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Molecular epidemiology of *Giardia duodenalis* in an endangered carnivore – The African painted dog

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ABSTRACT

The African painted dog (*Lycaon pictus*) is an endangered carnivore of sub-Saharan Africa. To assist in conservation efforts a parasitological survey was conducted on wild and captive populations. Faecal samples were collected and examined for the presence of parasites using traditional microscopy techniques. The protozoan *Giardia duodenalis* was identified at a prevalence of approximately 26% in the wild populations and 62% in the captive population. Molecular characterisation of these isolates using three loci, 18S rRNA, β-giardin and the glutamate dehydrogenase gene revealed the zoonotic assemblages A and B existed in high proportions in both populations. The dog assemblages C and D were rarely observed. The identification of the zoonotic genotype suggests this species has the potential to act as a reservoir for human infections. Zoonotic transmission may be possible in captive populations due to the close interaction with humans however, in wild populations anthropozoonotic transmission seems more likely. This study is the first to observe *G. duodenalis* in the African painted dog and to identify a possible emerging disease in this wild carnivore.

1. Introduction

The impact that parasites and other infectious agents have on wildlife has been increasingly recognized within conservation programs. Stressors such as human encroachment and habitat destruction are altering the incidence and effect that these pathogens have on wildlife populations, especially those endangered and under stress (McCallum and Dobson, 2002; McCallum and Dobson, 1995; Smith et al., 2009a,b; Wyatt et al., 2008). Habitat destruction results in reduced species ranges and increased interactions between populations, which in turn raises the risk of disease transmission between these populations (Lyles and Dobson, 1993; Scott, 1988). Perhaps of greater significance is human encroachment and the resultant increased interaction of humans and their domestic animals with cohabiting wildlife. Approximately 80% of domestic animal pathogens can infect wildlife (Cleaveland et al., 2001) so the risk of disease spillover into wild populations is potentially great. Examples of this have been seen with rabies and canine distemper, which were introduced from domestic dogs and decimated local populations of the African lion, spotted hyaena and the African painted dog (Alexander and Appel, 1994; Haas et al., 1996; Kat et al., 1996; Roelke-Parker et al., 1996).

The African painted dog (*Lycaon pictus*) is an endangered carnivore of sub-Saharan Africa (IUCN, 2010). This animal is a top level carnivore which once numbered approximately 300,000 individuals in 34 sub-Saharan countries. Viable populations now only exist in six countries and the current total number of animals in all of these countries is approximately 3000–5500 (IUCN, 2010). The African painted dog is

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from a very primitive canid lineage which is phylogenetically distinct from all other wolf-like canids (Bardeleben et al., 2005; Girman et al., 1993). Therefore, conservation of this unique and endangered carnivore is of great importance and requires a deep level of understanding of the processes which threaten the viability of wild populations. Diseases such as those caused by parasites are one such threatening process.

Parasite species previously observed in the African painted dog have included the macroparasites *Taenia* sp., *Ancylostoma caninum*, *Dipetalonema reconditum* and *Toxocara* sp. (van Heerden, 1986; van Heerden et al., 1995), and protozoan parasites *Babesia canis*, *Hepatozoon* sp. and *Sarcocystis* sp. (Bwangamoi et al., 1993; Colly and Nesbit, 1992; Peirce et al., 1995; Penzhorn, 2006). To date, species of *Giardia* have not been recorded in the African painted dog.

Giardia duodenalis (syn *G. intestinalis*; *G. lamblia*) is a ubiquitous protozoan parasite found in a wide range of animals which can cause diarrhoea and ill thrift in a wide range of host species (Farthing et al., 1986; Thompson and Monis, 2004). Molecular characterisation of *G. duodenalis* isolates has identified seven genetically distinct genotypes which appear to be host specific (Monis et al., 2009; Thompson, 2000). These genotypes have been named assemblages A through to G. Of interest to this study are Assemblages C and D which are specific to dogs and assemblages A and B which affect humans, but are also found in a variety of other mammals. Assemblages A and B are thus of particular significance as they are considered potential zoonotic agents. For this reason, there is an emerging interest in domestic animals and wildlife as possible reservoirs for this parasite. There have been many published studies of *G. duodenalis* in domestic dogs but relatively few for wild canids, reflecting the paucity of parasitological studies in wildlife (Applebee et al., 2005; Thompson et al., 2010).

