Evidence of high exposure to *Toxoplasma gondii* in free-ranging and captive African carnivores

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ABSTRACT

Toxoplasma gondii is an ubiquitous intracellular protozoan parasite. Mammals and birds are intermediate hosts and felid species are definitive hosts. In most human altered habitats the domestic cat is the predominant definitive host. Current knowledge of *T. gondii* infection in African ecosystems is limited. This study aimed to assess exposure to *T. gondii* in wild carnivores in the Serengeti ecosystem in East Africa. Carnivores can be infected by the consumption of tissue cysts when feeding on infected animals and by incidental ingestion of oocysts from environmental contamination. Incidental ingestion should occur regardless of a species' diet whereas the consumption of cysts should increase the chance of infection in carnivorous species. This predicts higher seropositivity in carnivorous than in insectivorous carnivores and lower seropositivity in juvenile carnivores with a long dependency on milk than in adults. We found high seropositivity in carnivorous species: 100% (15 of 15 samples) in adult African lions, 93% (38 of 41 samples) in adult spotted hyenas and one striped hyena sample was positive, whereas all four samples from the insectivorous bat-eared fox were negative. Juvenile hyenas (11 of 19 sera) had significantly lower seropositivity than adults (38 of 41 sera). Long-term monitoring of spotted hyenas revealed no significant difference in seropositivity between two periods (1988–1992 and 2000 to 2016). Identical results were produced in lion and hyena samples by a commercial multi-species ELISA (at serum dilution 1:10) and an in-house ELISA based on a recombinant *T. gondii* protein (at serum dilution 1:100), making the latter a useful alternative for small amounts of serum. We suggest that diet, age and lifetime range are factors determining seropositivity in carnivores in the Serengeti ecosystem and suggest that the role of small wild felids in the spread of *T. gondii* in the African ecosystem warrants investigation.

1. Introduction

Toxoplasma gondii is an intracellular apicomplexan parasite with a worldwide distribution. All mammal and bird species are thought to be potential intermediate hosts and all known definitive host species belong to the family Felidae (Tenter et al., 2000). Humans can also be infected and although the outcome is typically asymptomatic, infection can cause serious health problems, and therefore *T. gondii* is considered to be an important zoonotic pathogen (Dubey, 2010; Robert-Gangneux

and Darde, 2012).

Infection with *T. gondii* occurs orally either via ingestion of oocysts shed by infected felids or via the consumption of raw meat containing viable tissue cysts (Dubey, 2010). In the majority of urban landscapes worldwide, the domestic cat, *Felis catus*, is the most abundant definitive host species and primarily responsible for the contamination of urban areas with *T. gondii* oocysts (Dabritz et al., 2007; Torrey and Yolken, 2013). In landscapes where domestic cats are scarce or absent, environmental contamination by *T. gondii* oocysts stems mostly from wild

felid species (Afonso et al., 2013; Bevins et al., 2012; Lewis et al., 2017). After an initial acute infection, domestic cats shed oocysts for a limited period of days or weeks (Afonso et al., 2006; Zulpo et al., 2018). Antibodies induced by infection are presumed to persist throughout life (Dubey et al., 1995; Afonso et al., 2006; Zarnke et al., 2001) but may decline without further antigenic restimulation, as reported in humans (Rougier et al., 2017).

Toxoplasma gondii oocysts are resilient, particularly in moist environments, thus inadvertent ingestion of oocysts in water or on food may frequently occur in highly contaminated environments (Tenter et al., 2000; VanWormer et al., 2016). This transmission route most likely explains the variable levels of *T. gondii* seropositivity reported in several wild herbivore species worldwide (Bartova et al., 2007; Gauss et al., 2006; Hove and Mukaratirwa, 2005; Jardine and Dubey, 1996; Kutz et al., 2001; Riemann et al., 1975). The consumption of *T. gondii*-infected carcasses is also an important transmission route to carnivorous mammalian and avian species, as is the consumption by humans of insufficiently cooked infected meat (Jones and Dubey, 2012). Vertical transmission of *T. gondii* (in utero) has also been reported from several mammals, including cats and humans (Calero-Bernal et al., 2013; Parameswaran et al., 2009; Powell and Lappin, 2001; Robert-Gangneux and Darde, 2012; Sato et al., 1993; Vargas-Villavicencio et al., 2016; Verma et al., 2016).

