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HELMINTHOLOGY - ORIGINAL PAPER

Age-specific gastrointestinal parasite shedding in free-ranging cheetahs (*Acinonyx jubatus*) on Namibian farmland

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Abstract

The cheetah (*Acinonyx jubatus* Brookes 1828) is classified as "vulnerable" by the International Union for Conservation of Nature (IUCN). Threats to cheetah populations are a decrease of suitable habitats, an increase of conflicts with livestock farmers and potentially pathogens. While there is some information on the viral and bacterial pathogens circulating in cheetah populations, information on gastrointestinal parasites is scarce. Here, we investigate the gastrointestinal parasites in 39 free-ranging cheetahs in east-central Namibia using a coproscopical parasitological method. Most cheetahs (82%) shed eggs from *Ancylostoma* which comprised the majority of the total eggs in feces. Eggs and oocysts from *Toxascaris* (21% of cheetahs), Coccidia (13%), *Physaloptera* (8%), Taeniidae (5%), *Dipylidium* (3%), and Diphyllobothriidae (3%) were present at a lower prevalence. Parasite richness and *Ancylostoma* egg load were higher in juveniles and adults compared to cubs, but were not associated with sex. To our knowledge, this is the first study that assessed gastrointestinal parasites in free-ranging cheetahs and is a key starting point for future studies on the effect of parasites in this threatened species.

Keywords *Acinonyx jubatus* · *Ancylostoma* · Gastrointestinal parasites · Protozoa · Cestoda · Nematodes

Introduction

Throughout Africa, populations of large carnivores are declining (Bauer et al. 2015; Packer et al. 2009; Ripple et al. 2014; Woodroffe and Ginsberg 1998), including cheetahs (*Acinonyx jubatus* Brooke 1828), which are considered as "vulnerable" by the International Union for Conservation of Nature (IUCN) (Durant et al. 2015; Weise et al. 2017). Besides habitat fragmentation and conflict with livestock farmers, pathogens might be an additional threat to declining populations (Leclaire and Faulkner 2014). Even though cheetahs have reduced variability in the major histocompatibility complex (MHC) locus (Castro-Prieto et al. 2011), free-ranging cheetahs do not seem to be highly susceptible to viral and bacterial infections (Molia et al. 2004; Munson et al. 2005; Thalwitzer et al. 2010). Knowledge on the parasites infecting cheetahs is

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scarce (Mény et al. 2012; Round 1968). Parasites can have a negative impact on fitness (Scott 1988), in terms of reduced reproductive success and/or increased mortality in a range of species (Anderson and May 1978; Hudson et al. 1992; Krams et al. 2013; Lynsdale et al. 2017; Stirnadel and Ebert 1997). Consequently, when the effects of habitat fragmentation or conflicts with humans are combined with those of infection with parasites, resulting diseases might have an additive detrimental impact on populations and lead to increased extinction risk for vulnerable species (Kobbekaduwa et al. 2017). Therefore, information on parasite prevalence and diversity in threatened species is crucial to understand the role of parasites in the endangerment of these species.

Free-ranging wildlife populations are typically infected with various parasite taxa, which is influenced by both intrinsic factors such as age, sex, and body condition (Leclaire and Faulkner 2014; Setchell et al. 2007; Thurber et al. 2011) and extrinsic factors such as diet and habitat (Chasar et al. 2009; Gillespie et al. 2005; van Houtert and Sykes 1996; Vaz et al. 2007). Although parasites may also play an important role in the life history of free-ranging cheetahs, knowledge about parasites in cheetahs is mostly limited to studies in captive animals (Mény et al. 2012), case reports (Briggs et al. 1993; Zinkl et al. 1981), and taxonomic descriptions of individual parasite species (Round 1968). Obtaining baseline data of parasite prevalence, abundance and diversity in free-ranging cheetahs might be important to contribute to disease management and conservation efforts.

The objective of this study was to identify gastrointestinal parasites in free-ranging cheetahs in Namibia, a country that hosts one of the largest remaining cheetah populations worldwide (approximately 3600 cheetahs live in Namibia, Botswana, South Africa, and Zimbabwe) (Durant et al. 2017; Weise et al. 2017). Further, we aimed to identify individual host traits (age, sex, and body condition) that may potentially affect parasite richness and load. Since all investigated cheetahs had a very good body condition, we focused on age and sex.

