0%) said they fed their dogs raw internal organs.

Livestock were present on 127 (81.4%) of the homesteads; these included cattle ($n = 112$), goats ($n = 53$), pigs ($n = 31$), and sheep ($n = 23$). Home slaughter was practiced on 30 (23.6%) of the homesteads with livestock; of these, 19 (63.3%) did not have the home-slaughtered carcass inspected by a meat inspector prior to consumption. Livestock that died on the homestead were either buried ($n = 30/121$; 24.8%), fed to the dogs ($n = 19/121$; 15.7%), eaten by the owners and family members (n = 5/121; 4.1%) or skinned (n = 2/121; 1.7%); 64 participants (52.9%) said no animals had died on their homestead. Dogs frequently interacted with the livestock present on the homestead ($n = 105$; 72.9%), and some of the dogs ($n = 57$; 40.1%) also accompanied the livestock when grazing.

Dog faeces were disposed of in the garden or compost pit ($n = 45/142$; 31.7%), buried in the soil ($n = 27/142$; 19.0%), or left where deposited (n = 19/142; 13.4%); only 36 participants (25.4%) reported depositing the faeces in toilets where it could not contaminate the surroundings. While 70 (44.3%) participants said they dewormed their dogs, only 27 of these dewormed their dogs every 3 months. When asked which drugs they used for deworming, 1 reported using albendazole, 1 reported using levamisole, and 2 reported using a niclosamide-oxybendazole compound product; the remaining participants could not remember or did not know the name of the deworming drug they used.

3.2. Dog sampling

The majority of the 159 dogs included in the study were adult dogs (i.e., 1–5 years; $n = 113$; 71.1%) with fewer older (i.e., >5 years; $n = 32$; 20.1%) and younger (i.e., <1 year; $n = 14$; 8.8%) dogs. The dogs' sex distribution was roughly equal, with 88 (51.6%) female dogs, of which 9 were spayed, and 77 (48.4%) male dogs, of which 27 were castrated. Eighty dogs out of 159 interacted closely with humans at the homesteads. Moreover, 105 dogs from the 144 homesteads that also had livestock, interacted closely with the livestock.

Faecal samples were available for 155 dogs. Of these, 102 (65.8%) had one or more intestinal parasites detected on microscopy. The most frequently detected were hookworms ($n = 92$; 59.4%) and ascarids ($n = 15$; 9.7%). Other identified parasites included *Spirometra* spp. ($n = 2$), *Trichuris* spp. (n = 1), and *Entamoeba* spp. (n = 1); unknown ova were detected in three faecal samples.

One dog from Mayanja in Bungoma County showed infection with taeniid eggs. Over 50 eggs were isolated microscopically from the faecal sample of which 13 eggs were positively identified by PCR. Upon sequencing they were further specified to be *E. canadensis* (G6/7) (n = 1), which is a member of *Echinococcus granulosus* sensu lato group, the zoonotic *Taenia multiceps* (n = 1), and zoonotic *Taenia serialis* (6); the remaining sequences were indeterminate. Of the 77 samples from Busia tested by Copro-antigen ELISA, 64 (83.1%) were negative, 12 (15.6%) were positive, while 1 (1.3%) was classified as suspicious. Of the 13 dogs that were either Coproantigen-ELISA positive or suspicious, 11 belonged to homesteads that also owned livestock and, of these, 9 were reported to regularly interact with the livestock.

3.3. Movement data and regression analysis

Movement data were available for 73 of the 79 dogs sampled in Busia; of these, 71 also had Copro-antigen ELISA results and were therefore included in the analysis. The median recording period for these 71 dogs was 122.6 h (ranging between 25.6 and 151.6 h). The dogs travelled a median of 13.5 km daily (ranging between 2.0 and 24.5 km) and traversed median core and extended Home Ranges of 0.4 and 9.4 ha, respectively.