Currently little is known about *T. gondii* infection in large carnivores in protected landscapes in Africa, where domestic cats are absent or scarce. One of the largest protected landscapes in East Africa is the 25,000 km^2 Serengeti-Mara ecosystem and adjacent Ngorongoro Conservation Area (Sinclair and Arcese, 1995), hereafter termed the Serengeti ecosystem. This landscape holds 26 wild carnivore species, including six felid species. The largest felid is the African lion, *Panthera leo* (hereafter lion), which mostly preys on wildebeest (*Connochaetes taurinus*), plains zebra (*Equus quagga*), Thomson's gazelle (*Eudorcas thomsoni*), African buffalo (*Syncerus caffer*), topi (*Damaliscus korrigum*), and kongoni (*Alcelaphus buselaphus*) and warthog (*Phacochoerus aethiopicus*) (Scheel and Packer, 1995). The spotted hyena (*Crocuta crocuta*, family Hyaenidae), is the most numerous large carnivore in the Serengeti ecosystem (Hofer and East, 1995). It is an efficient predator and scavenger that consumes a wide range of species (East and Hofer, 2013). In the Serengeti ecosystem it predominantly consumes wildebeest, plains zebra and Thomson's gazelle (Hofer and East, 1993a), and in comparison to other large carnivores, maternal input in terms of lactation is high and the period of lactation long (Hofer et al., 2016). The striped hyena (*Hyaena hyaena*, family Hyaenidae), is predominantly a scavenger that occurs at low densities in the Serengeti ecosystem (Kruuk, 1976). The bat-eared fox (*Otocyon megalotis*, family Canidae), is a small insectivorous species, that occasionally consumes small mammals and birds (Lamprecht, 1978). Currently little is known about *T. gondii* infections in these four species in the Serengeti ecosystem, beyond reports that none of 112 faeces from lions contained *T. gondii*-like oocysts (Müller-Graf, 1995) or that 12% of 33 faeces from lions contained *T. gondii*-like oocysts (Bjork et al., 2000), and that a single serum sample from a lion was positive for *T. gondii* neutralizing antibodies (Riemann et al., 1975). Measures of seropositivity in carnivores are considered a useful general index of the combined infection caused by both the presence of *T. gondii* in the environment (oocysts) and the consumption of tissue cysts by carnivorous species (Burrells et al., 2013; Millán et al., 2013; Zarnke et al., 2001). Inadvertent infection as a result of the ingestion of oocysts from the environment is expected in all four carnivore species, but infection from the consumption of infected prey is less likely in the insectivorous bat-eared fox than the carnivorous species and in juvenile carnivores than in adults.

Our study aims to (1) determine the proportion of wild carnivores in the Serengeti ecosystem with anti-*T. gondii* antibodies (2) determine whether the occurrence of anti-*T. gondii* antibodies was lower in juvenile spotted hyenas than adults; (3) contribute new but limited data on the number of African carnivores in European zoological gardens (zoos)

with anti-*T. gondii* antibodies, and (4) compare results from a commercial multispecies ELISA for detecting anti-*T. gondii* antibodies with those from an in-house ELISA with a recombinant antigen from *T. gondii*, using commercially available anti-cat antibodies, with the aim of assessing whether or not the in-house ELISA with defined properties might require less serum, and hence be an attractive proposition when only limited serum is available.

2. Material and methods

2.1. Sample collection

In the Serengeti ecosystem, serum and plasma samples were mostly collected by veterinarians from animals that were anaesthetized for other purposes, such as the removal of wire snares set by bushmeat hunters (Hofer et al., 1993) between 1988 and 2016. Serum and plasma samples were also obtained opportunistically from fresh carcasses of animals killed by predators or vehicles between 1988 and 2016. All samples from species other than the spotted hyena were obtained from adult animals. In spotted hyenas, juveniles were aged at first sighting to an accuracy of ± 7 days and were categorized as juvenile when less than 24 months old and as adult when 24 months of age or older (Golla et al., 1999). Juvenile spotted hyenas are dependent on milk for at least the first six months of life (Hofer et al., 2016). Infection with *T. gondii* via milk has been reported (but see Costa and Langoni, 2010; Powell et al., 2001) but we do not know if this occurs hyenas.