Material and methods

Sample collection

From April 2009 to April 2016, we collected fecal samples from 39 free-ranging cheetahs (9 females, 28 males, 2 unknown) on commercial farmland in east-central Namibia. They were classified in three age categories, i.e., cubs (< 5 months of age, $n = 5$), juveniles (5 months to 24 months, $n = 5$), and adults (> 24 months, $n = 29$). The age of cheetahs was estimated as described in Caro (1994). Cheetahs were sampled rectally after capture, immobilization, and collaring ($n = 29$) within our long-term cheetah research project, after we retrieved a dead cheetah legally killed by a farmer ($n = 5$) or when handled cubs defecated ($n = 5$). The capture and handling is described in detail in Thalwitzer et al. (2010), Wachter et al. (2011), and Heinrich et al. (2017). The samples were primarily collected for other research questions (e.g., endocrinology and dietary analyses) that required storage at temperatures below 0 °C. We stored the fecal samples in liquid nitrogen (− 196 °C) until transported to Germany, in full compliance with the Convention on International Trade in Endangered Species (CITES), and stored them at − 80 °C until laboratory analyses.

Laboratory analyses

We morphologically identified and quantified parasite egg or oocyst load as eggs/oocysts per gram of feces (EPG/OPG) in fecal samples using a modified McMaster egg floatation technique. We used the final dilution of 1:15 diluted feces, thoroughly mixing 1 g of feces with 14 ml of Sheather's sucrose solution with the density of 1.3 g/ml and filtered it using a sieve (East et al. 2015; Foreyt 2001). In case less than 1 g of feces was available, we adjusted the ratio accordingly. Four McMaster slide counting chambers were loaded and left for 5 min before parasite eggs were identified using veterinary parasitology reference manuals (Bowman 2002; Foreyt 2001; Zajac and Conboy 2012). Identified parasite eggs and oocysts were counted and measured using a compound microscope (Axioplan, Carl 207 Zeiss AG, Oberkochen, Germany) at × 100 magnification. Since we exclusively identified parasites by morphology, identification was restricted to the genus/family or subclass level. All counts of parasite eggs and oocysts were conducted blind with respect to animal age and sex.

Statistical analyses

We use the term prevalence to describe the percentage of cheetahs with a present parasite taxa. Mean intensity of infection describes the average value of parasite load (eggs/oocysts per gram) of a particular parasite taxa among infected hosts and mean abundance describes the average value of parasite load (eggs/oocysts per gram) of a particular parasite taxa among all examined hosts (Bush et al. 1997; Margolis et al. 1982). Parasite richness was defined as the number of parasite taxa found in a sample from one individual.

We used the statistical software R version 3.4.2 for all statistical analyses and creating figures (R Core Team 2017). We conducted two-tailed tests and set the level of significance to $\alpha = 0.05$. Due to small sample sizes and unbalanced numbers of individuals by age and sex group, we tested the effect of sex on parasite richness and *Ancylostoma* egg load using the non-parametric Mann-Whitney *U* test. We tested the effect of age on parasite richness and *Ancylostoma* egg load using the non-parametric Kruskal-Wallis test followed by Dunn's test for post hoc analyses (R package "dunn.test"; Dinno 2015). We used the Bonferroni method to perform multiple-comparison adjustments for Dunn's test.

Results

We detected seven different gastrointestinal parasite taxa in fecal samples of the 39 cheetahs with large variations in abundance and intensities (Table 1). We detected eggs of gastrointestinal parasites in 85% of individuals (33 out of 39). Individuals were infected with 0 to 4 parasite genera (i.e., parasite richness, median = 1, mean = 1.33). Parasite richness was not associated with sex (Mann-Whitney U test, $W = 155$, $p = 0.27$, $n_{\text{females}} = 9$, $n_{\text{males}} = 28$; Fig. 1), but varied with age categories (Kruskal-Wallis test, Chi-squared = 12.19, df = 2, $p = 0.002$, $n = 39$). Parasite richness was highest in juveniles (median 3, $n = 5$), intermediate in adults (median 1, $n = 29$), and lowest in cubs (median 0, $n = 5$; Dunn's tests: juveniles vs. cubs $z = 3.49$, $p = 0.0002$; juveniles vs. adults $z = 2.33$, $p = 0.01$; cubs vs. adults $z = -2.23$, $p = 0.01$, Fig. 2).

