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Gastrointestinal Parasites in Reptiles from a Portuguese Zoo

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Abstract: The growing popularity of reptiles has contributed to their reproduction in captivity. When subjected to stressful environments, such as the presence of a higher number of humans and animals, reptiles may become more susceptible to parasites. Endoparasites in captive animals may cause several clinical signs ranging from mild to severe: lethargy, anorexia, diarrhea, cloacal/penile prolapse, infertility, intestinal malabsorption syndrome, and weight loss, among others. This study aimed to assess the presence of gastrointestinal parasites in fecal samples of reptiles from a Portuguese zoo through two techniques: a fecal flotation test (using a saturated sodium chloride solution) and Mini-FLOTAC. Ninety-nine samples belonging to 22 different animal species were collected and analyzed. Parasites were identified in 53.5% of the samples. Chelonians had a higher frequency (100%), followed by lizards (56.8%) and snakes (47.4%). The eggs/oocysts found were oxyurids (36.4%), strongylids/*Kalicephalus* sp. (8.1%), *Eimeria* sp. (5.1%), *Hymenolepis* spp. (5.1%), ascarids (4.0%), and *Isospora* sp. (2.0%). Both techniques presented the same results for each sample. The high prevalence of oxyurids, as well as of other parasites, can be explained by possible environmental contamination as these reptiles are kept in captivity. This study indicated the importance of assessing parasitic infections in reptiles in zoos, where routine coprological examinations should always be considered, as well as adequate prophylaxis.

Keywords: endoparasites; *Hymenolepis*; *Lampropeltis getula californiae*; oxyurid; One Health; *Pogona vitticeps*; strongylid

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1. Introduction

The growing popularity of reptiles has contributed to their captive breeding. However, there are few studies regarding the prevalence of diseases, particularly parasitic ones, which affect these animals in zoos. Reptiles, when subjected to stressful environments (such as those with a high concentration of animals and humans in restricted habitats), can become more immunologically susceptible to parasites that are often pathogenic to them, including possibly to other animals, such as humans [1,2]. Therefore, this topic has to be investigated using a One Health perspective. Reptiles kept in captivity have shown a higher prevalence of parasites and more efficient transmission of various monoxenous parasites compared to wild reptiles [2,3]. Additionally, in captive animals that were captured from their natural habitats, the absence of intermediate hosts, dietary changes, and other factors have been demonstrated to promote the adaptation of parasites to new conditions, potentially threatening other zoo animals, since there are different susceptibilities among different reptiles [1].

Endoparasites can cause different clinical outcomes depending on the species and their life cycle, habitat conditions, infection severity, and the host (age, sex, and health) [3]. Although mild helminth infections are generally well tolerated by reptiles, severe infections can result in clinical pathology, especially in young or immunocompromised animals [4]. An oxyurid infection may lead to lethargy, anorexia, diarrhea, cloacal/penile prolapse, growth disturbances, infertility, and, in high burdens, it can cause intestinal malabsorption syndrome, which can result in severe clinical conditions [5,6]. *Kalicephalus* sp. is the most common nematode in snakes and may cause weight loss, enteritis, lethargy, and anorexia [7]. Like helminths, reptiles also host a variety of intestinal unicellular pathogens, such as *Blastocystis* spp., *Cryptosporidium* spp., *Eimeria* spp., *Entamoeba* spp., *Giardia* spp., and *Isospora* spp. [8]. An infection by *Entamoeba invadens* can cause anorexia, dehydration, bloody diarrhea, hepatitis, gastritis, and colitis. Conversely, an infection by *Eimeria* spp. can result in weight loss and enteritis [4,9]. In Table 1, some epidemiological studies from European countries (with special emphasis on Portugal), with the prevalence of different helminths and protozoa identified in fecal samples from reptiles, are presented.

Table 1. Prevalence (%) of helminths and protozoa identified in fecal samples from reptiles.

