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Frequently observed parasites in pet reptiles' feces in Tehran

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Abstract:

BACKGROUND: Many wild-caught reptiles harbor some kind of parasite. Captivity with the negative effect of poor sanitary and husbandry management may lead to clinical disease. With the increasing trend in keeping non-native reptile species in the last decade, a need for the specification of reptile parasites and their hosts has emerged. **OBJECTIVES:** The study aims to gain data on intestinal parasites of reptiles kept as pets or in small private collections in close contact with people. **METHODS:** A combination of native and iodine stained direct smears along with flotation concentration were used to investigate parasites in pet reptiles' feces. All samples were investigated macroscopically and a smear was prepared and stained by modified Ziehl Neelsen for detection of Cryptosporidium. **RESULTS:** Stool samples from 100 pet or small zoological reptile collections (Lacertilia=36, Serpentes=20, Chelonii=11, Corocodilia=1) were collected. The total occurrence of parasite was 52%. 64.8% of the examined Lacertilia, 35.3% of Serpentes, 45.5% of Chelonii were infected. *Eimeria, Isospora, Cryptosporidium, Trichomonas, Balantidium*, Strongylid and Oxyurid eggs and amoeba were identified. *Cryptosporidium* was detected in Lacertilita, Serpentes and Chelonii. In the only sample from a Nile crocodile no parasites were detected. Eimeria was detected in Bearded dragon, Indian python, Albino python and king cobra and *Isospora* was identified in Bearded dragon and the alien Cheloniid species Red-eared slider. Amoeba was identified in *Iguana iguana* and Horsfield tortoise. **CONCLUSIONS:** Trichomonads, *Balantidium, Cryptosporidium, Isospora, Eimeria*, amoebae and nematode eggs were identified in the investigated samples. *Cryptosporidium* were detected by specific stains in 14 samples. Sauria was the most infected suborder (64.8%) while 32.4% of snakes and 45.5% of chelonians were infected. Parasites are common in pet reptiles but the parasite species, the degree of infestation and hygienic management will determine the ultimate clinical outcome of the existing parasite infections. Hence, examination for endoparasites should be recommended for checking the health status of all captive or newly entering reptiles.

Introduction

Reptilia with more than 6000 species, which are increasingly being kept as pets in recent years, are host to a diverse range of parasites (Rataj et al. 2011). Many wild-caught reptiles harbor some kind of parasites. Although harboring parasites does not always result in clinical disease, in captivity with the negative effect of poor sanitary and husbandry management the concentration of parasites may increase. Some of the parasites are harmless but others can be dangerous if left untreated (Pappini et al. 2011). Depending on the parasite species and the degree of infestation endoparasites may cause different clinical symptoms (Pasmans et al. 2008). Some of these parasites may not affect the animal but can cause health problems in people (Pasmans et al. 2008).

Although there are many case reports, zoo reptile parasitic surveys or comprehensive parasitological description of a single parasite species recovered from reptiles (Fernando et al. 2009; Abdel-Baki et al. 2013; Díaz et al. 2013), there are very few surveys on the prevalence of fecal parasites in pet reptiles worldwide (Pasmans et al. 2008; Pappini et al. 2011; Rataj et al. 2011). Considering the lack of knowledge about the normal microbiota of most reptilian taxa (Pasmans et al. 2008), as well as the increasing trend in keeping non-native reptile species in the last decade, there is evidently a need for the specification of parasites and their hosts. These data will improve the understanding of the ecology of both the parasites and their hosts (Marschang 2015), besides helping veterinarians to perform routine screening and prescribe preventive/therapeutic measures (Pasmans et al. 2008). The present study was thus undertaken to gain data on intestinal parasites of reptiles kept as pets or in small private collections in close contact with people.

Materials and Methods

Between April 2013 and September 2014, fresh fecal samples from 100 captive reptiles not showing any clinical signs, representing 28 species were collected. These included 11 Saurian species (n=54), 12 Ophidia (n=34), four Chelonian species (n=11) and a single sample from crocodilian suborder (Table 1). The samples were investigated immediately after arriving in the laboratory or preserved in SAF and refrigerated for subsequent examination. A combination of native and iodine stained direct smears together with flotation saturated salt solution (CNF) was performed on each sample (Pasmans et al. 2008; Wolf et al. 2014). Nematode infestations according to egg morphology were characterized as strongylid, strongyloides and oxyurid (Fig. 1). Besides all samples were investigated macroscopically and by modified Ziehl-Neelsen (MZN) staining for detection of gross parasites and *Cryptosporidium*, respectively. Detected oocysts were sporulated in 2.5% potassium dichromate for genus identification.

