Helminth parasites in the endangered Ethiopian wolf, *Canis simensis*

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Abstract

Ethiopian wolves, *Canis simensis*, are an endangered carnivore endemic to the Ethiopian highlands. Although previous studies have focused on aspects of Ethiopian wolf biology, including diet, territoriality, reproduction and infectious diseases such as rabies, little is known of their helminth parasites. In the current study, faecal samples were collected from 94 wild Ethiopian wolves in the Bale Mountains of southern Ethiopia, between August 2008 and February 2010, and were screened for the presence of helminth eggs using a semi-quantitative volumetric dilution method with microscopy. We found that 66 of the 94 faecal samples (70.2%) contained eggs from at least one group of helminths, including *Capillaria*, *Toxocara*, *Trichuris*, ancylostomatids, *Hymenolepis* and taeniids. Eggs of *Capillaria* sp. were found most commonly, followed by *Trichuris* sp., ancylostomatid species and *Toxocara* species. Three samples contained *Hymenolepis* sp. eggs, which were likely artefacts from ingested prey species. Four samples contained taeniid eggs, one of which was copro-polymerase chain reaction (copro-PCR) and sequence positive for *Echinococcus granulosus*, suggesting a spillover from a domestic parasite cycle into this wildlife species. Associations between presence/absence of *Capillaria*, *Toxocara* and *Trichuris* eggs were found; and egg burdens of *Toxocara* and ancylostomatids were found to be associated with geographical location and sampling season.

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Introduction

Ethiopian wolves, *Canis simensis*, are medium-sized territorial canids endemic to the Ethiopian highlands (Sillero-Zubiri & Gottelli, 1994). They live in family packs consisting of 2–13 adult wolves, and 0–6 yearlings and pups (Sillero-Zubiri *et al.*, 1996a). Packs occupy and defend territories (Sillero-Zubiri & Gottelli, 1995b), and all adult pack members participate in daily territory boundary patrols, and defend their pack territory from wolves in neighbouring packs (Sillero-Zubiri & Macdonald, 1998). Although Ethiopian wolves live in packs, they mainly forage solitarily and specialize in hunting Afroalpine rodents, especially the endemic giant molerat, *Tachyoryctes macrocephalus*, which is the Ethiopian wolf's main prey in the Bale Mountains (Sillero-Zubiri & Gottelli, 1995a). Ethiopian wolves are co-operative breeders, and generally only the dominant female in a pack breeds (Sillero-Zubiri *et al.*, 1996a). Breeding is seasonal, with pups (1–6) usually born at the end of the rainy season (October–January, Sillero-Zubiri & Gottelli, 1994). Ethiopian wolves are considered to be the world's rarest canid (Marino *et al.*, 2006), and are listed as 'endangered' by the International Union for Conservation of Nature (IUCN), with fewer than 500 adults surviving (Marino & Sillero-Zubiri, 2011). Threats to Ethiopian wolf survival include habitat destruction and fragmentation (Sillero-Zubiri & Macdonald, 1997), but the most immediate threat is infectious disease spread by domestic dogs, notably rabies (Haydon *et al.*, 2002; Randall *et al.*, 2004; Johnson *et al.*, 2010).

As the world's rarest canid, and a flagship species for its unique habitat (Sillero-Zubiri & Macdonald, 1997), the Ethiopian wolf has long been the focus of scientific studies, including those on diet (Sillero-Zubiri & Gottelli, 1995a), territoriality (Sillero-Zubiri & Macdonald, 1998), breeding system (Sillero-Zubiri *et al.*, 1996a) and reproductive physiology (van Kesteren *et al.*, 2012, 2013). However, to date, no detailed studies on Ethiopian wolf parasites have been published. A publication by Jebessa (2009) describes eight Ethiopian wolf faecal samples and records eggs of *Trichuris vulpis*, taeniid species, *Ascaris lumbricoides* and an adult *Echinococcus granulosus*. M. Anwar (pers. comm. in Sillero-Zubiri & Gottelli, 1994) reported finding nematodes, trematodes and *Taenia* sp. in the intestines of several carcasses. In the current study we aimed to study the helminth fauna of Ethiopian wolves in more detail by analysing faecal samples collected from 94 individual wolves. We further aimed to assess the effects of wolves' location, sex and age on their helminth fauna, and to assess relationships between the different parasites found.