Countries	Reptiles	Protozoa	%	Cestodes	%	Nematodes	%	Reference
Several countries	Snakes	*Cryptosporidium* sp.	1.9	Unidentified	-	Strongylida	20.4	[2]
						Ascarididae	7.4	
		Cyclospora sp.	1.9			*Strongyloides* sp.	5.6	
		Nyctotherus sp.	1.9			*Capillaria* sp.	3.7	
		Tetratrichomonas sp.	1.8			Oxyuridae	1.9	
	Lizards	*Nyctotherus* sp.	10.0	*Oochoristica* sp.	3.0	Oxyuridae	57.1	
		Balantidium sp.	2.4			Strongylida	11.8	
		Cryptosporidium sp.	0.9			Ascarididae	6.9	
		Isospora sp.	0.9			*Physaloptera* sp.	6.3	
		Eimeria sp.	0.6			Filarioidea	5.4	
						Capillaria sp.	0.3	
		Tetratrichomonas sp.	0.6			*Strongyluris* sp.	0.3	
						Strongyloides sp.	0.3	
	Chelonians	*Balantidium* sp.	26.2	Unidentified genus	0.3	*Tachygonetria* sp.	81.8	
						Strongylida	43.7	
		Nyctotherus sp.	1.6			*Angusticaecum* sp.	20.3	
						Strongyloides sp.	3.7	
Italy	Snakes	Eimeriidae	27	*Hymenolepis nana*	2	*Strongyloides* spp.	2	[6]
						Capillaria spp.	2	
	Lizards	Eimeriidae	36	Unidentified	-	Oxyuridae	48	
Italy	Chelonians	*Nyctotherus* sp.	18.6	Unidentified genus	0.5	Oxyuridae	74.4	[10]
		Cryptosporidium spp.	12.6			Ascarididae	2.8	
		Balantidium sp.	0.9					
		Coccidia	0.9			Strongylida	0.9	
		Giardia spp.	0.5					
Germany	Chelonians	*Hexamita* sp.	0.007	Unidentified	-	Oxyuridae	43.2	[5]
		Balantidium spp.	0.007			*Angusticaecum* spp.	0.01	
		Entamoeba spp.	0.005			*Strongyloides* spp.	0.003	
		Trichomonas spp.	0.004					
		Blastocystis spp.	0.002			*Heterakis* spp.	0.001	
		Hartmanella spp.	0.001					
		Trimitus spp.	0.001					

Table 1. *Cont.*

Countries	Reptiles	Protozoa	%	Cestodes	%	Nematodes	%	Reference
Germany	Lizards	*Isospora amphiboluri* *Entamoeba* spp. *Choleoeimeria* spp. *Trichomonas* spp. *Cryptosporidium* spp.	17 0.8 0.5 0.3 0.3	Unidentified	-	Oxyuridae *Strongyloides* spp.	41.2 0.1	[11]
Germany	All	Coccidia	4	Unidentified genus	1.6	Oxyuridae	18.7	[12]
		Cryptosporidium spp.	0.1			Ascarididae	5.2	
		Balantidium sp.	0.1			Strongylida	4.0	
						Hookworms	0.6	
						Strongyloides spp.	0.6	
						Capillaria sp	0.4	
						Lungworms	0.1	
Spain	Lizards	Coccidia	33	Unidentified	-	Oxyuridae	56	[13]
	Chelonians	*Nyctotherus* sp. *Balantidium* sp.	22 11	Unidentified	-	Oxyuridae	28	
Portugal	All	Coccidia *Isospora* spp. *Nyctotherus* spp. *Eimeria* spp.	23.0 10.8 5.0 2.9	Unidentified genus	0.7	Oxyuridae Strongylida Ascarididae *Strongyloides* spp. *Rhabdias* spp.	41.0 10.8 1.4 0.7	[14]
Portugal	Chelonians	*Nyctotherus* sp. *Amoeba* spp.	31.3 25.0	Unidentified	-	Oxyuridae	37.5	[15]
	Lizards	*Nyctotherus* sp.	25.0	Unidentified	-	Oxyuridae	75.0	
Portugal	Snakes	Eimeriidae	6.2	Unidentified	-	Nematoda	6.2	[16]
	Lizards	*Entamoeba* sp. *Balantidium* sp. Coccidia *Nyctotherus* sp. *Isospora amphiboluri* *Choleoeimeria* sp.	34.2 18.2 12.1 9.0 9.0 6.0	Unidentified	-	Nematoda	51.3	
	Chelonians	*Entamoeba* sp. *Balantidium* spp.	33.3 66.6	Unidentified	-	Pharyngodonidae	100	

There are several methods for quantitative parasitological analysis, such as McMaster, FLOTAC, and Mini-FLOTAC. The last method provides faster results with higher repeatability and sensitivity while requiring less labor work and no centrifugation [17,18]. Although techniques like Mini-FLOTAC can be applied to samples from any host, their use in reptiles is uncommon [3]. This study aims to assess the presence of gastrointestinal parasites in reptiles kept in a Portuguese zoo using two techniques: a flotation test (using a saturated sodium chloride—NaCl—solution) and Mini-FLOTAC.