Samples were stored in liquid nitrogen (at −196 °C) or a freezer (−15 °C) in the Serengeti until transported frozen to Germany where they were stored (at −80 °C) until analysed. Sera from 7 captive carnivores from six zoos from Germany and the Netherlands (including Tierpark Berlin, Hodenhagen Zoo, Schwerin Zoo, Opel Zoo and Leipzig Zoo in Germany and Amersfoort Zoo in the Netherlands) were collected by zoo veterinarians primarily during health examinations of animals, including 3 spotted hyenas, 3 lions and one brown hyena, *Hyaena brunnea*.

2.2. Commercial immunological assays

A total of 35 sera (27 adults, 8 juveniles) collected from spotted hyenas in the Serengeti ecosystem between 1988 and 1992 were tested for anti-*T. gondii* antibodies in 1992 at the Animal Health Diagnostic Centre at Cornell University (Ithaca, New York), using protocols established by J.P. Dubey and the application of a commercial indirect haemagglutination (IHA ELISA) test (Toxoplasmosis TPM-Test indirect haemagglutination kit, Wampole Laboratories, Princeton, NJ, USA.) Subsequent comparison of this indirect haemagglutination test with a modified commercial multi-species IgG ELISA (Toxo IgG II ELISA kit, Wampole Laboratories, Princeton, NJ, USA) showed very good agreement between the results produced by the two assays using sera from 6 domestic species, including cats and dogs (Schaefer et al., 2011).

Furthermore, we screened 45 samples of serum or plasma collected from wild carnivores in the Tanzanian section of the Serengeti ecosystem between 1997 and 2016, including 25 samples (14 adults and 11 juveniles) from spotted hyenas, 15 samples from adult lions, 4 samples from adult bat-eared foxes, and one from an adult striped hyena. To these samples and to the samples from European zoos we applied the ID Screen® Toxoplasmosis Indirect Multi-species ELISA (IDVet, Grabels, France). The ID Screen® Toxoplasmosis Indirect Multi-species ELISA was used per supplier's instructions. Serum and plasma samples were diluted 1:10. Samples were considered positive if the Sample/Positive control ratio (S/P %) was higher than 50%, doubtful if the S/P % was between 40 and 50% and negative if the S/P % was below 40%. Controls were provided in the kit. We conducted blind screening with regard to species and age.

Table 1

Anti-*Toxoplasma gondii* antibodies in African lions in zoos and in natural populations worldwide. N, number of samples tested. IFAT, indirect fluorescent antibody test; LAT, latex agglutination test; MTA, modified agglutination test; ELISA, Enzyme-Linked ImmunoSorbent Assay; * ID-Screen *T. gondii* ELISA also used in this study; N, number of samples tested.

Location	Positive/N (%)	Test used	Reference	Captive/Wild
Germany	3/3 (100)	ELISA*	Current study	Captive
Brazil	5/9 (56)	IFAT	André et al. (2010)	Captive
Brazil	14/27 (52)	MAT	Silva et al. (2001)	Captive
China	6/6 (100)	MAT	Yang et al. (2017)	Captive
Czech Republic	2/2 (100)	IFAT	Sedlak and Bártová (2006)	Captive
Italy	13/14 (93)	IFAT	Marková et al. (2018)	Captive
Mexico	7/7 (100)	MAT	Alvarado-Esquivel et al. (2013)	Captive
Romania	3/3 (100)	ELISA*	Dărăbuş et al. (2014)	Captive
Senegal	3/7 (43)	ELISA*	Kamga-Waladjo et al. (2009)	Captive
South Africa	10/14 (71)	IFAT	Cheadle et al. (1999)	Captive
Thailand	1/7 (14)	LAT	Thiangtum et al. (2006)	Captive
USA	12/22 (55)	MAT	de Camps et al. (2008)	Captive
USA	8/10 (80)	IFAT	Spencer et al. (2003)	Captive
Tanzania (Serengeti NP)	15/15 (100)	ELISA*	Current study	Wild
South Africa (Kruger NP)	12/12 (100)	IFAT	Penzhorn et al. (2002)	Wild
Zimbabwe	21/21 (100)	IFAT	Penzhorn et al. (2002)	Wild
Botswana	49/53 (92)	IFAT	Penzhorn et al. (2002)	Wild
South Africa (Hluhluwe-Umfolozi NP)	30/30 (100)	IFAT	Penzhorn et al. (2002)	Wild
Namibia	65/66 (98)	ELISA	Spencer and Morkel (1993)	Wild