Materials and methods

Study site

This study was conducted in the Bale Mountains National Park (BMNP) in southern Ethiopia (7°N, 39°40′E). Samples were collected in the Web Valley (WV, 7°01′N, 39°69′E), Morebawa (MB, 6°92′N, 39°63′E) and the Sanetti Plateau (SP, 6°85′N, 39°88′E). The Web Valley and Morebawa are both located at 3450–3550 m above sea level (asl), and the Sanetti Plateau is located at 3800-4300 m asl, with all three locations supporting wolf densities of approximately 1.2 wolves/km^2 (Sillero-Zubiri *et al.*, 1996a), although disease outbreaks have periodically reduced this density (e.g. Haydon *et al.*, 2002; Randall *et al.*, 2004; Johnson *et al.*, 2010). The climate in the higher altitudes of the Bale Mountains is characterized by an 8-month wet season (approximately March–October), with rainfall maxima of 1150 mm/year, and a 4-month dry season (approximately November–February, see Hillman, 1988; Atickem *et al.*, 2009). Temperatures in the dry season range from −15°C at night to +26°C during the day, with more modest temperature fluctuations during the wet season (Hillman, 1988). The vegetation is typical of Afroalpine meadowland and is dominated by short alpine grasses and herbs, including African sage, *Artemesia afra*, *Alchemilla* sp. and bushy everlasting, *Helichrysum* species. Areas with poor drainage are swampy and dominated by grasses such as *Cyperus* sp. and *Scirpus* sp. (Malcolm, 1997). The mammalian fauna in BMNP includes endemic species such as the giant molerat, Starck's hare, *Lepus starcki*, mountain nyala, *Tragelaphus buxtoni* (Yalden & Largen, 1992), and Menelik's bushbuck, *Tragelaphus scriptus meneliki* (Blower, 1968), as well as non-endemic species including klipspringer, *Oreotragus oreotragus*, common duiker, *Sylvicapra grimmia*, rock hyrax, *Procavia capensis* (Blower, 1968), serval cats, *Felis serval*, spotted hyenas, *Crocuta crocuta*, and honey badgers, *Mellivora capensis* (Sillero-Zubiri *et al.*, 1996b). In addition, humans, domestic dogs and livestock, including cattle, sheep and goats, are present inside BMNP (Vial *et al.*, 2010).

Collection and processing of faecal samples

Faecal samples were collected between 7 August 2008 and 20 February 2010 (see van Kesteren *et al.*, 2012, 2013). The sampling time included two field seasons, one from August 2008 to February 2009 and one from July 2009 to February 2010, with two samples collected opportunistically in April and May 2008. A total of 94 wolves were sampled. This included 51 wolves from the Web Valley, including 47 from eight known packs and 4 wolves not belonging to any known pack (including one adult male dog–wolf hybrid, Gottelli *et al.*, 1994). A total of 28 wolves from four packs in the Sanetti Plateau were included, as well as 15 wolves from six packs in Morebawa. Of the 94 sampled wolves, 37 were female, 56 were male and one was of unknown sex. Of wolves sampled, 75 were adult (classed as ≥2 years old), 11 were sub-adult (≥1 ≤ 2 years), 7 were juvenile (≥0.5 ≤ 1 year) and one was a puppy (<6 months).

Ethiopian wolves were followed on foot with the assistance of local field assistants. Whenever a wolf was seen defecating, the faecal sample was collected within minutes of defecation. The age, sex and identity of the wolf were recorded. In addition, 10 samples were collected from wolves that defecated while foot-trapped as part of the 2008 emergency rabies vaccination (Johnson *et al.*, 2010). Individual wolves were identified by ear tags ($n = 41$), individual markings ($n = 43$) or from foot traps and subsequent ear tagging ($n = 10$). Upon collection,

faecal samples were put in 50 ml tubes and stored in a cooler box on ice until return to the research camp, where a subsection of each sample was preserved in 95% ethanol in 35-ml or 15-ml tubes. Samples were stored at room temperature until transport to the UK, where they were stored at −20°C until analysis.