The median distance between each homestead and the slaughterhouse was 443.6 m (ranging between 14.1 and 1077.7 m). The majority (>95%) of the homesteads included in the study were therefore within a 1 km distance from the slaughterhouse. The dogs spent a median of 30.2% of the recording period within their homestead, though this ranged between 0.2 and 79.4%. Of the 71 dogs, 43 (60.6%) did not have any GPS recordings within the slaughterhouse area; the remaining 28 (39.4%) dogs spent between 0.3 and 24.6% of the recording period within the slaughterhouse compound. There was a weak inverse correlation ($\rho = -0.4$) between the household-slaughterhouse distance and percentage time spent within the slaughterhouse compound.

While daily distance travelled was normally distributed, both the core and extended Home Ranges were not and were transformed using a square-root and logarithmic transformation, respectively. The proportion of time spent at the household was normally distributed, but the proportion of time spent at the slaughterhouse was right-skewed and no transformation improved its distribution. It was therefore categorized as follows: 0% = no; >0% = yes. For the purpose of the regression analysis, the young (<1 year) and adult (1–5 years) age groups were collapsed into one category as there were only 5 dogs in the young category, and previous analysis (Muinde et al., 2021) had shown that their behaviour did not differ significantly from that of adult dogs; the Copro-antigen ELISA suspicious result was assumed to be positive.

In the univariable analysis, only the variable 'spent time at the slaughterhouse' was marginally associated with the outcome of interest (Table 1). Specifically, 7/28 (25.0%) of the dogs that spent time at the slaughterhouse were copro antigen-positive, compared to 4/43 (9.3%) of the dogs that did not spend any time at the slaughterhouse. Consequently, the odds that dogs spending spent time at the slaughterhouse were copro-positive was 3.25 (0.85–12.39), compared to that of dogs that did not spend time at the slaughterhouse. Since only the variable 'spent time at the slaughterhouse' remained statistically significant when assessed for conditional associations with the outcome, no final multivariable analysis is presented.

4. Discussion

This study sought to determine the infection levels of taeniid cestodes and other parasites infecting dogs in western Kenya using various targeted diagnostic techniques. We also sought to explore whether the movement behaviour of dogs that live in homesteads near slaughterhouses is associated with possible *Taenia* spp. and *E. granulosus* s. l. parasite burden. This, in turn, could shed light on the potential role of *Canis lupus familiaris* (domestic dogs) in contaminating the environment with infectious parasites and the potential propagation of a novel parasitic life cycle of CE given the reported interactions between dogs and livestock. This study would partially guide other studies in countries and regions with undocumented dog parasite transmitted diseases. The

Table 1
Descriptive statistics and unconditional associations between explanatory variables and coproantigen results of 71 free-roaming owned dogs in Busia County, western Kenya.

Explanatory variable	Median (25th - 75th Percentile)	Odds Ratio	95% Confidence Intervals	P-value
Daily distance travelled (km)	13.5 (11.2–16.3)	0.99	0.86–1.15	0.94
Core Home Range (ha)	0.4 (0.3–0.7)	0.53	0.04–7.32	0.64
Extended Home Range (ha)	9.4 (4.6–17.5)	1.15	0.55–2.40	0.71
Time spent in household (%)	30.2 (17.0–45.9)	1	0.96–1.03	0.93
Time spent in slaughterhouse		3.25	0.85–12.39	0.08*
Yes	7/28 (25.0%)			
No	4/43 (9.3%)			
Dog age		2.13	0.47–9.56	0.33
≤5 years	8/59 (13.6%)			
>5 years	3/12 (25.0%)			
Dog sex		0.61	0.16–2.31	0.47
Female	7/38 (18.4%)			
Male	4/33 (12.1%)			

study is timely as over the last few years, an increasing live animal trade of ruminants destined for slaughter and location consumption has developed in the study region, with significant movements of animals in to western Kenya originating in more northerly regions of the country (Watson and Binsbergen, 2008) where these helminths are known to be endemic (Griffith et al., 2020).