Results

Overall, 52 fecal samples were infested with protozoas and nematodes. Single protozoan and helminthic infestations were detected in 10 and 23 of the samples, respectively. Dual/multiple protozoan infection and concurrent infection of protozoans and helminths were observed in six and 13 samples, respectively. Trichomonads,

Table 1. Reptile species examined for fecal parasites.

	Scientific name	Common name	Number examined	Number infested
Sauria	*Iguana iguana*	Iguana	33	25
	Eublepharis macularius	Leopard gecko	8	4
	Trapelus agilis	Steppe agama	1	1
	Varanus griseus caspius	Trans Caspian desert monitor	2	0
	Agamura persica	Blunt tailed spider gecko	1	0
	Lacerta media	Three-lined Lizard	1	1
	Pogona vitticeps	Bearded dragon	3	3
	Tiliqua scincoides	Blue tongue skink	1	1
	Sceloporus malachiticus	Green spiny lizard	1	0
	Cryptopodion scabrum	Rough-tailed Gecko	1	0
	Pseudopus apodus	European legless lizard	2	0
Ophidia	*Python molurus*	Indian Python	6	2
	Macrovipera lebetina obtusa	*Levantine viper*	8	2
	Eryx jaculus	Common sand boa	2	1
	Echis carinatus sochureki	Eastern saw-scaled viper	2	1
	Agkistrodon intermedius caucasicus			
Caucasian pit viper	*1*	0		
	Eryx johnii persicus	Persische sand boa	2	0
	Spalerosophis diadema cliffordi	Clifford's snake/Diadem Snake	2	0
	Rhynchocalamus melanocephalus	Black-headed Snake	2	0
	Ophiophagus hannah	king cobra	4	3
	Malpolon monspessulanus	Montpellier snake	1	0
	Coluber nummifer	Coin Snake	3	2
	Micrurus fulvius	Coral snake	1	0
Chelonian	*Geochelone elegans*	Indian star	1	0
	Testudo graeca	Thigh tortoise	2	0
	Trachemys scripta elegans	Red-eared slider	3	1
	Testudo horsfieldii	Russian tortoise	5	4
	Crocodylus niluticus	Nile crocodile	1	0
Total			100	52

Balantidium, *Cryptosporidium, Isospora, Eimeria* and amoebae were the identified protozoas (Fig. 2) (Table 2). All three types of nematode eggs were identified in the infested samples. Fecal wet smear revealed 34 animals harboring parasites while floatation augmented it to 36. Amoeba and *Cryptosporidium* were detected by specific stains in seven and 14 samples, respectively.

Sauria was the most infected suborder (64.8%) and oxyurid eggs were the most frequently identified parasites (37%) herein. Wet smear revealed 40.7% (22/54) parasitic infections while the use of floatation increased the infection frequency to 50% (27/54) ($p=0.01$, $\chi2= 11.0$). Seven of the 11 inspected saurian species, were infested. *Cryptosporidium* and amoebae were identified in 16.7% and 11.1% of these samples. In a blue tongue skink MZN staining revealed acid-fast organisms larger than *Cryptosporidium* oocysts (13-17×10-13 µm) resembling *Cyclospora* sp (Fig 3).

32.4% of snakes were infested and use of floatation method did not significantly increase the detection rate ($p=0.09$, $\chi2= 2.8$).

Table 2. Frequency of parasites detected in fecal samples.