Domestic dogs are often infected with *G. duodenalis* assemblages C and D, but assemblages A and B are also common (Ipankaew et al., 2007; Leonhard et al., 2007; Traub et al., 2004; van Keulen et al., 2002). Studies of wildlife species have also observed infections with assemblages A and B and these have raised questions on the transmission dynamics of this parasite (Fayer et al., 2006; Graczyk et al., 2002; Thompson et al., 2009). Zoonotic transfer has been suggested between dogs and humans living in close conditions (Ipankaew et al., 2007; Lalle et al., 2005; Pelayo et al., 2008; Traub et al., 2004; Winkorth et al., 2008) however the total risk appears to be low (Berrilli et al., 2004; Hopkins et al., 1997). Alternatively, anthropozoonotic transfer has been suggested, whereby humans may infect other animals, particularly wildlife (Applebee et al., 2005; Caccio et al., 2005; Graczyk et al., 2002). Overall the transmission dynamics and subsequent risks to human health or wildlife are not well understood (Caccio et al., 2005).

The aim of this study was to examine wild and captive populations of the African painted dog for the presence of *Giardia* species, and to genetically characterise any isolates obtained from these populations using three loci, 18S rRNA, β-giardin and the glutamate dehydrogenase gene. This will enable identification of *G. duodenalis* at the assemblage and sub-assemblage levels and subsequently any health risk this parasite may pose within wild and captive environments of this endangered carnivore.

2. Materials and methods

2.1. Sample collection

Faecal samples were collected while tracking wild populations in Zambia and Namibia during three field trips conducted in 2007, 2008 and 2009. In most cases these samples were collected within moments of being deposited. The captive samples came from a population in a zoo in Australia ($n = 17$) which was also sampled in 2007, 2008 and 2009. Additionally, two human faecal samples were obtained from zoo keepers working with the captive population, which had been approved by Murdoch University's Human Research Ethics Committee. All samples were given a consistency score of 1–5 with 1 being firm and 5 being liquid. Two grams of faecal matter were placed into two 10 ml centrifuge tubes containing 8 ml of either 10% formalin for subsequent microscopy analysis or 70% ethanol for molecular characterisation. If possible, individual animal identification was obtained while collecting faecal samples. In the field this was possible by identification of coat patterns of animals which had previously been recorded, while in the captive population, coloured plastic beads added to the animals feed were employed as faecal markers.

2.2. Parasitological techniques

All formalin fixed samples were screened via microscopy using malachite green stain (Elliott et al., 1999) followed by sodium nitrate and zinc sulphate flotations. The use of different flotation media provided different specific gravities which ensured that all parasite ova would be floated and observed under the microscope. All parasite ova and cysts observed were recorded, although only data for *Giardia* are reported here. All samples were then analysed using PCR protocols specific for *Giardia*, as described below. See Table 1 for samples which tested positive for *Giardia* through microscopy and/or with PCR amplification.

2.3. DNA extraction

A 2 ml aliquot of each ethanol-preserved sample was centrifuged and ethanol eluted. The remaining solid matter was used for DNA extraction using the Maxwell® 16 Instrument (Promega, Madison, USA) as per manufacturer's instruction. In an attempt to reduce the impact of inhibitors in carnivore faecal matter, the final elution was diluted to a 5:1 ratio with nuclease free water.

2.4. Amplification of 18S rRNA locus

A nested PCR was used for amplification of a 130 bp product of the 18S rRNA locus. The primary reaction utilized the primers RH11 and RH4 (Hopkins et al., 1997) and in the nested reaction primers GiarF and GiarR (Read et al., 2004) were used. The PCR reaction was performed in 25 μl

Table 1
Prevalence of parasites (with 95% confidence intervals in parentheses) detected in faecal samples from captive and wild populations of the African painted dog.

Prevalence of *Giardia* sp.	Captive ($n=16$)	Wild – Zambia ($n=43$)	Wild – Namibia ($n=28$)	Wild – total ($n=71$)	Cap vs. wild *P*-value
Total prevalence(CI 95%)	62% (37–82%)	28% (16–43%)	25% (12–44%)	27% (17–38%)	0.009

volumes consisting of 1 μl of extracted DNA, 2.0 mM $MgCl_2$, 1× reaction buffer (20 mM Tris–HCl, pH 8.5 at 25 C, 50 mM KCl), 200 μM of each dNTP, 10 ρmol of each primer, 0.5 units of TAQ-T*i* DNA polymerase (Fisher Biotec, Perth Australia), and H_2O. DMSO 5% was added to the primary round and 0.5 μl BSA (10 mg/mL) was added to the nested round. Amplification conditions were modified from Hopkins et al. (1997) and are as follows; the primary reaction was initiated with a denaturing step of 96 °C for 5 min, then 35 cycles of 96 °C for 45 s, 58 °C for 30 s and 72 °C for 45 s, followed by a final extension of 72 °C for 7 min. The nested reaction was altered by a decrease in annealing temperature to 55 °C.