Co-infection occurred in 14 individuals (36%). The most common parasite responsible for co-infection was *Ancylostoma*. Ten individuals were infected with two parasites, with *Ancylostoma* being one of them. The second parasite was either *Toxascaris* (5 individuals), Coccidia (2 individuals), *Physaloptera* (2 individuals), or Diphyllobothriidae (1 individual). Three individuals were infected with three parasites. They were infected with *Ancylostoma* and *Toxascaris*, with the third parasite being either Coccidia (2 individuals) or Taeniidae (1 individual). One individual was infected with four parasites: *Ancylostoma*, *Dipylidium*, Taeniidae, and *Physaloptera*.

Ancylostoma EPG was present in 82% of individuals (32 out of 39) and was not associated with sex (Mann-Whitney U test, $W = 120.5$, $p = 0.86$, $n_{\text{females}} = 9$, $n_{\text{males}} = 28$, Fig. 3), but varied with age categories (Kruskal-Wallis test, Chi-squared = 6.59, df = 2, $p = 0.037$, $n = 37$). *Ancylostoma* EPG was highest in juveniles (median 3600, $n = 5$), intermediate in adults (1325, $n = 29$) and lowest in cubs, with juveniles and adults not differing significantly from each other (median 0, $n = 5$; Dunn's tests: juveniles vs. cubs $z = 2.55$, $p = 0.006$; juveniles vs. adults $z = 1.41$, $p = 0.08$; cubs vs. adults $z = -1.92$, $p = 0.028$, Fig. 4).

Discussion

To our knowledge, this study is the first assessment of gastrointestinal parasites in a free-ranging cheetah population. We found that 85% of 39 individuals were infected with parasites, with *Ancylostoma* being the most prevalent taxa (in 82% of cheetahs). Parasite richness and *Ancylostoma* egg load were highest in juveniles, intermediate or similarly high, respectively, in adults and lowest in cubs, while none of these parameters were associated with sex.

Similar to other studies on free-ranging African carnivore species, using similar methods, most cheetahs were infected with gastrointestinal parasites (85% in our study, 78% in spotted hyenas (*Crocuta crocuta* Kaup 1828), 93% in lions (*Panthera leo* Linnaeus 1758), 100% in wild dogs (*Lycaon pictus* Brookes 1827), all in Zambia, Southern Africa; Berentsen et al. 2012). The total number of parasite taxa ($n = 7$) in our study is similar to a study in spotted hyenas in Kenya, Eastern Africa ($n = 9$; Engh et al. 2003). In lions, the parasite diversity varied by region: in Mozambique, Southern Africa, Lajas et al. (2015) identified six parasite species, whereas Bjork et al. (2000) identified 19 in Northern Tanzania, Eastern Africa.

Table 1 Parasite screening results from fecal samples ($n = 39$) with prevalence (%), mean abundance of parasites (average value of parasite load (eggs/oocysts per gram of feces) among all cheetahs), mean and maximum intensity of infection (average value of parasite load among infected cheetahs), and mean egg/oocyst size (μm) of the parasites. EPG/OPG: eggs/oocysts per gram

Parasite	Prevalence (%)	Mean abundance (EPG/OPG)	Mean intensity (EPG/OPG)	Maximum intensity (EPG/OPG)	Size (μm)
Protozoa					
Coccidia	5 (12.8)	119.2	178.8	3600	18.0–22.5 × 18.0–36.0
Cestoda					
Taeniidae	2 (5.1)	96.2	138.1	3650	27.0 × 27.0
Dipylidium	1 (2.6)	1.9	75.0	75.0	45.0 × 31.5
Diphyllobothriidae	1 (2.6)	0.6	25.0	25.0	27.0 × 50.0
Nematoda					
Ancylostoma	32 (82.1)	2082.4	2426.1	12,350	30.0 × 50.0
Toxascaris	8 (20.5)	72.4	139.5	1500	72.0 × 72.0
Physaloptera	3 (7.7)	74.4	74.4	1800	55.0 × 32.0