Samples were analysed for parasite eggs using the volumetric dilution method, as described by Ashford *et al.* (1981). In brief, a small portion of faeces (~1 ± 0.1 g) previously fixed in 95% ethanol was sieved through a small mesh with 4 ml of distilled water. The slurry was collected in a 15-ml centrifuge tube and distilled water was added to make up to 15 ml in total volume. Tubes were centrifuged at 2000 rpm for 2 min, the supernatant was discarded and the pellet was re-suspended in 4 ml of distilled water. A drop of re-suspended sample was placed on a microscope slide and examined under a compound microscope for the presence of parasite eggs. Five slides were examined per faecal sample, and the total number of eggs per slide were counted, and recorded as mean eggs per gram (epg). Helminth eggs were identified to genus level using Ash & Orihel (1997) and Soulsby (1974).

When taeniid eggs were identified, we attempted to determine the species present using two polymerase chain reaction (PCR) approaches. First, we attempted to isolate and extract DNA directly from the taeniid eggs using a Qiagen® DNEasy blood&tissue kit (Qiagen, Hilden, Germany), which was then subjected to a generic cestode PCR protocol (von Nickisch-Rosenegk *et al.*, 1999). Second, DNA was extracted from faecal samples positive for taeniid eggs using a Qiagen® Qiamp Stoolkit, following the manufacturer's instructions but using 1 g of faeces. This DNA was subjected to amplification with generic cestode primers (von Nickisch-Rosenegk *et al.*, 1999) and a copro-PCR protocol was performed for *E. granulosus* sensu lato (Abbasi *et al.*, 2003; with modifications by Boufana *et al.*, 2008). Positive controls (DNA from adult *E. multilocularis* and *E. granulosus*) and negative controls (PCR-grade water) were included in all PCRs. A Stratagene (La Jolla, California, USA) Robocycler was used for cycling profiles and PCR products were separated by electrophoresis on a 1.5% agarose gel (Bioline, London, UK) in 1 × Tris–borate–EDTA buffer (Severn Biotech, Kidderminster, UK), stained with gel red (Cambridge Biosciences, Cambridge, UK). Gels were visualized using Syngene G:Box gel documentation system (Cambridge Biosciences). Samples that were successfully amplified were sequenced by Beckman Coulter (Essex, UK) and the resulting sequences analysed using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Data analysis

Data were analysed using R statistical software version 3.0.1 (R Development Core Team, 2013). For initial analysis and for logistic regression modelling all faecal samples were dichotomously classified as positive (at least one egg recorded) or negative for each type of helminth egg. Data were available for five potential risk factors: location of wolf (MB, SP, WV); age of wolf; sex of wolf and sampling season (wet season 2008, dry season 2008–2009, wet season 2009, dry season 2009–2010). Additionally, the dichotomized status (positive/negative) of the sample in relation to each of the other three types of nematode eggs identified were considered as possible risk factors of interest. Cestodes were excluded from the analysis due to the very low number of outcomes ($n < 5$). The one puppy sampled was removed from further analysis.

Initial univariable analysis of associations between different nematode species and risk factor variables (including presence of other nematodes) was conducted using Fisher's exact test. In order to quantify levels of association between nematodes, phi coefficients were also calculated.