The PCR product was purified using a Wizard SV gel and PCR Clean-up system (Promega, Madison, USA) after the manufacturer's instructions, except the final elution was reduced to 20 μl from 50 μl. Sequence reactions were performed using the Big Dye Terminator Version 3.1 cycle sequencing kit (Applied Biosystems) according to the manufacturer's instructions. PCR products were sequenced in the reverse direction only (GiarR), as initial sequencing of forward reactions was unsuccessful in this study as seen in others (Leonhard et al., 2007; Palmer et al., 2008). Reactions were electrophoresed on an ABI 3730 48 capillary machine. Assemblages were identified via single nucleotide polymorphisms (SNP's) within the 130 bp sequence as previously described by Hopkins et al. (1997).

2.5. Amplification of β-giardin locus

A nested PCR was used for amplification of a 492 bp product of the β-giardin gene. The primary reaction utilized the primers G7 and G759 (Caccio et al., 2002) and in the nested reaction primers as described by Lalle et al. (2005) were used. The PCR reaction was performed in 25 μl volumes consisting of 1 μl of extracted DNA, 2.0 mM $MgCl_2$, 1× reaction buffer (67 mM Tris–HCl, 16.6 mM $(NH_4)_2SO_4$, 0.45% Triton X-100, 0.2 mg/ml gelatin), 200 μM of each dNTP, 10 ρmol of each primer, 0.5 units of T*th* Plus DNA polymerase (Fisher Biotec, Perth Australia), and H_2O. The primary reaction was initiated with a denaturing step of 95 °C for 5 min, then 40 cycles of 95 °C for 30 s, 65 °C for 30 s and 72 °C for 60 s, followed by a final extension of 72 °C for 7 min. The nested reaction was altered by a decrease in annealing temperature to 55 °C and 35 cycles. The PCR product was purified and sequenced as described above for the 18S rRNA locus. Assemblages were identified via SNP's within the 292 bp sequence when compared with reference sequences AY072723, AY072727, AY072726, AY072725 and AY45646 (Caccio et al., 2002; Lalle et al., 2005).

2.6. Amplification of the glutamate dehydrogenase locus

A semi-nested PCR was used for amplification of a 432 bp product of the glutamate dehydrogenase (*gdh*) gene. The primary reaction utilized the primers GDHeF and GDHiR with GDHeR and GDHiR in the semi-nested reaction as described by Read et al. (2004). The PCR reaction was performed in 25 μl volumes consisting of 1 μl of extracted DNA, 3.0 mM $MgCl_2$, 1× reaction buffer (67 mM Tris–HCl, 16.6 mM $(NH_4)_2SO_4$, 0.45% Triton X-100, 0.2 mg/ml gelatin), 200 μM of each dNTP, 10 ρmol of each primer, 0.5 units of T*th* Plus DNA polymerase (Fisher Biotec, Perth Australia), and H_2O. DMSO 5% was added to the primary reaction only. Cycling conditions for both reactions were denatured at 94 °C for 5 min, then 45 cycles of 94 °C for 30 s, 60 °C for 30 s and 72 °C for 1 min, followed by a final extension of 72 °C for 4 min. The PCR product was purified, sequenced and analysed as previously described for the 18SrRNA locus. Assemblages were identified via SNP's within the 432 bp sequence when compared with reference sequences AY178735, AY178737, AF069059, AY178739, L40508 and AY178738 (Monis et al., 1996, 1999).

2.7. Data analysis

Of the 17 samples found positive with microscopy 12 were amplified with PCR along with 27 found negative with microscopy. Prevalence of infection was expressed as percentage of positive samples found either by microscopy or PCR with 95% confidence intervals calculated assuming a binomial distribution, using the software Quantitative Parasitology 3.0 (Rozsa et al., 2000). Faecal consistency scores were compared between dogs testing positive or negative using a Wilcoxon rank sum test. Differences in prevalence between populations were tested using the Fisher exact test. To compare prevalences between wild and captive populations, data from year one (2007) of the captive population was tested against the total individual animals sampled from the wild. Sampling of the captive population was duplicated each year, but within wild populations animals were sampled only once as it was found that the composition of packs altered significantly from season to season with many deaths recorded and therefore duplicate sampling was extremely rare. Differences in prevalence of infection among years in the captive population were tested by a Cochran χ^2 test, with individual dogs as the blocking factor.