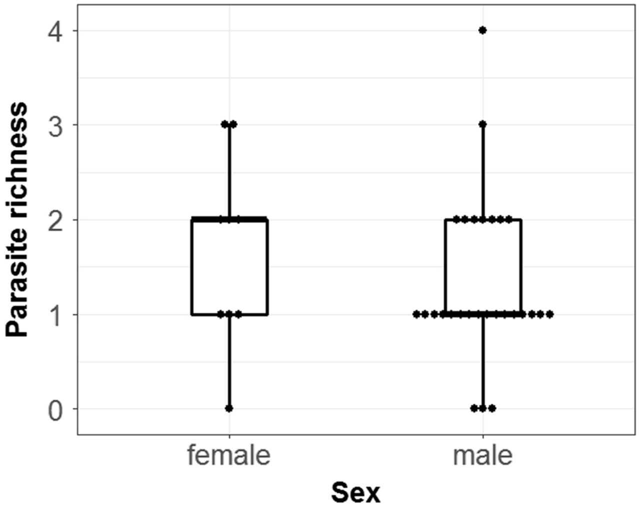

Fig. 1 Parasite richness in female and male cheetahs. The figure shows data points (dots), medians (bold line), and 25th–75th percentiles (box). The whiskers extend from the 25th (lower)/75th (upper) percentile to the data point no further than × 1.5 interquartile range

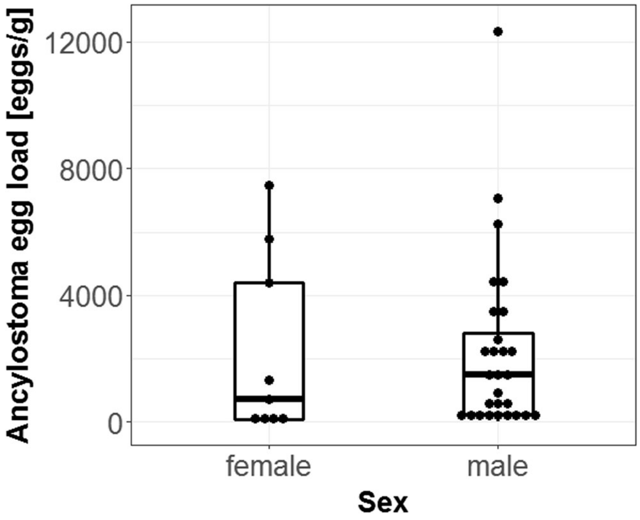

Fig. 3 *Ancylostoma* egg load in female and male cheetahs. The figure shows all data points (dots), medians (bold line), and 25th–75th percentiles (box). The whiskers extend from the 25th (lower)/75th (upper) percentile to the data point no further than × 1.5 interquartile range

The number of co-infecting parasite taxa was similar in our study (mean 1.33) compared with the study of Engh et al. (2003) on spotted hyenas (mean 3) and the study of Bjork et al. (2000) on lions (mean 3), although fecal samples were frozen in our study that may have destroyed some parasite eggs and oocysts (Schurer et al. 2014). Freezing of the fecal samples may have led to an underestimation of the abundance and prevalence of parasite taxa (Nielsen et al. 2010). Especially thin-shelled oocysts are vulnerable to harsh environmental conditions (Schurer et al. 2014). However, Schurer et al. (2014) found that parasite egg numbers of several parasite genera typical for canids, including *Toxascaris leonina*, *Toxocara canis*, and Taenidae eggs, were relatively robust to freezing, probably due to relatively thick outer shells. In contrast, strongylid egg counts significantly decreased after freezing (Schurer et al. 2014).

Despite the high prevalence and load of parasite eggs and oocysts (Table 1), all animals were in a good health state and none of the animals showed clinical signs of infections such as fever, anorexia, or diarrhea.

Parasite richness and the *Ancylostoma* egg load were lowest in cubs, which might be surprising because cubs, similarly to kittens of domestic cats and puppies of domestic dogs,

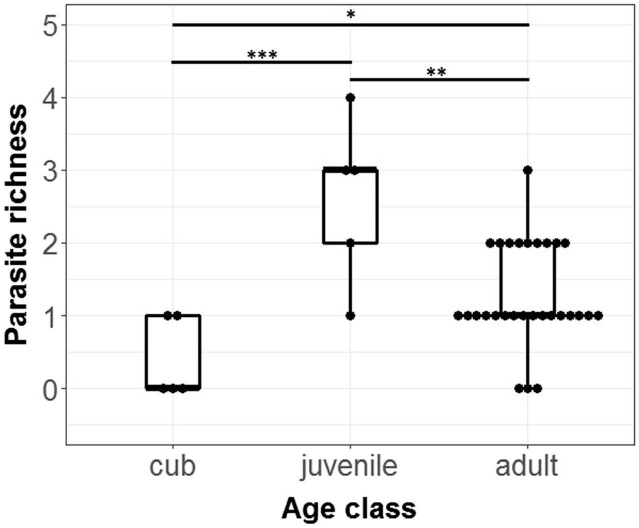

Fig. 2 Parasite richness in cubs, juveniles, and adult cheetahs. The figure shows data points (dots), medians (bold line), and 25th–75th percentiles (box). The whiskers extend from the 25th (lower)/75th (upper) percentile to the data point no further than × 1.5 interquartile range. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