Generalized linear models were created for nematode data, using either the presence/absence of each of the four nematode eggs (logistic regression), or egg counts (negative binomial regression) as dependent variables. Logistic regression was conducted using the 'glm' command from the core 'stats' package in R, and negative binomial regression was conducted using the 'glm.nb' command from the 'MASS' package (Venables & Ripley, 2002). The same model selection protocol was used for all nematodes and model types. After parameterizing the model with all seven independent variables, a stepwise backward elimination process was used to create a suitably parsimonious model. Variables were removed sequentially according to their estimated impact on the residual deviance of the model (as assessed using a chi-square test), and likelihood ratio tests were used to assess whether the variables were contributing to the model. The 'season' variable was found *post hoc* to improve the final model fit. Therefore the effect of the two constituent variables (wet/dry season and sampling year) was investigated using a stepwise elimination process, which included first replacing the original variable with the two constituent variables, and including the interaction between them. Except for this case, interactions between other variables were not assessed in the current investigation due to the low number of outcomes and the potential risk of model instability. Comparison of the parameter estimates for different levels of those variables with more than two levels was achieved using the 'glht' command in the 'multcomp' package (Hothorn *et al.*, 2008), and factor levels were collapsed if warranted.

The final model was then compared to a null model containing only an intercept, and if the likelihood ratio P value was greater than 0.05 it was concluded that there were no significant predictors of the nematode in question. Additionally, all final negative binomial regression models were compared with equivalent Poisson regression models using a likelihood ratio test with a P value cut-off of 0.05.

Results

Eggs of at least one of six different helminths were found in 66 faecal samples (70.2%), namely species of *Capillaria*, *Trichuris*, *Toxocara*, *Hymenolepis*, ancylostomatids and taeniids. *Capillaria* eggs were the most commonly found helminth eggs ($n = 49$ faecal samples), with between 1 and 2504 epg. *Trichuris* eggs were found in 21 faecal samples, *Toxocara* eggs were found in 14 samples and ancylostomatid eggs were found in 21 samples. Only 3 samples contained *Hymenolepis* sp. and 4 contained

Table 1. The prevalence (%) of helminth eggs per gram (epg) in 94 faecal samples of the Ethiopian wolf from 2008 to 2010. Median and interquartile range estimates are based upon positive samples only.

Helminth species (sp.)	% of positive samples	Maximum epg	Median epg and interquartile range
Nematoda			
Capillaria	52.1	2504	30 (9–84)
Trichuris	22.3	46	9 (3–18)
Ancylostomatid	22.3	22	2 (1–5)
Toxocara	14.9	9	3 (2–3.75)
Cestoda			
Taeniid	4.3	2	1.5 (1–2)
Hymenolepis	3.2	3	3 (2–3)

taeniid eggs (table 1). Most samples ($n = 34$) contained eggs of only one type of helminth, with some containing two ($n = 22$) or three ($n = 7$) types. Two samples contained eggs of four helminth types, and one sample contained eggs of five types.

Attempts to extract and amplify DNA directly from taeniid eggs using a generic cestode PCR (von Nickisch-Rosenegk *et al.*, 1999) were not successful. Similarly, the faecal DNA samples did not yield a diagnostic band when tested with the generic cestode primers (von Nickisch-Rosenegk *et al.*, 1999), possibly because this protocol is not optimized for copro-PCR. However, faecal DNA extracted from one of the four samples positive for taeniid eggs was copro-PCR positive and, after sequencing, gave a 98% match to *E. granulosus* (NCBI database number DQ157697.1).

Due to the very low numbers of positive samples, analysis was not conducted for *Hymenolepis* or taeniid species. There was some evidence of positive associations between the presence of species of *Capillaria*, *Toxocara* and *Trichuris* (table 2).

Of the five non-parasite model variables (wolf age, sex, location, season and year), none was found to be significantly associated with the presence of species of *Capillaria*, *Toxocara*, ancylostomatids or *Trichuris*. However, there was evidence of a positive association between the presence of *Capillaria* and each of *Toxocara* and *Trichuris*, as had been identified from simple pairwise comparison with odds ratio estimates from the final logistic regression models (table 3).