The close dog-human-livestock interactions in western Kenya, coupled with unsafe disposal of dog faeces, facilitate disease transmission between dogs, ruminants and to humans. Dogs act as definitive hosts of several helminthic worms with the potential to contaminate the environment by shedding worms or eggs which subsequently mature in the soil (FAO, 2014). In this study, 47% (70/148) of the participants reported deworming their dogs, though fewer did so regularly. The high frequency of hookworms identified in the sampled dogs could be due to this poor uptake of deworming practices, with a consequent heightened risk of zoonotic disease transmission.

Dogs' infection with *Echinococcus* spp. and *Taenia* spp. has been reported previously from different parts of Kenya (Mulinge et al., 2018; Mulinge et al., 2020; Nungari, 2020). However, no study had been done in western Kenya, a region that lies between the two *Echinococcus* spp. prevalent regions of Turkana in the north and Maasailand in the south. The dog infection with *E. canadensis* (G6/7) reported in this study is a notable finding, further supporting the emergence of this disease through the establishment of a life cycle of *E. granulosus s.* l. in the western Kenya region. Mulinge et al. (2018) reported finding of *E. canadensis* (G6/7) from dogs in Turkana, Maasai Mara, and Isiolo regions of Kenya, as well as *E. granulosus s. s., E. felidis and E. ortleppi*. Similarly, Kagendo et al. (2014) reported *E. granulosus s. s.* and *E. felidis* from the sylvatic cycle in Kenya (lions and hyena). Most recently, Nungari (2020) reported the presence of *E. equinus*, *E. felidis* and four *Taenia* spp. *(T. hydatigena*, *T. multiceps T. ovis* and unknown *Taenia* spp.*)* from dogs in Kajiado west in Maasailand, Kenya. This study records the second instance finding of *E. canadensis* in dogs (Mulinge et al., 2018) from a region previously considered non-endemic due to lack of reports on *Echinococcus* spp. and *Taenia* spp. infection.

The finding of eggs of *T. multiceps* indicates the presence of coenurosis in livestock in western Kenya, while *T. serialis* in dog faeces as confirmed through sequencing may indicate the presence of a sylvatic cycle in the region, triggered potentially by dog predation of rabbits or wild hares. These zoonotic parasites pose the risk of coenurosis in humans (Deplazes et al., 2019). Mulinge et al. (2020) has reported seven different *Taenia* spp. among domestic dogs from other regions in Kenya. The establishment of a parasitic life cycle in western Kenya was indicated by the infection of the Mayanja dog with *E. canadensis* (G6/7) and *Taenia* spp. as shown by the Copro-PCR method. Mitochondrial DNA markers aid in demonstrating multi-species infection of taeniids in individual canids (Štefanić et al., 2004; Xiao et al., 2006; Zhang et al., 2006; Schurer et al., 2014), as confirmed in this study. *Echinococcus granulosus s.* l. in western Kenya would be maintained through a putative synanthropic life cycle involving dogs and sheep/cattle, similar to what is experienced in the Turkana region of East Africa. The same is reported in studies in Algeria (Kouidri et al., 2012) and Libya (Buishi et al., 2005).

The Coproantigen ELISA technique detects the presence of a specific antigen in a sample. It involves the use of a capture and a detection antibody where the intestinal release of metabolic products by the parasite are immunologically detected, even in instances where eggs are undetected in the faeces (Allan and Craig, 2006). The presence of 15.6% coproantigen-ELISA positive dogs in Busia, even without concomitant detection of taeniid eggs by flotation and microscopy, may denote previous infections that had not been treated, though one must note that the Copro-antigen method does not discriminate between *Echinococcus* spp. and *Taenia* spp. infections. The Copro-antigen technique has been successfully used for surveillance of infection levels in dogs in Brazil, 11% (de la Rue, 2008), Chile, 3.5–11.7% (Acosta-Jamett et al., 2010), Peru, 46% (Moro et al., 1999), Uruguay, 4.3% (Irabedra and Salvatella, 2010), Turkey, 8.9% (Guzel et al., 2008), and China 18% in 2008 and 15.9% in 2009 (WHO, 2011). Since the Copro-antigen protocol is not yet locally available in Kenya, this study was only able to apply this diagnostic technique to a small sub-sample of the surveyed dog population after export of the processed samples. A more extensive surveillance effort is undoubtably necessary, and development of a local Copro-antigen ELISA surveillance protocol on dog faeces would aid effective surveillance toward eventual control of CE.