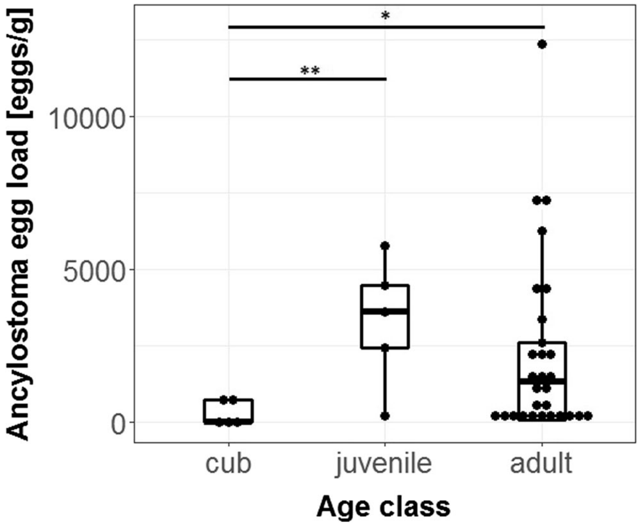

Fig. 4 *Ancylostoma* egg load in cubs, juveniles, and adults cheetahs. The figure shows all data points (dots), medians (bold line), and 25th–75th percentiles (box). The whiskers extend from the 25th (lower) / 75th (upper) percentile to the data point no further than × 1.5 interquartile range. $^*p < 0.05$, $^{**}p < 0.01$

might be infected with hookworms (including *Ancylostoma)* due to transplacental and/or transmammary transmission (Burke and Roberson 1985). In our opportunistic and cross-sectional study design, we cannot test vertical transmission since we lack data on parasite infections of the respective mothers. Moreover, most of adult females samples were non-reproducing, thus further research in cheetahs is warranted to better understand both effects of reproductive status on female hookworm infections and the potential implication in vertical transmission of hookworms. However, when considering only horizontal transmission, cubs are likely to be less exposed to parasites as they stay hidden in the lairs until the age of 2 months (Wachter et al. 2018). Offspring also benefit from the maternal immunity obtained via placenta and/or during lactation (Kumar et al. 1989; Ross et al. 1993; Tyson 1977; Adamski and Demmer 2000). These combined effects likely explain the low parasite diversity and egg load in cubs in our study. After weaning at age of 4 months (Wachter et al. 2018), the maternal immunity is fading and the own immune system of the young is not yet fully functional (Blecha and Kelley 1981; Holsapple et al. 2003). In this time window, the individuals are more susceptible to parasites, which might explain the increased parasite richness and *Ancylostoma* egg loads in juveniles compared to the other age groups. Furthermore, the juvenile age also coincides with diversification of diet, i.e., prey species, resulting in exposure to higher levels and diversity of parasite species. With increasing age, mammals develop specific immunity towards these parasite species (e.g., Milner et al. 2010), which would lead to lower egg/oocysts load in adult cheetahs compared with juveniles. In line with our findings, juvenile spotted hyenas shed more often eggs of *Dipylidium caninum* in their feces than adults (East et al. 2013), while age was not associated with parasite richness in African lions (Bjork et al. 2000).

Neither parasite richness nor the *Ancylostoma* egg load was related with sex. This could be explained by the similar group size of both sexes in cheetahs (Caro 1994). Males live either solitary or in coalition groups of two to three males, whereas females are either solitary or with their offspring (Caro 1994). Thus, males and females have contact with only a few conspecifics and may be exposed to parasites at a similar degree.