Parasite genus	*Host species (Number inspected)*	Number of Positive samples
Balantidium	*Iguana iguana (33)*	1
	Testudo horsfieldii (5)	1
Trichomonads	*Iguana iguana (33)*	2
	Eryx jaculus (2)	1
Amoebae	*Iguana iguana (33)*	4
	Eublepharis macularius (8)	2
	Testudo horsfieldii (5)	1
Isospora	*Pogona vitticeps (3)*	1
	Trachemys scripta elegans (3)	1
Cryptosporidium sp.	*Iguana iguana (33)*	7
	Pogona vitticeps (3)	1
	Tiliqua scincoides (1)	1
	Macrovipera lebetina obtusa (8)	1
	Echis carinatus sochureki (2)	1
	Ophiophagus hannah (4)	1
	Testudo horsfieldii (5)	2
Cyclospora sp.	*Tiliqua scincoides (1)*	1
Eimeria sp.	*Pogona vitticeps (3)*	1
	Python molurus (6)	1
Strongylid egg	*Iguana iguana (33)*	11
	Trapelus agilis (1)	1
	Pogona vitticeps (3)	1
	Coluber nummifer (3)	1
	Testudo horsfieldii (5)	1
Oxyurid egg	*Iguana iguana (33)*	6
	Eublepharis macularius (8)	4
	Lacerta media (1)	1
	Pogona vitticeps (3)	1
	Macrovipera lebetina obtusa (8)	1
	Testudo horsfieldii (5)	1
Strongyloides egg	*Tiliqua scincoides (1)*	1
	Macrovipera lebetina obtusa (8)	1
	Ophiophagus hannah (4)	1

Three of those samples were infected with *Cryptosporidium* sp. oocysts. No amoeba was identified in Ophidia. King cobra was the most infested species. In a sample from Coluber nummifer mite eggs originating from a prey were detected.

45.5% of chelonians were infected, among which *Testudo horsfieldii* had the most diverse range of fecal parasites. *Cryptosporidium* sp. and amoeba were identified in *Testudo horsfieldii* (Table 2). *Blantidium*, Strongylid and oxyurid eggs were the identified parasites in investigated chelonians.

Discussion

In the present study, 54 Saurians, 34 Ophidias, 11 Chelonians and a crocodile were investigated coprologically for fecal parasites. The most frequent detected fecal

Figure 1. An oxyurid egg in floated fecal wet smear from Lacerta media (40x).

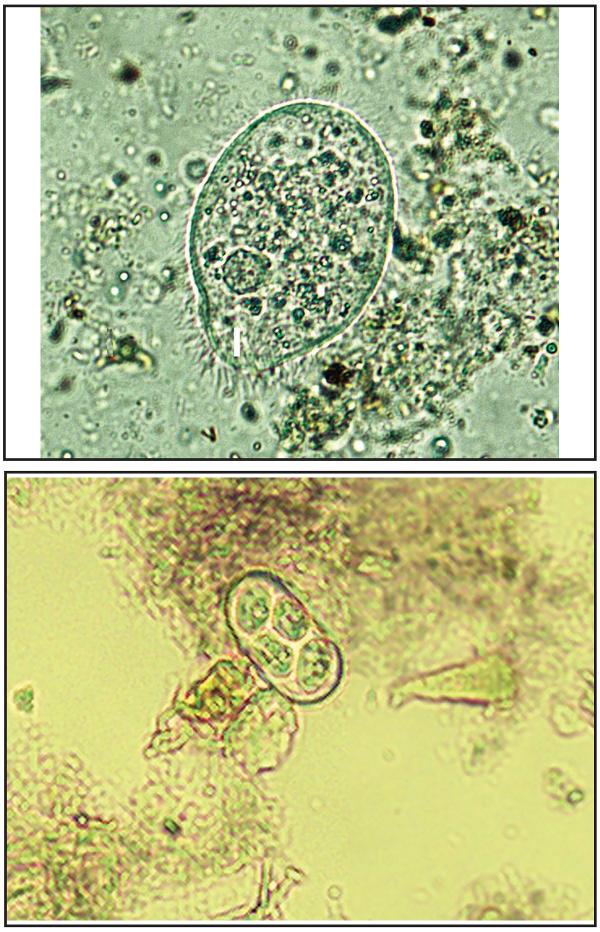

Figure 2. a: Balantidium in floated fecal wet smear from a Green iguana, 20x; b: Eimerian oocyst from a Bearded dragon, 20x.