Genotypic information was obtained using the 18S rRNA locus. The multicopy nature and strong sequence conservation of 18S rRNA provide the sensitivity and specificity required to determine basic assemblage information (Caccio and Ryan, 2008; Weilinga and Thompson, 2007).

However, to obtain sub-assemblage information the loci β-giardin and *gdh* loci were used, as has been done in many other studies (Abe et al., 2003; Lalle et al., 2005; Leonhard et al., 2007; Palmer et al., 2008; Read et al., 2004; Traub et al., 2004). Resultant nucleotide sequences were compared with published sequences on NCBI. Sequences were aligned against reference sequences for β-giardin and *gdh* loci using the sequence alignment program Sequencer™ 4.8 (Gene Codes Corporation, Ann Arbor, USA), allowing assemblage and sub-assemblage assignment.

3. Results

3.1. Prevalence of infection

Table 1 shows the prevalence of infection with *Giardia* spp. within the two wild populations and one captive population of painted dogs. Prevalence of infection with *Giardia* did not differ significantly between the two wild populations ($P = 1.00$), but was significantly greater in the captive population than in either wild population ($P = 0.03$ for comparison of captive and Zambian populations; $P = 0.02$ for comparison of captive and Namibian populations). Over all populations, there was no significant difference in faecal consistency scores between infected (mean score = $2.4 \pm$ SE 0.19) and uninfected (2.3 ± 0.13) dogs ($P = 0.78$), with only three ($n = 87$) samples presenting with diarrhoea (i.e. consistency score of 4 or 5).

3.2. Molecular characterisation of G. duodenalis isolates

All samples were initially screened at the 18S rRNA locus and identified as *G. duodenalis*. Samples successfully amplified at the 18S locus and/or found to be positive with microscopy were also amplified at the β-giardin and *gdh* loci. Within the captive population, isolates from 16 individual animals were successfully amplified at one, two or three loci. Amplification was successful for isolates from 14 animals at the 18S rRNA locus, 3 at β-giardin and 6 at *gdh*. Within the wild population, isolates from 14 individual animals were amplified; 13 at 18S rDNA, 7 at β-giardin and 1 at *gdh* (Table 2). Isolates from both captive and wild populations displayed variation in assemblage and sub-assemblage type depending on the locus used (Table 2).

Assemblage information obtained from the 18SrRNA gene showed a dominant proportion of samples in the captive population to be assemblage B (86%) with mixed assemblages A/B and B/C/D both being 7%. Within the wild population assemblage B was also dominant with 38% of isolates; however, greater variability was seen in the remaining isolates with several mixed assemblages.

Longitudinal data were obtained from the captive population as sampling was conducted every year for 3 years (Table 3). *G. duodenalis* was observed in each year, with prevalences ranging between 50% and 69% (Table 3). All animals tested positive at least once throughout the sampling period and there was no significant difference in prevalence among years ($\chi^2 = 0.19$, $P = 0.16$). Assemblages A and B were seen in high proportions in each year (Table 3).

Isolates obtained from the two zoo keepers were also amplified at the 18SrDNA and β-giardin loci. Sequencing of the 18SrRNA gene revealed assemblage B and BIII/BIV at the β-giardin locus for both isolates.

4. Discussion

This study is the first to identify *G. duodenalis* in the African painted dog. Previous parasitological studies on this animal have not found this protozoan (Colly and Nesbit, 1992; Peirce et al., 1995; Penzhorn, 2006; van Heerden, 1986; van Heerden et al., 1995).

The prevalence of *G. duodenalis* in the wild, 28% in the Zambian population and 25% in the Namibian population, was similar to other reports in wild canids, e.g. 12.5–32% in coyotes (Gompper and Williams, 1998; Thompson et al., 2009; Trout et al., 2006), and 46% in wolves (Klock et al., 2005). The greater prevalence found in this study in the captive population (62.5%) is most likely indicative of their captive environment. Animals are housed over long periods in the same area, allowing environmental build up and continued cycling of this protozoan within the population. This effect is reduced in wild populations as they are extremely nomadic.