All negative binomial regression models were a superior fit to the data than the equivalent Poisson regression models (LRT P values < 0.001 in all cases), suggesting that the distribution of egg counts between different wolves was overdispersed. Wolf age, sex, location, sampling season and sampling year were not significant predictors for *Capillaria* or *Trichuris* egg counts. Location was a significant predictor for *Toxocara* sp. egg counts, with higher egg counts amongst wolves in the Sanetti Plateau compared to Web Valley and Morebawa. As there was no evidence of a difference between the coefficients for Web Valley and Morebawa, these factor levels were combined for the final model. Location was also a significant predictor for ancylostomatid egg count, with samples collected in the Web Valley associated with higher egg counts than those collected from the Sanetti Plateau. A similar pattern was observed for Morebawa as compared to Sanetti, but did not reach significance. Sampling year was also a significant predictor for ancylostomatid egg count, with samples collected during the second field season (2009–2010), being associated with higher egg counts. In terms of associations between different nematode species, the presence of *Capillaria* was associated with higher egg counts of both *Toxocara* and *Trichuris*. A positive association between ancylostomatid presence and *Trichuris* egg counts was also identified. No associations with *Capillaria* egg count were identified. The possibility that this was due to the effect of a single high recorded egg count (2504 epg) was investigated by repeating the model selection process with this observation removed. However, there remained no evidence to select any model other than that with an intercept only (data not shown). Table 4 shows the results of the negative binomial regression model, with coefficients presented on the log scale and the intercept set to zero.

Discussion

Helminth eggs of species of *Trichuris*, *Capillaria*, *Toxocara*, ancylostomatids, *Hymenolepis* and taeniids, including *E. granulosus*, were identified from Ethiopian wolf faecal samples. These helminth genera have all been recorded in closely related grey wolves, *Canis lupus* (Craig & Craig, 2005). Faecal samples from Arctic foxes, *Vulpes lagopus,* (Meijer *et al.*, 2011; Elmore *et al.*, 2013), and coyotes, *Canis latrans* (Gompper *et al.*, 2003), have been found to contain *Capillaria* species. *Trichuris* spp., including *T. vulpis*, have been found in Arctic foxes (Meijer *et al.*, 2011) and maned wolves, *Chrysocyon brachyurus* (Curi *et al.*, 2012). Ancylostomatid helminths

Table 2. Measures of association (phi coefficients) and Fisher's exact test (P values) between different nematode species.

Comparison of species (sp.)		Phi	Fisher's P value
Capillaria	*Trichuris*	0.31	0.01
Toxocara	*Capillaria*	0.22	0.04
Trichuris	Ancylostomatid	0.14	0.24
Toxocara	*Trichuris*	0.13	0.30
Toxocara	Ancylostomatid	0.01	1.00
Capillaria	Ancylostomatid	0.00	1.00

Table 3. Significant risk factors for presence/absence of the four nematodes (no significant risk factors found for ancylostomatids) using the final logistic regression model. OR denotes the odds ratio, and the LRT *P* value is the likelihood ratio test *P* value of the final model compared to a null (intercept-only) model.

Nematode (sp.)	Risk factor	OR	95% CI	LRT *P* value
Toxocara	*Capillaria*	3.96	1.13–18.48	0.03
Capillaria	*Toxocara*	3.55	0.9–17.17	0.002
	Trichuris	4.99	1.62–18.93	0.002
Trichuris	*Capillaria*	5.31	1.76–19.89	0.002

have also been recorded in maned wolves (Curi *et al.*, 2012) and red foxes (Dalimi *et al.*, 2006). Taeniid species, including *Taenia* sp. and *Echinococcus* sp., have been found in several canid species, including Arctic foxes (Meijer *et al.*, 2011; Elmore *et al.*, 2013), red foxes (Vervaeke *et al.*, 2005; Dalimi *et al.*, 2006), coyotes (Gompper *et al.*, 2003) and golden jackals (Dalimi *et al.*, 2006). *Hymenolepis* sp. do not usually use canids as definitive hosts (Woodland, 1924), and the three samples containing *Hymenolepis* eggs found here are probably artefacts from ingested rodent prey. *Hymenolepis* eggs were also recorded in Arctic fox faecal samples (Elmore *et al.*, 2013).