parasite was oxyurid egg (12%). In lizards pinworm eggs were the most frequent parasite showing two morphologically distinct eggs as reported by Rataj et al (2011). One morphotype with dark pitted egg wall identified as *Pharyngodon* sp. was detected in leopard gecko, *Lacerta media* and bearded dragon while the other unidentified species of pinworm egg was more elongate and translucent, mostly found in Iguanas (Fig. 4a, b). Oxyurid eggs are rarely detected in snakes (Okulewicz et al. 2014). Rataj et al. (2011) reported oxurid eggs from a Platyceps karelini. In this study pinworm eggs were detected in a *Levantine viper* (Fig. 4c). Although most pseudoparasitic pinworms from snakes are *Syphacia* (Souza et al. 2014), the eggs detected in the present study morphologically resembled lizard's pinworms (Pharyngodonidae) (Wright 2009). The actual identification of these eggs requires isolation of the adult worm or further molecular investigations. Reptiles with oxyurid infections are generally asymptomatic. Pathologic changes are rare, but heavy infections might be one of the causes of anorexia in tortoises coming out of hibernation (Mitchell 2007) and a positive correlation has been reported between oxyurid and salmonella infection in Cheloniids (Dipineto et al., 2012). Strongylid eggs as the second most prevalent detected parasite were mostly seen in lizards while others reported it as the most prevalent parasite in Ophidia (Pasmans et al. 2008; Rataj et al. 2011).

Trichomonads were identified in iguanas and a snake (3%) and *Balantidium* was recovered from a Russian tortoise and an iguana (2%). Our efforts in culturing the trichomonads in diamond's medium at 37 ◦C and at room temperature were unsuccessful. Ciliates and flagellates are commonly found in herbivorous lizards and also in turtles and snakes (Papini et al. 2011; Rataj et al. 2011). Endoparasites are an important cause of disease in captive reptiles. Some of the

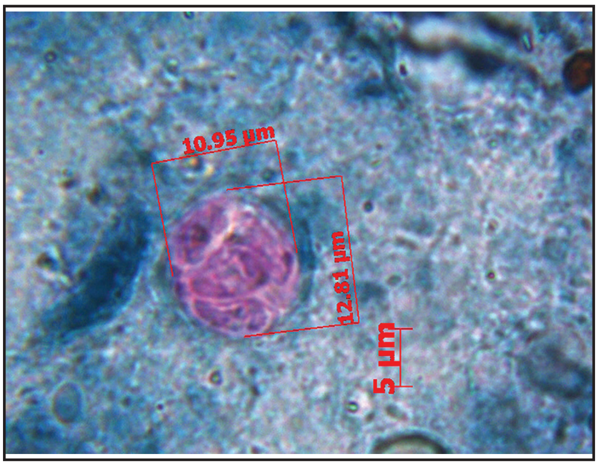

Figure 3. Acid-fast organism larger than *Cryptosporidium* oocysts from a blue tongue skink (MZN staining, 100x).

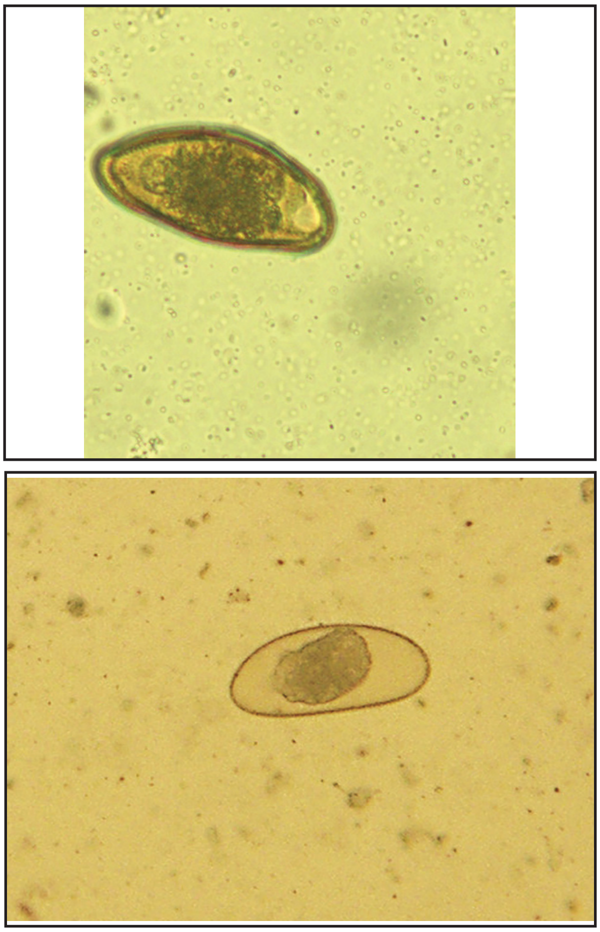
Figure 4. Two types of pinworm eggs detected in lizard's feces. a: Pinworm egg with dark pitted wall identified as *Pharyngodon* sp. from *Eublepharis maculariu* (10x); b: Pinworm egg being more elongate and more translucent found in the feces of most inspected Green iguanas (10x).