2.3. In-house ELISA

We applied an in-house ELISA to all lion, spotted hyena and stripped hyena samples, including the samples from European zoos. Expression and purification of recombinant SAG1 (rSAG1-6H) was done as follows. Briefly, C-terminally 6His-tagged SAG1 (amino acids 31–289) from pSAG1-GPI (Seeber et al., 1998) was cloned into plasmid pASG-IBA33 (IBA, Göttingen, Germany) according to the manufacturer's instructions. For expression the resulting plasmid pASG33-SAG1 was transformed into *Escherichia coli* SHuffle® T7 Express cells (New England Biolabs, Frankfurt am Main, Germany) together with plasmid pMJS9 (Nguyen et al., 2011) to aid in proper disulphide bonding of rSAG1-6H. After induction of expression with 0.5% arabinose and 200 ng/ml anhydrotetracycline for 18 h at 30 °C, rSAG1-6H protein was purified using a HisTrap FF 1 ml column on an ÄktaPurifier FPLC system essentially as described by the manufacturer (GE Healthcare, Chicago, USA). Finally, buffer was exchanged to PBS on a PD10 column (GE Healthcare) before the protein was stored at −20 °C until further use. Protein concentration was determined using the BCA assay (Thermo Fisher, Darmstadt, Germany). Protein purity was assessed by SDS-polyacrylamide gel electrophoresis, silver staining and immunoblot using anti-His tag antibodies and found to be ~95% pure.

For the ELISA MaxiSorp® plates (Thermo Fisher) were coated overnight at 4 °C with 100 ng of rSAG1-6H per well or PBS as control. All further steps were performed at room temperature. Unspecific binding of serum to the plate was blocked by incubation with 5% soluble milk powder in PBS for 1 h. Then sera from lions and hyenas were serially diluted 1:100, 1:200 and 1:400, added in duplicates to wells and incubated for 90 min. As positive and negative controls we used seropositive plasma and seronegative serum from domestic cats (kindly provided by G. Schares; Friedrich-Loeffler-Institut, Riems, Germany). Controls were used at a dilution of 1:2000. As secondary antibody we used a peroxidase-conjugated goat anti-cat IgG (H + L) at a dilution 1:4000 (KPL, Gaithersburg, MD, USA). SureBlue® TMB Peroxidase substrate (KPL, Gaithersburg, MD, USA) was added and the reaction stopped after 10 min by adding sulphuric acid. The resulting colour signal measured at 450 nm (650 nm reference) at a Tecan Infinite M200 PRO reader. Samples were considered positive when the value was higher than the mean from two independent experiments + 3 standard deviations of negative cat or hyena sera of the same dilution.

2.4. Data analysis

Statistical analyses were performed in R, version 3.3.0 (R core team, 2016). Proportions were calculated with the Clopper-Pearson confident intervals (CI) at confidence level of 0.95 using the package DescTools version 0.99.24 (Signorell et al., 2018). We tested for differences between groups using the Pearson's chi-squared test (R core team, 2018). In all statistical tests, the significance threshold was fixed at 5% and all tests were two-tailed.