Coccidia comprise several genera including *Besnoitia*, *Neospora*, *Toxoplasma*, *Hammondia*, *Cryptosporidium*, *Sarcocystis*, and *Cystoisospora* (previously called *Isospora*) (Junker et al. 2015). The life cycle of *Besnoitia* is not fully understood, although a recent study suggests that felids might be potential candidate as definitive host for this taxa (Verma et al. 2017). *Neospora*'s definitive hosts are canids (Donahoe et al. 2015) and based on morphological features (e.g., size, number of sporozoites), we can rule out *Toxoplasma*, *Hammondia*, *Cryptosporidium*, and *Sarcocystis* (Foreyt 2001), making it most likely that the recorded oocysts in our study belong to the genus *Cystoisospora*. *Cystoisospora* can infect wildlife, domestic animals, and humans and cause substantial clinical signs (coccidiosis, Lindsay et al. 1997). The oocyst prevalence of Coccidia was 13% in our study. *Cystoisospora* was previously described in cheetahs. *I. rivolta* has been found in captive cheetahs in Namibia (Mény et al. 2012) and free-ranging ones in South Africa (Penzhorn et al. 1994) and *I. felis* has been reported in captive cheetahs in Namibia (Mény et al. 2012). The oocyst prevalence of Coccidia in this study was intermediate compared with other free-ranging African carnivore species. In spotted hyenas, the oocyst prevalence of *Cystoisospora* was higher (25.7%, Kenya, Engh et al. 2003 and 33.3%, Zambia, Berentsen et al. 2012), in wild dogs it was lower (8%, Zambia, Berentsen et al. 2012), and in lions, ranged between 0 and 48% (Bjork et al. 2000; Berentsen et al. 2012). We cannot exclude that due to freezing the samples in our study, the prevalence of Coccidia in cheetahs may be underestimated; however, other factors can influence the prevalence such as species susceptibility, diet, and study location. *Cystoisospora* oocysts were also found in one of two fecal samples of caracals (*Caracal caracal*) and two out of three samples of banded mongooses (*Mungos mungo*) in South Africa (Lukášová et al. 2018).

Infections with Taeniidae in definitive hosts, such as humans, felids, and canids, often do not cause any distinct clinical signs, although they may be associated with abdominal pain, with diarrhea or constipation (Raether and Hänel 2003). We detected an egg prevalence of Taeniidae in 5% of 39 cheetahs. In previous studies, *Taenia acinonyxi* and *Taenia hlosei* were identified in free-ranging cheetahs in Iran and South Africa (Hosseini et al. 2011; Round 1968). Compared with other free-ranging African carnivore species, the egg prevalence of Taeniidae in cheetahs was low (11% out of 9 in spotted hyenas in Zambia, Berentsen et al. 2012; 15% to 80% in lions in Zambia, Tanzania, and Mozambique, Bjork et al. 2000; Berentsen et al. 2012; Kavana et al. 2015; Lajas et al. 2015; 23% out of 13 in wild dogs in Zambia (Berentsen et al. 2012).

In humans and domestic dogs, infections with *Dipylidium* are subclinical (Raether and Hänel 2003). The parasite is common in dogs and side-striped, golden, and black-backed jackals (*Canis adustus* Sundevall 1847, *Canis aureus* Linnaeus 1758, *Canis mesomelas* Schreber 1775) in Kenya (Nelson et al. 1965). The egg prevalence of *Dipylidium* was 3% in our study. In other studies of free-ranging felids, *Dipylidium caninum* occurred at a higher prevalence (20% in spotted hyenas, Zambia and Kenya, Engh et al. 2003; Berentsen et al. 2012) or was absent (0% in lions, Tanzania, Bjork et al. 2000 and wild dogs, Zambia, Berentsen et al. 2012). Juvenile spotted hyenas from the Serengeti National Park, Tanzania, showed significantly higher infection prevalence compared to adults (55% and 15.8%, respectively), indicating that predominantly juveniles maintained infections

(East et al. 2013). Moreover, the likelihood of infection was associated with the number of adults and younger juveniles in the clans and also with prey availability (East et al. 2013).

The family Diphyllobothriidae comprises two heteroxenous parasite genus with similar egg morphology, whose life cycle involves two intermediate hosts. The first is a copepod crustacean and the second a fish in the case of *Diphyllobothrium* or any vertebrate in the case of *Spirometra* that has fed on an infected copepod, before it infects canids and felids as definitive hosts (Holodniy et al. 1990; Mehlhorn and Gerhard 2002; Qiu and Qiu 2009). Infections are usually subclinical in definitive hosts. The egg prevalence of Diphyllobothriidae was 3% in our study. In other free-ranging African wildlife species, the egg prevalence of *Spirometra* varies largely from 8% in wild dogs in Zambia (Berentsen et al. 2012) to 74% in spotted hyenas in Kenya (Engh et al. 2003) and from 25 to 100% in lions in Zambia, Tanzania, and Mozambique (Bjork et al. 2000; Smith and Kok 2006; Berentsen et al. 2012; Kavana et al. 2015; Lajas et al. 2015).