2. Materials and Methods

Between February 2021 and February 2022, fecal samples were collected from reptiles in a Portuguese zoo. In zoos, the aim is to carry out minimal animal manipulation. To achieve this, most animals have never been dewormed or previously dewormed animals are not dewormed for at least a year. All the samples collected were from animals placed in individual enclosures. For this study, 99 reptile samples were collected from 22 species of reptiles kept at Zoo da Maia (Portugal) and analyzed for the presence of gastrointestinal parasites (Table 2).

Table 2. Species (scientific and common name) and number of reptiles examined.

Reptiles	Common Name	Number (N = 99)
	Chelonians/Order Testudines	
Testudo graeca	Greek tortoise	5
	Lizards/Suborder Lacertilia	
Pogona vitticeps	Bearded dragon	12
Phelsuma madagascariensis	Madagascar gecko	4
Uromastix geyri	Geyr's dabb lizard	4
Salvator merianae	Southern black-and-white teju	4
Ophisaurus apodus	Sheltopusik	4
Hemitheconyx caudicinctus	African gecko	2
Iguana iguana	Iguana	3
Zonosaurus maximus	Southeastern girdled lizard	3
Oplurus cuvieri	Collared iguana	1
	Snakes/Suborder Serpentes	
Lampropeltis getula californiae	California kingsnake	12
Python reticulatus	Reticulated python	7
Python bivittatus	Burmese python	6
Python regius	Ball python	6
Pantherophis obsoletus	Western rat snake	5
Epicrates angulifer	Cuban boa	5
Boa constrictor	Boa constrictor	4
Pantherophis guttatus	Corn snake	4
Lampropeltis triangulum hondurensis	Honduran milk snake	3
Lampropeltis getula splendida	Desert kingsnake	2
Morelia spilota	Carpet python	2
Epicrates cenchria maurus	Brown rainbow boa	1

The samples were collected from the environment immediately after defecation, during the normal daily activities of animal management and facility hygiene. After collection, the samples were stored in identified plastic bags at 4 °C and processed within 48 h through coprological methods in the laboratory of the Escola Superior Agrária of the Instituto Politécnico de Viana do Castelo (ESA—IPVC), Portugal. Each fecal sample was macroscopically checked for tapeworm proglottids and adult roundworms and then analyzed through a flotation test (a saturated sodium chloride solution, specific gravity of 1.2) [4] and Mini-FLOTAC techniques. Concerning Mini-FLOTAC, two flotation solutions were used—NaCl (specific gravity of 1.2) and zinc sulphate (ZnSO4) (specific gravity of 1.35)—following the instructions reported in the original description by Cringoli et al. [19]. A dilution factor of 1:20 was used in Mini-FLOTAC (2 g of sample was added to 38 mL of solution). To the best of our knowledge, a Mini-FLOTAC protocol for reptiles has not yet been defined; therefore, only qualitative results were considered for both techniques: Mini-FLOTAC and the traditional flotation test. To detect the presence of eggs/oocysts, a conventional optical microscope was used with total magnifications of 100× and 400×. Eggs and oocysts were identified according to the descriptions of Zajac et al. [4].

3. Results

Of the 99 samples examined, we detected the presence of parasites (eggs/oocysts) in 53 (53.5%). All of the chelonians investigated presented parasites (100%, $n = 5/5$), while the prevalence of parasites decreased in lizards (56.8%, $n = 21/37$), followed by snakes (47.4%, $n = 27/57$) (Tables 3 and 4). Both techniques showed the same parasites for each sample.

Table 3. Species and number of lizards examined and frequency of parasites.

Species	*n* (N = 37)	Frequency of Parasites	
		Number of Samples	%
Pogona vitticeps	12	11	91.7
Phelsuma madagascariensis	4	0	0.0
Hemitheconyx caudicinctus	2	2	100
Iguana iguana	3	2	66.7
Uromastix geyri	4	3	75.0
Salvator merianae	4	1	25.0
Zonosaurus maximus	3	0	0.0
Ophisaurus apodus	4	1	25.0
Oplurus cuvieri	1	1	100

Table 4. Species and number of snakes examined and frequency of parasites.