Many helminths found in Ethiopian wolves have been recorded in domestic dogs from Debre Zeit (Yacob *et al.*, 2007), Ambo (Zewdu *et al.*, 2010) and Jimma (Degefu *et al.*, 2011), all located within approximately 325 km north-west of BMNP. Domestic dogs are closely related to Ethiopian wolves (Gottelli *et al.*, 1994) and are sympatric in the Bale Mountains (Laurenson *et al.*, 1998; Vial *et al.*, 2010). As such, they may have some parasites in common, and transmission of parasites between domestic dogs and Ethiopian wolves may occur. *Ancylostoma caninum* was the most common dog helminth recorded in Debre Zeit and Jimma, and the second most common in Ambo (Yacob *et al.*, 2007; Zewdu *et al.*, 2010; Degefu *et al.*, 2011). Other commonly found helminths in dogs in all three studies were *Toxocara canis* and *Trichuris vulpis* (Yacob *et al.*, 2007; Zewdu *et al.*, 2010; Degefu *et al.*, 2011), with taeniids, including *E. granulosus*, reported in Jimma (Degefu *et al.*, 2011) and Ambo (Zewdu *et al.*, 2010). *Capillaria* sp. have been reported in domestic dogs in several world locations (e.g. Fok *et al.*, 2001; Di Cesare *et al.*, 2012b), although none were reported from the three Ethiopian study sites (Yacob *et al.*, 2007; Zewdu *et al.*, 2010; Degefu *et al.*, 2011).

Infection of Ethiopian wolves with the four nematode species reported here could result from direct transmission from other wolves or domestic dogs (Urquhart *et al.*, 1996); or, in the case of *Toxocara* and *Capillaria*, through consumption of infected small mammals (e.g. Warren, 1969; Ceruti *et al.*, 2001). In domestic dogs, infections with *T. vulpis* (Urquhart *et al.*, 1996), *T. canis* (Glickman & Schantz, 1981) and ancylostomatids, such as *A. caninum* (see Traub *et al.*, 2004) and *Uncinaria stenocephela* (Urquhart *et al.*, 1996), are usually localized in the intestines. Most infections are light and asymptomatic, although clinical signs may become apparent in severe cases. In domestic dogs, clinical symptoms may include intestinal inflammation and haemorrhage (Kirkova & Dinev, 2005) and pneumonia (Urquhart *et al.*, 1996), and heavy ancylostomatid infections may cause anaemia, diarrhoea and anorexia, and lead to mortality, particularly in pups (see Traub *et al.*, 2004). Although symptoms are likely to be similar in Ethiopian wolves, exact clinical manifestations of nematode infections in Ethiopian wolves are not known. Canids may be infected with at least four species of *Capillaria*, including *C. aerophila, C. boehmi* (Di Cesare *et al.*, 2012a), *C. plica* (Senior *et al.*, 1980) and *C. hepatica* (syn. *Calodium hepaticum*, Crowell *et al.*, 1978; Lloyd *et al.*, 2002). However, none of these infections would be expected to result in the production of eggs in the faeces: *C. plica* usually occurs in the bladder (Senior *et al.*, 1980); *C. aerophila* usually occurs in the respiratory tract (Traversa *et al.*, 2011); *C. boehmi* usually occurs in the nasal cavities and sinuses (Di Cesare *et al.*, 2012a); and *C. hepatica* usually occurs in the liver (Soulsby, 1974) (Urquhart *et al.*, 1996). Based on this, it is likely that the *Capillaria* eggs represent those 'passing through' the intestine following consumption of prey species infected with this nematode. Although we could not identify the species of *Capillaria* found, *C. hepatica* occurs commonly in rodents (Soulsby, 1974), with at least 34 rodent species reported to be infected with *C. hepatica* (Spratt & Singleton, 2001). Although no *Capillaria* sp. were found by Jebessa (2009), this finding may be due to the low sample size ($n = 7$ rodents), and detailed studies on parasites of the rodent community of BMNP are lacking.

Statistical analysis of our data found no effect of wolf age, sex, location, sampling year or season on the

Table 4. Significant risk factors for differences in nematode species using the final negative binomial regression model; coefficients of log of egg counts (CF), 95% confidence intervals (CI) and likelihood ratio test (LRT) *P* values.