3. Results

3.1. Anti-T. gondii antibodies in carnivores in the Serengeti ecosystem

Results from a commercial IHA ELISA revealed that most serum/plasma samples from spotted hyenas between 1988 and 1992 had anti-*T. gondii* antibodies, including 6 of 8 samples (75%, CI: 35–97%) from juveniles and 24 of 27 samples (89%, CI: 71–98%) from adults. Results from a commercial ID Screen® indirect multi-species ELISA for samples from spotted hyenas obtained between 2000 and 2016 revealed that 5 of 11 samples (45%, CI: 17–77%) from juveniles and 14 of 14 samples (100%, CI: 77–100%) from adults had anti-*T. gondii* antibodies. As we found no significant difference in the results from adults sampled in these two periods (Chi-squared test, $\chi^2 = 0.44$, df = 1, p = 0.51) or for those from juveniles in these two periods ($\chi^2 = 0.67$, df = 1, p = 0.41) we combined the results for adults from the two periods, and the results from juveniles in the two periods. Overall, these combined results revealed that 11 of 19 juvenile (58%, CI: 33–80% %) and 38 of 41 adult (93%, CI: 80–98%) spotted hyenas had anti-*T. gondii* antibodies. Juveniles were less likely to have anti-*T. gondii* antibodies than adults ($\chi^2 = 8.30$, df = 1, p = 0.004).

In total we investigated 20 serum samples from adults in three additional carnivore species in the Serengeti ecosystem between 1997 and 2005, using the ID Screen® indirect multi-species ELISA. The results revealed that all 15 sera (100%, CI: 78–100%) from adult lions and one sample from a striped hyena had anti-*T. gondii* antibodies whereas sera from four bat-eared foxes were all negative (0% CI: 0–60%). There was no difference in the occurrence of anti-*T. gondii* antibodies in samples from adult spotted hyenas and adult lions ($\chi^2 = 0.17$, df = 1, p = 0.68).

Results from the ID Screen® indirect multi-species ELISA for seven samples from captive carnivores (3 spotted hyenas, 3 lions and one

Table 2

Anti-*Toxoplasma gondii* antibodies in Hyaenidae previously reported in the literature. N, number of samples tested; SFDT, Sabin-Feldman dye test; IFA, immuno-fluorescence assay; MAT, modified agglutination test; ELISA, Enzyme-Linked ImmunoSorbent Assay; * ID-Screen *T. gondii* ELISA. Spotted hyena (*Crocuta crocuta*), Brown hyena (*Parahyaena brunnea*), Striped hyena (*Hyaena hyaena*).

Hyena species	Location	Positive/N (%)	Test	Reference
Spotted hyena	Zoos in Germany and Netherlands	3/3 (100)	ELISA*	Current study
Brown hyena	Zoo in Germany	1/1 (100)	ELISA*	Current study
Spotted hyena (juveniles)	free ranging, Serengeti NP (Tanzania)	11/19 (58)	ELISA*	Current study
Spotted hyena (adults)	free ranging, Serengeti NP (Tanzania)	34/41 (93)	ELISA*	Current study
Spotted hyena	free ranging, Kenya	6/6 (100)	SFDT	Bakal et al. (1980)
Brown hyena	Zoos in Czech Republic	3/3 (100)	IFA	Sedlak and Bártová (2006)
Striped hyena	Zoo in France	1/2 (50)	MAT	Alerte (2008)
Spotted hyena	Zoo in France	1/1 (100)	MAT	Alerte (2008)
Striped hyena	Breeding Centre for Endangered Arabian Wildlife, UAE	3/6 (50)	MAT	Dubey et al. (2010)
Spotted hyena	Zoos in Australia	5/10 (50)	IFA	Wait et al. (2015)

brown hyena) in European zoos were all positive (Tables 1 and 2).

3.2. Comparison of an in-house ELISA assay with a commercial multi-species ELISA

We assessed a total of 48 samples obtained from lions, spotted hyenas and a striped hyena between 2000 and 2016 in the Serengeti ecosystem and from captive lions, spotted hyenas and a brown hyena, for the presence or absence of anti-*T. gondii* antibodies using the commercial ID Screen® indirect multi-species ELISA kit and our in-house ELISA. The results in terms of the antibody being detected or not for each of these 48 samples were identical, even though for the commercial ID Screen® indirect multi-species ELISA we applied a serum dilution of 1:10 whereas for the in-house ELISA we used a serum dilution of 1:100.

4. Discussion

Our study revealed that a high proportion of large carnivores in the Serengeti ecosystem had anti-*T. gondii* antibodies but none of the four samples from the insectivorous bat-eared fox had positive titres. Juvenile spotted hyenas had positive titres less often than adults. The proportion of adult spotted hyenas with anti-*T. gondii* antibodies in the study population was similar during two sampling periods: from 1988 to 1992 and from 2000 to 2016. In line with previous studies (Tables 1 and 2) we found high *T. gondii* seropositivity in large African carnivores in zoos.