Unfortunately, this study was not successful in amplifying all isolates at all loci. Previous studies have also reported lower than expected amplification of *Giardia* DNA in dogs (Caccio and Ryan, 2008; Leonhard et al., 2007; Palmer et al., 2008; Read et al., 2004; Robertson et al., 2006; Traub et al., 2004). The inability to amplify at all loci may be attributed to several factors. First, β-giardin and *gdh* loci require high cyst numbers for amplification success (Castro-Hermida et al., 2007). In addition, faecal samples from carnivores in general have low PCR success, due to the presence of inhibitors (Kohn and Wayne, 1997; Piggott and Taylor, 2003). Finally the majority of samples in this study were not fresh but placed in a fixative; this was necessary to preserve DNA and to comply with quarantine restrictions for sample importation, but probably reduced the success of DNA amplification due to degradation. Even when amplification was successful, some isolates showed differences in assemblage and sub-assemblage typing depending on the locus used. This has also been found in previous studies and may be due to meiotic recombination or preferential amplification of one assemblage over another in mixed infections (Caccio and Ryan, 2008; Caccio et al., 2005; Cooper et al., 2007; Teodorovic et al., 2007).

Despite the difficulties mentioned above, we have been able to obtain some assemblage and sub-assemblage information for both wild and captive populations of the African painted dog. Genotypic information did not reveal any novel genotypes, but the zoonotic assemblages B and to a lesser degree A were prevalent within both populations. The dominance of these zoonotic assemblages was surprising especially as the dog assemblages C and D were rarely seen. Assemblages A and B have been reported in studies on domestic dogs, coyotes and wolves but the dog assemblages have also been reported at similar prevalences (Table 4). The lack of assemblages C and D seen in both wild and captive populations of this study perhaps indicate that the African painted dog is not as susceptible for this genotype as members of the genus *Canis*. Interestingly assemblage A has been the dominant zoonotic genotype

Table 2
Genotypic characterisation of *Giardia duodenalis* isolates from individual African painted dogs at the18SrRNA, β-giardin and *gdh* loci.

Captive population ($n=16$)						Wild population ($n=14$)					
ID No.	Sex	Age	18S	β-g	*gdh*	ID No.	Sex	Age[a]	18S	β-g	*gdh*
C1	M	1	A/B	–	–	W1	F	A	A	–	–
C2	M	1	A/B	–	–	W2	M	1	B	A	
C3	M	3	B	–	A2	W3	?	?	B	BIII	–
C4	M	1	B	BIII/BIV	–	W4	F	A	B	–	–
C5	M	2	B	–	BIV	W5	M	A	A/B	A2	–
C6	M	2	B	–	–	W6	F	A	A/D	A2	–
C7	M	2	A/B	–	–	W7	M	A	B	A2	–
C8	M	3	B/C	–	–	W8	M	A	A/B/C	–	–
C9	M	7	B	–	–	W9	?	A	B	–	–
C10	M	9	B	–	–	W10	?	?	A/B	–	–
C11	M	3	–	–	BIV	W11	?	?	A/B	–	–
C12	F	2	–	BIII/BIV	BIV	W12	?	A	–	A2	–
C13	F	1	B	–	–	W13	M	A	C/D	–	–
C14	F	4	B	BIII/BIV	A2	W14	F	A	A/B/C	BIII	BIV
C15	F	4	B	–	D						
C16	F	8	B	–	–						

[a] Wild animals over 1 year of age were allocated as adult (A).

Table 3
Prevalence (with 95% confidence intervals in parentheses) and genotypic characterisation of *Giardia* isolates obtained in a captive population of African painted dogs over a 3 year period.

Sample year	Prevalence	18S rRNA	β-Giardin	*gdh*
2007 $n=16$	62%(37–82%)	B = 5 A/B = 3	BIII/BIV = 1	BIV = 1
2008 $n=14$	50%(24–76%)	B = 5B/C/D = 1	BIII = 1BIII/BIV = 1	
2009 $n=13$	69%(41–88%)	B = 6	BIV = 1	AII = 2BIII = 1 BIV = 1A/B/D = 1

reported in the literature in studies of both wild canids and domestic dogs, as opposed to this study, where assemblage B was the dominant genotype (Table 4).

As noted in other wildlife studies, the presence of the zoonotic assemblages found in the African painted dog raises the question of their potential to act as reservoirs for human infection (Graczyk et al., 2002; Thompson et al., 2009; Trout et al., 2006). The answer can perhaps be explained by their ecology and environment. Close interaction between dogs and humans living in rural communities in India has shown evidence of zoonotic transmission of *Giardia* (Traub et al., 2004) and indeed this kind of transmission could well be possible within captive populations of the African painted dogs. The zoo keepers in our study had direct contact with the African painted dog's confined environment and were at risk of exposure through regular cleaning of water features and removing excrement from the enclosure. Isolates from the two keepers typed to the same sub-assemblage as one of the captive animals in their care, suggesting that zoonotic transmission may have occurred.