Nematode species (sp.)	Risk factor	CF	95% CI	LRT *P* value
Toxocara	Web Valley/Morebawa	0.00	–	0.002
	Sanetti Plateau	2.25	0.81–3.69	
	Capillaria	2.23	0.68–3.79	
Trichuris	*Capillaria*	3.01	1.61–4.42	0.002
	Ancylostomatid	1.72	0.15–3.29	
Ancylostomatid	Web Valley	0.00	–	<0.001
	Morebawa	0.07	−1.77–1.63	
	Sanetti Plateau	2.17	−3.88 to −0.05	
	1st season (2008–2009)	0.00	–	
	2nd season (2009–2010)	1.58	0.08–3.08	

dichotomized presence/absence status of each of the four nematode species investigated. Care should be taken when interpreting the results of the negative binomial regression due to the relatively low number of positive outcomes, but these models suggested that location was a significant predictor for *Toxocara* egg counts, with higher egg counts in the Sanetti Plateau than in the Web Valley and Morebawa. In contrast, ancylostomatid egg burdens were higher in the Web Valley than in the Sanetti Plateau (with a similar pattern observed for Morebawa and Sanetti, which may have not reached significance due to the relatively low number of samples collected from Morebawa). This could be related to differences in habitats between the three locations. The Web Valley and Morebawa are geographically closer to each other than to the Sanetti Plateau and are at a similar altitude (~3500 m asl). The Sanetti Plateau, however, is further away and located at approximately 4200 m asl. The micro-climate differs somewhat between these two altitudes, and this could potentially affect wolf exposure to nematode eggs, as temperature and humidity are known to affect development of nematode eggs (e.g. Matthews, 1985; Gamboa, 2005). Similarly, the two locations have different domestic dog densities, with higher densities in the Web Valley (Vial *et al.*, 2010). However, as the Web Valley and Morebawa are located at a lower altitude than the Sanetti Plateau (and are therefore warmer), and generally have more domestic dogs, the finding of higher *Toxocara* egg burdens in the Sanetti Plateau is of interest, and could be due to localized exposures. Ancylostomatid egg burdens were higher during the second field season, which may also be associated with climatic effects, or increasing dog populations (Vial *et al.*, 2010).

Associations between different nematodes were commonly identified in the regression models. A positive association was found between the presence of *Capillaria* eggs and both the presence and numbers of *Toxocara* and *Trichuris* eggs. The presence of ancylostomatid eggs was also associated with higher *Trichuris* egg counts. Possible reasons for these associations are the presence of a biological association between infections with these helminths, residual confounding in the model or random variation. As *Capillaria* eggs were suspected to represent artefacts rather than patent infections, a biological association between presence of eggs and presence of *Toxocara* or *Trichuris* (e.g. due to competition within the intestine) would be unlikely. It is therefore more likely that either this pattern is due to random variation (a relatively low number of samples were positive for *Toxocara* or *Trichuris*), or represents residual confounding by an unrecorded common exposure, such as micro-climatic effects or differences in local dog densities. The association between *Trichuris* egg count and the presence of ancylostomatids may also be due to residual confounding by a common exposure – although differences in pre-patent periods (Urquhart *et al.*, 1996) may need to be accounted for if this were to be investigated further.