Our long-term survey of anti-*T. gondii* antibodies in spotted hyenas in the Serengeti ecosystem revealed that 93% of 41 sera from adults were positive. The only previous report we are aware of found that all of six sera from wild spotted hyenas in Kenya were positive (Bakal et al., 1980). We found that juvenile spotted hyenas in the Serengeti ecosystem were less often seropositive (58% of 19 sera from juveniles) than adults. This in part may be because juveniles in our study population are dependent on milk for the first six months of life and are weaned between 12 and 18 months of age (Hofer et al., 2016; Hofer and East, 1995). As a result, juveniles are less likely to consume *T. gondii* infected tissue than adults. Also, the likelihood of exposure to a pathogen can increase with age and life-time range, as illustrated by the increase with age in seropositivity to rabies specific virus-neutralizing antibodies in our study population (East et al., 2001). Juveniles remain at communal dens until approximately 12 months of age (Hofer and East, 1993b) and hence their life-time range during their first year of life is small. Incidental contamination of communal den areas with oocysts is probably lower than in other areas in the ecosystem where felids more often deposit faeces, thus chance encounters with contaminated areas should increase with life-time range. In other mammalian (humans, sheep) intermediate hosts and definitive hosts (domestic cats and wild felids), age-dependent increases in seropositivity has been reported (Afonso et al., 2010; Dubey, 2009; Wilking et al.,

2016; Zarnke et al., 2001). An age-dependent increase in seropositivity has also been reported in European avian species (Cabezón et al., 2011). We speculate that the consumption of infected tissue probably induces anti-*T. gondii* antibodies in spotted hyenas more often than the incidental ingestion of oocysts.

Toxoplasma gondii seropositivity in wild populations of the two other carnivorous species in the family Hyaenidae, the striped hyena and brown hyena, is unknown. We found that serum from one adult striped hyena in the Serengeti ecosystem was positive and that most spotted, striped and brown hyenas held in zoos (Table 2) have anti-*T. gondii* antibodies. Sera from all four bat-eared foxes, an insectivorous canid, in the Serengeti ecosystem were seronegative. To our knowledge, *T. gondii* seropositivity in the insectivorous member of the family Hyaenidae, the aardwolf (*Proteles cristatus*) is unknown, but it may be that seropositivity in aardwolves is lower than in carnivorous hyena species. Consistent with the idea of the importance of diet, carnivorous birds have higher *T. gondii* seropositivity than other bird species (Cabezón et al., 2011), and carnivorous mammals in zoos and circuses in Italy have higher seropositivity than herbivorous mammals (Marková et al., 2018).

In line with studies on lion sera from other locations in Africa (Table 1) we found that all of 15 sera from adult lions in the Serengeti ecosystem contained anti-*T. gondii* antibodies. Whether lions shed oocysts during a relatively short period after an initial acute infection, as in the domestic cat (Afonso et al., 2006; Zulpo et al., 2018) is not known. Standard coprological examination of samples from lions in the Serengeti ecosystem has revealed either the presence of *T. gondii*-like oocysts in 12% of fecal samples (Bjork et al., 2000) or the absence of oocysts in faeces (Müller-Graf, 1995). Studies on both the Eurasian lynx (*Lynx lynx*) and Canadian lynx (*Lynx canadensis*), also report an absence of *T. gondii* oocysts in faeces (Ryser-Degiorgis et al., 2006; Simon et al., 2013). Little if anything is known on *T. gondii* infection and oocyst shedding by the three smaller felid species in the Serengeti ecosystem: the serval (*Felis serval*), the caracal (*Felis caracal*) and the African wild cat (*Felis sylvestris*) or in the rodent and bird prey species of these three species (Hunter and Bowland, 2013; Stuart and Stuart, 2013; Stuart et al., 2013).