The most common parasite genus detected in our cheetah population was the hookworm *Ancylostoma* (82%). *Ancylostoma* inhabit their host's small intestine where they feed on the mucosa and blood (Coop and Kyriazakis 1999; Zajac and Conboy 2012). Some genera can cause weakness, fatal anemia, and loss of body mass in their hosts (Bowman and Nelson 2014; Farid et al. 1965; Lefkaditis et al. 2006; Mehlhorn and Gerhard 2002; Seguel and Gottdenker 2017). *Ancylostoma* is one of the most energetically costly gastrointestinal parasite taxa that infects the spotted hyena in the Serengeti National Park (East et al. 2015). The pathologies caused by *Ancylostoma* increase with the number of adult hookworms present (Fujiwara et al. 2006). Infection occurs when infective larvae penetrate the skin of a host and, in some parasite species, when larvae enter a host through the intrauterine route or transmammary route during lactation (Burke and Roberson 1985).

This genus was also most common in captive cheetahs in Namibia with 44% prevalence of *Ancylostoma* eggs in all samples (Mény et al. 2012), and in free-ranging spotted hyenas in Kenya with 90% egg prevalence (Engh et al. 2003). Previously, it has been reported that free-ranging cheetahs in Zambia were infected with *A. iperodontatum* and *A. paraduodenale* and captive cheetahs in Senegal with *A. braziliense* and *A. caninum* (Round 1968). *A. caninum* was also detected in 19% of fecal samples of free-ranging wild dogs in South Africa (van Heerden et al. 1995).

We detected an egg prevalence of *Toxascaris* in 21% of cheetahs. In cats and dogs, infections with some *Toxascaris* genera can result in loss of body mass, diarrhea, a dull fur coat, or even death (Bowman and Nelson 2014; Lefkaditis et al. 2006; Mehlhorn and Gerhard 2002). In a study on captive cheetahs in Namibia, *T. leonina* was identified (Mény et al. 2012), thus it is possible that individuals in our study were infected with the same species. *T. leonina* was also identified in cheetahs from several zoos in Africa, North America, and Europe (Round 1968).

The egg prevalence of *Physaloptera* was 8% in our study. Infections with *Physaloptera* were less common (1%) in lions from Tanzania (Mueller-Graf 1995). In chacma baboons (*Papio ursinus* Kerr 1792) in South Africa, the prevalence of *Physaloptera* varied largely by region from 8% in Cape Peninsula to 78% in a wildlife reserve on the mainland (Damiana et al. 2012). Concerning the rare cases in which humans were infected with *Physaloptera* sp., no clinical signs were reported (Soriano and Pan 1955).

Conclusion

Our study is the first detailed report on gastrointestinal parasite diversity and prevalence in a free-ranging cheetah population. In terms of parasite richness, our results are similar to other studies on free-ranging African carnivore species. Although gastrointestinal parasitic infections are rarely lethal, they might have important fitness consequences by potentially reducing reproductive outcomes and decreasing survival probabilities, and thus are of conservation concern in combination with other pathogens and/or environmental stressors (Pedersen et al. 2007). Our study contributes to a deeper understanding of the gastrointestinal parasite diversity in cheetahs in different life history stages and can be used as a baseline for comparison in future studies. However, future studies may include a combination of classic parasitological methods with molecular identification of species (Traversa et al. 2008) and/or metabarcoding approaches (Heitlinger et al. 2017). More in-depth research of the subclinical effects of these parasites and the host responses is clearly needed to understand their impact on fitness in individuals and extinction risk for populations.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All experimental procedures described in the material and methods were approved by the Internal Ethics Committee of the Leibniz Institute for Zoo and Wildlife Research (Leibniz-IZW) (permit number #2002-04-01) and the Ministry of Environment and Tourism of Namibia (permit numbers 1300/2008, 1392/2009, 1514/2010, 1514/2011, 1689/2012, 1813/2013, 1914/2014, and 2067/2015). All experiments were carried out in compliance with the approved guidelines of the IZW and the laws of Germany and Namibia.

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