Species	*n* (N = 57)	Frequency of Parasites	
		Number of Samples	%
Lampropeltis getula californiae	12	6	50.0
Python reticulatus	7	0	0.0
Python bivittatus	6	0	0.0
Python regius	6	2	33.3
Epicrates angulifer	5	4	80.0
Pantherophis obsoletus	5	5	100
Boa constrictor	4	4	100
Pantherophis guttatus	4	1	25.0
Lampropeltis triangulum hondurensis	3	2	66.7
Morelia spilota	2	1	50.0
Lampropeltis getula splendida	2	1	50.0
Epicrates cenchria maurus	1	1	100.0

Overall, the most frequent parasites were oxyurids (36.4%, *n* = 36/99) and strongylid/*Kalicephalus* sp. (8.1%, *n* = 8/99) (Table 5 and Figure 1).

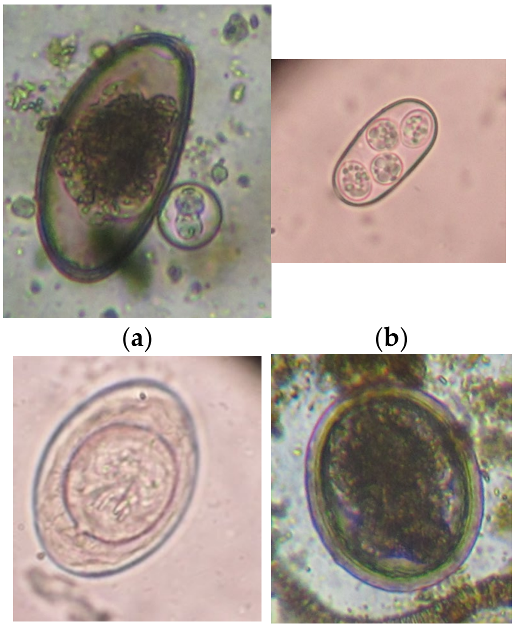

Figure 1. Microphotographs of reptile gastrointestinal parasites. (**a**) Oxyurid egg (on the left) and *Isospora* sp. oocyst (on the right); (**b**) *Eimeria* sp. oocyst; (**c**) *Hymenolepis nana* eggs; (**d**) Ascarid egg. All images were taken using a total magnification of 400×.

Table 5. Frequency of parasites in examined chelonians, lizards, and snakes.

Parasites	Chelonians ($n = 5$)	Lizards ($n = 37$)	Snakes ($n = 57$)	Frequency (n)	Frequency (%)
Oxyurids	1	21	14	36	36.4
Strongylids/*Kalicephalus* sp.	1	1	6	8	8.1
Eimeria sp.	0	0	5	5	5.1
Hymenolepis spp.	0	0	5	5	5.1
Ascarids	4	0	0	4	4.0
Isospora sp.	0	2	0	2	2.0

Among the samples where parasites were identified ($n = 53$), mixed parasitic infections were found in six (11.3%) reptiles. Only one chelonian out of the five investigated presented mixed parasitic infections (20.0%), followed by 2 lizards out of 37 (5.4%) and 3 snakes out of 57 (5.3%) investigated.

4. Discussion

Gastrointestinal parasites, primarily protozoa and nematodes, are commonly found in reptiles in captivity, possibly due to the monoxenous life cycle of several species and the high resistance of oocysts, eggs, or larvae, allowing them to survive in captive habitats [3]. Furthermore, as stated by Panayotova-Pencheva et al. [1], the confined environment of zoo habitats promotes geo-helminth development and maintenance, leading to frequent re-infections of reptiles.

According to Jacobson [20], parasites from the superfamily Oxyuroidea are usually observed as intestinal parasites in chelonians and lizards, often developing a commensal relationship with the host. The high frequency of oxyurids in this study in lizards and snakes can be explained by the fact that these parasites do not require an intermediate host to complete their life cycle [21]. Moreover, animals with an herbivorous and insectivorous diet are frequently parasitized by oxyurids [20], with at least twelve different genera of oxyurids already reported in snakes, lizards, and chelonians [21]. Moreover, the eradication of oxyurids may not always be successful [22]. Several studies reported a high prevalence of these parasites [2,5,6,10–15].