intestinal parasites are considered normal residents of the gut flora but with predisposing factors they may lead to gastrointestinal diseases. The parasite species, the degree of infestation and conditions in captivity such as overcrowding or hygienic management will determine the ultimate clinical outcome of the existing parasite infections. Consequently, examination for endoparasites has been recommended for checking the health status of all captive reptiles (Pasmans et al. 2008). Furthermore, precise morphologic and taxonomic description of reptile parasite species regarding reptile species, the lifecycle and their health impact are not widely identified or described. Performing more detailed research on these aspects will certainly improve the understanding of the ecology of both the parasites and their hosts and may contribute to improving the safety and welfare of these animal species.

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بررسی آلودگی های انگلی مدفوعی در خزندگان در اسارت شهر تهران

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چکیده

زمینه مطالعه: خزندگان به ویژه انواعی که از حیات وحش صید می‌شوند ممکن است آلوده به انواع انگل‌ها باشند. در شرایط اسارت به دلیل وجود استرس‌هایی مانند تراکم بالا و عدم تغذیه صحیح، حیوان ضعیف شده و علایم بالینی در حیوان ظاهر می‌گردد. **هدف:** با توجه به افزایش تمایل به نگهداری خزندگان به عنوان حیوان خانگی و با توجه به معدودبودن مطالعات در زمینه انگل‌های موجود در خزندگان در ایران و دنیا و با نظر به این‌که اطلاعات در این زمینه در کشور غالباً محدود به گزارشات موردی می‌باشند، در این طرح تلاش شد تا شیوع انگل‌های قابل ردیابی در مدفوع انواع خزندگان که به نوعی در تماس نزدیک با جوامع انسانی هستند، مانند انواع گونه‌های موجود در مجموعه‌های خصوصی، مجموعه‌های زیستی عمومی و خزندگان خانگی منفرد استان تهران مورد برررسی و شناسایی قرارگیرند. **روش کار:** تعداد ۱۰۰ نمونه مدفوع از رده خزندگان، شامل مارها، مارمولک‌ها، تمساح‌ها و لاک‌پشت‌ها از نظر آلودگی به انواع انگل‌های گوارشی تک‌یاخته‌ای و پریاخته‌ای به روش گسترش مرطوب، شناورسازی، رنگ‌آمیزی زیل نیلسن و گسترش با لوگول مورد بررسی قرار گرفتند. **نتایج:** در این مطالعه نمونه مدفوع ۵۴ مارمولک، ۳۴ مار، ۱۱ لاک‌پشت و یک نمونه مدفوع از کروکودیل مشـتمل بر ۲۸ گونه خزنده، مورد بررسـی انگل‌شناسی قرار گرفتند. ۵۲٪ نمونه‌ها آلودگی انگلی داشتند. انواع تک‌یاخته‌های آپی‌کمپلکسا شامل کریپتوسپوریدیوم، ایزوسپورا و ایمریا، همچنین تک‌یاخته تاژک‌دار تریکومونادی، تک‌یاخته مژه‌دار بالانتیدیوم و نیز آمیب و انواع تخم‌های نماتود جنین‌دار، اُکسیورید و لارودار از انگل‌های شناسایی در نمونه‌های مورد بررسی بودند. **نتیجه‌گیری نهایی:** آلودگی انگلی در خزندگان در اسارت شیوع بالایی داشته و در صورت بروز علایم بالینی باید با مداخلات درمانی همراه گردند. به علاوه با توجه به غیربومی بودن برخی از گونه‌های بررسی شده در این مطالعه، یکی از نکاتی که در مورد خزندگان به عنوان حیوان خانگی باید مدنظر دامپزشکان باشد آگاه نمودن افراد در مورد خطرات احتمالی ناشی از رهاسازی عمدی یا فرار گونه‌های غیربومی در محیط زیست می‌باشد. در این راستا سازمان محیط زیست و سازمان دامپزشکی نیز دارای نقش غیرقابل انکاری بوده و نیاز است با سنجش مداوم بازار حیوانات خانگی و ارزیابی خطرات زیست محیطی آنها، نسبت به وضع قوانین مناسب و اطلاع‌رسانی‌های مناسب اقدام نمایند.

واژه های کلیدی: مدفوع، مارمولک، انگل گوارشی، خزندگان، مار

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