The density of the definitive hosts is key to transmission efficiency. The continuous growth in the dog population in Kenya (Kitala et al., 2001), as well as their roaming / straying nature, has become an impediment in the control of diseases for which they are vectors. The home range of the dog, together with its infection intensity, is epidemiologically significant in determining the dispersal distance of the parasite, thus facilitating transmission. The dogs in this study had a core home range of 0.4 ha and a median daily travel distance of 13.5 km. The dogs may roam to scavenge for food, or when accompanying owners in their daily engagements (Muinde et al., 2021). All these sites that dogs interact with can therefore act as potential point sources for spread of emerging parasites such as *E. granulosus s.* l. in this environment, with the potential for further spread through dog movements to neighbouring areas.

Analysis of movement data indicated that some dogs visited the slaughterhouse, and these dogs had a higher odds of being copro-antigen positive, though these results were only marginally significant. Other studies indicate that dogs can get infected with CE in a slaughterhouse disposal area where condemned organs are readily available (Ajlouni et al., 1984; Mulinge et al., 2018). Some of the smaller slaughterhouses in the study area did not have secured condemnation pits, providing dogs with potential access to infected offal as food. Cases of home slaughter were reported, and two-thirds of the slaughter done at home was not inspected by an authorised meat inspector, posing a risk for disease transmission. Some slaughterhouses did not meet the standard operational requirements and lacked appropriate facilities and hygiene, and owned dogs could easily access them to scavenge raw offal. Improved slaughterhouse infrastructure coupled with adherence to regulations governing condemnation are therefore key legislative requirements for effective control of CE.

Formalised and effective surveillance for *E. granulosus s.* l. transmission would require frequent Copro-antigen surveys of dogs (Craig et al., 2015). Though Copro-ELISA is a common protocol, the lack of established capacity in East Africa restricts routine and cost effective surveys. Building capacity for Copro-ELISA would allow for the establishment of baseline testing, followed by the more sophisticated Copro-

PCR and sequencing methods that help in species specificity. Promulgation of education programmes on control of echinococcosis and uninterrupted deworming of dogs with praziquantel are also recommended to disrupt establishment of the life cycles of CE and other *Taenia* spp. Such a surveillance and control programmes could complement the National Rabies control strategy (Bitek et al., 2018) geared toward elimination of dog-mediated human rabies, through the implementation of an intersectoral approach of dog population treatment and management leading to the coordinated control of multiple zoonoses.

Ethical statement

Ethical approval for this study was granted by the International Livestock Research Institute Institutional Animal Care and Use Committee (Reference number ILRI-IACUC-2017-10) and the Institutional Research Ethics Committee (IREC Reference No. ILRI-IREC-2018-02), which are approved by the Kenyan National Commission for Science, Technology and Innovation and also approved by the Federal wide Assurance for the Protection of Human Subjects in the USA. Approval to conduct this work was also granted by the Social Science Research Ethical Review Board at the Royal Veterinary College (Reference number URN SR2017-1084), and by the Department of Veterinary Services and relevant offices at the devolved government level.

Consent for publication

Not applicable.

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Author contributions

TM, AWTM, JM, EMF, LCF conceived and designed the study; TM, PM, LCF collected the data; TM, EM, LG, JMB, MR, LCF analysed and interpreted the data; TM, LCF wrote the first draft, and all authors revised and approved the final version.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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