In the present study, ascarids were only identified in chelonians; however, they can also be found in snakes and lizards [2]. Ascarids have a monoxenous life cycle but can be transmitted via feeding or cohabitation through a paratenic host, such as amphibians, small mammals, and other reptiles [22]. Although the number of chelonian samples in this study was small ($n = 5$), the frequency of these parasites was high (80.0%).

The prevalence of strongylids (8.1%) in this study was lower compared to previous studies that found a prevalence of 20.4% (11/55), mainly *Kalicephalus* spp. [2], or 19.7% (14/74) [23]. Both of these previous studies were carried out in pet reptiles, which may explain the higher prevalence of parasites. The zoo sanitary and medical prophylaxis measures are more efficient in preventing parasitic infections. Conversely, other studies reported a lower prevalence, such as Pasmans et al. [3]. Although the definite host of *Kalicephalus* spp. is snakes [20], a small percentage of chelonians and lizards could also be affected (possibly functioning as paratenic hosts). These parasites have a direct life cycle and an oral infection route, with the possibility of transcutaneous infection, although this has not been properly proven [24].

Coccidiosis is one of the main causes of morbidity and mortality in reptiles [25]. The most common coccidian genera in these animals are *Eimeria* and *Isospora* [26], as found in this study. These parasites have a direct life cycle and are transmitted via the fecal–oral route through food, fomites, and infected substrates present in the terrarium [20,22], thus increasing the likelihood of transmission.

In this study, only two bearded dragons (*Pogona vitticeps*) were parasitized by the genus *Isospora*. Although the number is small, this is considered by Heard et al. [9] to be the most commonly reported coccidiosis in this species. Despite the small number, the prevalence of this genus in lizards (5.4%, 2/37) was higher than in the study by Rataj et al. [2], who reported 0.9%. These differences could be explained by the sample size of the different studies, as Rataj et al. [2] analyzed 949 samples while we investigated 99 samples.

According to Jacobson [20], the genus *Eimeria* is usually found in snakes and lizards. In this study, *Eimeria* was not identified in lizards, and in snakes, it had a lower prevalence (5.1%) compared to other studies (27.0% and 6.2%, respectively) [6,16]. The differences found between the studies may be related to the origin of the samples (whether they were collected from pet reptiles or a zoo), the size of the sample, and also the enormous diversity of reptiles that exist, each with its physiology, management, and very particular microbiota.

Although *Hymenolepis* sp. was found in this study in boas (*Boa constrictor*), its definitive host is the field vole [27]. Rinaldi et al. [6] considered it a pseudoparasite. In the present study, as boas are carnivorous, this parasite may have been acquired through feeding on live voles. Although it does not harm reptiles, this parasite is zoonotic [27], which underlines the importance of maintaining good hygiene practices among zoo staff during feeding and cleaning activities.

Overall, parasites can infect reptiles in a zoo through the introduction of a new animal that has not been quarantined in the zoo, through intermediary and paratenic hosts, or, even more probably, through food contamination and human visitors or staff who might be infected with these parasites [1]. All these parameters can and should be as controlled as possible, aiming for minimal manipulation of these animals (for instance, to proceed to medical prophylaxis or treatment) to avoid stress and for animal well-being purposes. This could be achieved by promoting training of zoo staff on biosecurity measures, zoonoses, and the importance of good personal hygiene practices to reduce the cross-contamination between habitats and protect animals and human health using a One Health perspective. Frequent and regular cleaning of the environment must be practiced, which will undoubtedly reduce the number of infective stages of different parasites in the environment, hence controlling such parasites.

The Mini-FLOTAC technique used in this study proved to be sensitive, as described in the literature. In the future, it would be useful to define a Mini-FLOTAC protocol for reptiles, together with reference values of eggs/oocysts per gram of feces that would allow distinguishing parasitism from parasitosis.

5. Conclusions

This study highlights the importance of assessing the presence of gastrointestinal parasites in reptile enclosures in zoological gardens. The present study found that 53.3% of the reptiles were infected with gastrointestinal parasites, demonstrating that, even with the high management standards practiced at the zoo, along with regular fecal examinations, a non-negligible frequency of parasitic infections remains. It is considered that if the biosecurity measures were reduced, a higher frequency of infection would become evident. Routine coprological examinations should always be conducted, and if diagnosed, animals should be properly treated and their environment sanitized as soon as possible. Furthermore, it is crucial to promote training of zookeepers from a One Health perspective.

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Data Availability Statement: The original contributions presented in the study are included in the article; further inquiries can be directed to the corresponding author.

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