Information on *T. gondii* seropositivity in African wild ungulates provides information on environmental contamination, as herbivores are probably infected by the incidental consumption of oocysts in the environment while grazing or drinking water. In the Serengeti ecosystem, plains zebras are consumed by large predators (Grange et al., 2004) and 3.6% of 29 sera from plains zebra have anti-*T. gondii* antibodies (Riemann et al., 1975). In Zimbabwe, a wide range of seropositivity (between 10% and 90%) has been reported in wild ungulates (Hove and Dubey, 1999; Hove and Mukaratirwa, 2005). Research is required to investigate the factors determining the occurrence of both anti-*T. gondii* antibodies and tissue cysts in wild ungulate species, to better understand the relative importance of environmental contamination and the consumption of tissue cysts as transmission

routes for *T. gondii* to definitive felid hosts and intermediate carnivorous mammals and bird hosts. As none of four sera from the insectivorous bat-eared fox were seropositive we cautiously interpret the results from this small samples to suggest that environmental contamination with *T. gondii* oocysts in the habitats occupied by these bat-eared foxes was low and the chance of infection through the consumption of prey (mostly insects and occasional small birds and mammals) was also low.

Our current knowledge of *T. gondii* epidemiology in ecosystems, such as the Serengeti, that contain many wild felid species and few domestic cats is limited. If we assume that all six wild felid species in the Serengeti ecosystem are infected with *T. gondii* and that oocyst shedding is mostly restricted to a limited period following a primary infection, as in the domestic cat, then it could be argued that because of their shorter lifespans, smaller ranges and generally higher densities (Hunter and Bowland, 2013; Stuart and Stuart, 2013; Stuart et al., 2013), small felid species (the African wild cat, serval and caracal) might be expected to contribute more to environmental contamination than the larger felid species (lion, leopard and cheetah) (Caro, 2013; Hunter et al., 2013; West and Packer, 2013).

The current worldwide genetic diversity of *T. gondii* has been classified into three clonal lineages (named type I, II, or III), that are three of 16 described haplogroups belonging to six major clades (Su et al., 2012). Currently little is known about the genetic diversity of *T. gondii* strains in Africa, particularly from East Africa (Galal et al., 2017). This lack of information on *T. gondii* in this region of Africa is also apparent in the Global Mammal Parasite Database (Stephens et al., 2017). Of 568 entries specifying *T. gondii* as a parasite species, only 28 are from Africa, and none are from East Africa.

Even though experimental infection of laboratory mice with two *T. gondii* strains of different genotypes has been reported (Brandão et al., 2009; Burrells et al., 2013), it is currently unclear whether this occurs in wild mammalian hosts. Infection with more than one *T. gondii* genotype challenges the view that *T. gondii* induces life-long immunity (Zulpo et al., 2018). Analysing tissues known to harbour *T. gondii* cysts (brain, heart, muscles) from lions and hyena carcasses that potentially sampled a collection of genotypes from infected prey could give insights into the genetic diversity of *T. gondii* in the Serengeti ecosystem.

A commercial multi-species Toxoplasmosis ELISA kit that is based on the major *T. gondii* antigen SAG1 was previously shown to work with sera from domestic cats and dogs (Roqueplo et al., 2011) as well as lions (Dărăbuș et al., 2014; Kamga-Waladjo et al., 2009). We showed that this kit and our in-house ELISA, using recombinant SAG1 and commercially available anti-cat antibodies, provided identical results with respect to seropositivity. The in-house ELISA has the advantage of demonstrating seropositivity at dilutions of one or more orders of magnitude higher than the commercial kit. Our ELISA is both cost-effective since it only requires cheap reagents, i.e. antigen and antibody, and is resource-saving as much less serum is required to obtain identical qualitative results. The latter is of importance when serum from wild animals (which can be time consuming and costly to obtain) need to be screened for evidence of exposure to multiple pathogens. Given the recent advances in the use of smartphones as colorimetric readers for ELISA-based assays (Vashist et al., 2015; Vashist and Luong, 2018) together with smartphone apps that can subsequently analyse the data we envisage that cheap in-house ELISAs like ours could be used to provide useful results on the relationship between *T. gondii* and wildlife in the field.

5. Permits

The research was approved by The Ethics Committee of Leibnitz Institute for Zoo and Wildlife Research (permit number 1997-02-01). Samples from lions in Tanzania were transported with CITES permits issued by the relevant Tanzanian and German Authorities. Research in Tanzania was conducted under clearance granted by the Tanzania

Commission for Science and Technology (COSTECH) to HH, SCMF and MLE.

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