In addition to the four nematode species, two cestode species were found in our study. Three samples were found to have *Hymenolepis* sp. eggs, which probably represent artefacts from ingested rodent prey (Woodland, 1924; Elmore *et al.*, 2013). Four Ethiopian wolf faecal samples contained taeniid eggs, and one of these samples was copro-PCR and sequence positive using primers designed to detect *E. granulosus* sensu lato, although unfortunately this protocol does not allow for identification of the strain (Boufana *et al.*, 2008). Future studies could aim to further elucidate taeniid species present in the Bale Mountains and in Ethiopian wolves, especially since the complex of *E. granulosus* sensu lato is now generally considered to consist of different species, including *E. granulosus* sensu strictu, *E. equinus*, *E. ortleppi* and *E. canadensis* (Thompson, 2008), and there is some disagreement between authors on the specificity of the protocol used (Abbasi *et al.*, 2003; Naidich *et al.*, 2006; Zhang *et al.*, 2006; Boufana *et al.*, 2008). Jebessa (2009) reported finding adult *E. granulosus* tapeworms in one of eight faecal samples collected from Web Valley wolves, and unidentified taeniid eggs in all samples. Canids are definitive hosts of *E. granulosus* (Eckert & Deplazes, 2004), and are susceptible to more than ten *Taenia* species. These may be derived from ungulates (*T. hydatigena*, *T. ovis* (Gemmell, 1987) or *T. multiceps* (Varcasia *et al.*, 2006)); from rodents (*T. crassiceps* (Hoberg, 2002) and *T. polycantha* (Haukisalmi & Henttononen, 1993)); or from lagomorphs (*T. pisiformis* (Jenkins & Rickard, 1985)).

Rodents are the main prey for Ethiopian wolves (Sillero-Zubiri & Gottelli, 1995a) and occur at high density in BMNP, with many species being endemic (Yalden & Largen, 1992). Although very little is known about the parasites of rodents present in BMNP, it is reasonable to suspect that *Taenia* sp. infections in Ethiopian wolves could result from ingestion of infected rodents. *Echinococcus granulosus* is highly prevalent in Ethiopia, and both sheep and goats commonly act as intermediate hosts (for a review see Kassa, 2012). Data from slaughterhouses in Robe, located approximately 30 km east of BMNP, suggest that the prevalence of *E. granulosus* in sheep is greater than 60% (Fromsa & Jobre, 2011). Local livestock herders commonly graze sheep and goats inside the BMNP (e.g. Vial *et al.*, 2010), and *E. granulosus* life cycles could be maintained inside the Park. Sheep from the Ethiopian highlands have also been found to be commonly infected with *T. hydatigena* (Bekele *et al.*, 1988), a species which also has a high biotic potential (Gemmell, 1987). Although nominally protected within the BMNP, the Web Valley, Morebawa and the Sanetti Plateau are inhabited by people, livestock and domestic dogs (Laurenson *et al.*, 1998; Vial *et al.*, 2010). Ethiopian wolves occasionally hunt or scavenge sheep and goats (Sillero-Zubiri & Gottelli, 1995a; F. van Kesteren, pers. obs.), and could become infected with *E. granulosus* or *Taenia* sp. by consuming livestock offal. Little is known of the parasitic burdens of wild ungulates in the BMNP, although helminth transmission between domestic livestock and mountain nyala has been reported (Shiferaw & Laurenson, 2011), and it is possible that domestic taeniid cycles are spilling over into native ungulates, which, although uncommon, could also be consumed by Ethiopian wolves (Sillero-Zubiri & Gottelli, 1995a).

The Bale Mountains are a unique ecosystem, containing many endemic mammals (Hillman, 1988; Yalden & Largen, 1992), birds (Blower, 1968) and amphibians (Hillman, 1988).

However, currently this unique environment is also home to an estimated 35,000 people and their livestock and domestic dogs (Jebessa, 2009; Vial *et al.*, 2010). Livestock densities in the Web Valley are estimated at 250 head/km^2 for cattle and 120 head/km^2 for sheep and goats (Vial *et al.*, 2010) and domestic dog densities may be as high as 10/km^2 during the rainy season (Atickem *et al.*, 2009). This environment, where wild endemic species, humans, livestock and domestic dogs all co-exist, could be home to domestic and sylvatic parasite cycles and transmissions, which are currently poorly understood. The results presented here expand our knowledge on helminths present in the Bale Mountains, and shows that Ethiopian wolves share some helminth species with other wild carnivores, as well as with domestic dogs in Ethiopia.

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Conflict of interest

None.

Ethical standards

Ten samples were collected opportunistically from wolves foot-trapped during an emergency parenteral rabies vaccination campaign. This vaccination was approved by the Technical Committee of the Ethiopian Wildlife Conservation Authority.

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