In the wild however, human contact with the African painted dog would be minimal. They are not companion animals and have never been domesticated. Due to their nomadic nature they will roam outside national parks and move through human settlements, although they actively avoid human contact, making zoonotic transfer unlikely (Creel and Creel, 2002). However, there is a constant stream of tourists and locals into the national parks and as suggested by Graczyk et al. (2002) studying gorillas in Uganda, indiscriminate defecation by humans could be a source of *Giardia* infection. Other evidence for anthropozoonotic transmission has been seen between humans and beavers, where humans have been implicated in introducing *Giar-*

Table 4
Assemblages of *Giardia duodenalis* observed in both wild canids and domestic dogs noted in the literature and this report. Number of times reported in literature is presented as (*r*=).

Host	Assemblage	Reference
Canis familiaris	A ($r=11$)	Berrilli et al. (2004), Ipankaew et al. (2007), Itagaki et al. (2005), Lalle et al. (2005),
	B (r = 3)	Leonhard et al. (2007), Marangi et al. (in press), Palmer et al. (2008), Read et al. (2004),
	C/D ($r=11$)	Traub et al. (2004), van Keulen et al. (2002)
		Berrilli et al. (2004), Ipankaew et al. (2007), Itagaki et al. (2005), Lalle et al. (2005),
		Leonhard et al. (2007), Monis et al. (1998), Palmer et al. (2008), Souza et al. (2007)
Canis latrans and *Canis lupus*	A ($r=3$)	Gompper et al. (2003), Klock et al. (2005), Thompson et al. (2009), Trout et al. (2006)
	B ($r=1$)	
	C/D ($r=3$)	
Lycaon pictus	A	This publication
	B	

dia to beavers through sewage runoff upstream from the beaver colony (Bemrick and Erlandsen, 1988; Fayer et al., 2006).

While the transmission of *G. duodenalis* from humans to African painted dogs remains speculative, it appears that once established within these packs infection is maintained by dog to dog transmission. They are very social animals and physical contact is common in all aspects of pack life, providing ample opportunity for transmission to occur (Creel and Creel, 2002; Woodroffe et al., 1997). This was evident in the captive population as each individual animal displayed an infection with *G. duodenalis* at some point over the 3-year study period. As previously mentioned, prevalence was far lower in the wild populations and distribution among packs was not even. *G. duodenalis* was observed in several individuals in the same pack and in none in other packs, again suggesting that once the parasite is introduced into a pack, animal to animal transmission occurs.

The pathogenesis of *Giardia* spp. to the host is complicated as many infected hosts can be asymptomatic while others display severe diarrhoea (Eckmann, 2003). In this study, only two of the 85 faecal samples positive for *G. duodenalis* showed signs of diarrhoea, suggesting that parasitic infection in these animals was generally asymptomatic. Perhaps of more importance than clinical symptoms are the potential subclinical effects. Infections with *Giardia* spp. have been related to growth retardation and malabsorption in children, particularly those with poor nutrition (Astiazaran-Garcia et al., 2000; Berkman et al., 2002). With respect to African painted dogs, which often have concurrent enteric infections (this study, data not shown), subclinical impacts of *G. duodenalis* could be of great importance, particularly as pack sizes decrease and hunting efficacy declines (Courchamp et al., 2002; Creel and Creel, 1995).

Ultimately, the transmission dynamics of *G. duodenalis* within the African painted dog remains unclear. While this species could act as a reservoir for zoonotic infections, the lack of direct human contact in the wild currently makes this unlikely. However, increased human activity into their natural environment clearly has the potential to increase the prevalence of *G. duodenalis* infections in the African painted dog. With other pressures and stressors, this could potentially be an emerging disease, which will require monitoring in the future. This dynamic however, is altered within captive populations. Due to the necessary interaction between humans and their captive charges in this environment, the risk of zoonotic transfer is high and should be a consideration in future management protocols.

In summary *G. duodenalis* is a parasite of the African painted dog that may impact on both wild and captive populations. This highlights the need for continued research to be conducted on wildlife and their surrounding influences to more precisely ascertain the nature of any threats to the charismatic but endangered African painted dog.

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