ISSN 1852-771X

Rev. med. vet. (En línea) 2025, 106(3): 9 – 16

Artículo de investigación - Estudio observacional

A retrospective study of recorded parasitological findings on reptiles, 2006 to 2021

Estudio retrospectivo de hallazgos parasitológicos en reptiles, 2006 - 2021

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RESUMEN

El parasitismo en reptiles es un fenómeno común a nivel mundial. Los autores han realizado un estudio aleatorio simple de 15 años de endoparásitos recolectados en los exámenes post mortem con 85 reptiles diversos provenientes de la cría, cautiverio o vida silvestre. Los cadáveres desollados y congelados se sometieron a una inspección general en la necropsia, y los parásitos fueron recuperados de los órganos principales. La identificación de parásitos se realizó de acuerdo con la literatura especializada, aunque no siempre se pudo arribar a nivel de especie.

Las diferencias en las prevalencias y la familia taxonómica, la edad y el género de los reptiles se evaluaron mediante la prueba de Chi-cuadrado utilizando un nivel de significancia de 0,05. En general, no hubo lesiones macroscópicas asociadas con parásitos. No se observaron diferencias estadísticamente significativas entre la infestación de parásitos y la edad o el sexo por familias de reptiles. Los análisis de laboratorio mostraron varias especies de endoparásitos, mientras que las infecciones mixtas ocurrieron principalmente con dos o más especies de parásitos. Entre los parásitos identificados, la clase más prevalente perteneció significativamente a Trematoda (72,8 %), seguida de Cestoda (6,8 %) y Sporozoa (12,6 %). Telorchis clava y Opisthogonimus lecithonotus (Trematoda) en serpientes, Proterodiplostomum sp. (Trematoda) y Dujardinascaris sp. (Nematoda) en caimanes, y Prionosomoides scalaris (Trematoda) en tortugas, fueron los parásitos visualizados con mayor frecuencia. Se concluye, entonces, que una amplia variedad de parásitos, en su mayoría de la clase Trematoda, están presentes en el área de estudio con

ABSTRACT

Parasitism in reptiles is a common phenomenon worldwide. The authors have performed a simple randomized 15-year period study of endoparasites collected at the post-mortem examinations of 85 diverse reptiles coming from farming, captivity or wildlife. Pelted and frozen carcasses were submitted for general inspection at necropsy, and parasites were recovered from the main organs. Parasite identification was done according to specialized literature, although not always it could be made at species level.

Differences on the prevalences and relationships to family reptile, age and gender were evaluated using the Chi-square tests using 0.05 as the level of significance.

In general, there were no macroscopic lesions associated with parasites. No statistically significant differences between parasite infestation and age or sex by reptile families were seen. Laboratory analyses showed several endoparasite species meanwhile mixed infections occurred mostly with two or more species of parasites. Among the identified parasites, the most prevalent class significantly belonged to Trematoda (72.8%), followed by Cestoda (6.8%) and Sporozoa (12.6%). Telorchis clava and Opisthogonimus lecithonotus (Trematoda) in snakes, Proterodiplostomum sp. (Trematoda) and Dujardinascaris sp. (Nematoda) in caimans, and Prionosomoides scalaris (Trematoda) in tortoises, were the most frequent visualized parasites. It is concluded, then, that a wide variety of parasites, mostly of those of Trematoda class, are present in the una prevalencia relativamente alta y una infección de intensidad media baja a moderada. La importancia de explorar sistemáticamente las prevalencias de parásitos en el área de distribución silvestre podría mejorar nuestra percepción de los diferentes ciclos de vida involucrados y también de los posibles riesgos para la salud pública.

Palabras clave: reptiles, endoparásitos, prevalencia, Argentina.

study area with relatively high prevalence and low to moderate mean intensity infection. The importance of systematically exploring the prevalences of parasites in the wild might improve our perception of the different life cycles involved and the eventual public health risks.

Key words: reptiles, endoparasites, prevalence, Argentina.

INTRODUCTION

Reptiles comprise a diverse group of ectothermic vertebrates that play central roles in various ecosystems worldwide[1]. Knowledge of endoparasites of wildlife is important for understanding the ecology, natural history and evolution of both parasite and host species[2], as their prevalence might vary depending on environmental conditions (i.e., presence of intermediate hosts and suitable transmission routes)[3].

With their increased popularity as pets and display animals in zoological collections during the last decades, and their use as research subjects, there has been a tremendous body of biomedical information and literature which has been published on some reptiles[1,4]. Moreover, the high prevalence of parasites in wild reptiles found worldwide highlights the importance of understanding parasite life cycles and transmission pathways[3]. Also, another aspect to consider is public health and potential zoonotic risks. In fact, the increasing integration of snakes and other reptiles into captive or research settings, driven by conservation, the exotic pet trade, and medical research, has heightened concerns about underlying parasitic infections which may have zoonotic potential[5].

Overall, recent studies from different countries have demonstrated that endoparasites in reptiles are quite common and often at a high prevalence[3].

A recent state of knowledge on reptile parasites in Argentina has been done providing an updated taxonomic and systematic checklist of nematodes, as a total of 40 nematode taxa, belonging to 2 orders, 5 suborders, 12 families and 19 genera, were found to parasitize 54 reptile species across 11 families[6], but similar studies for Cestoda or Trematoda are still lacking. The purpose of this article is to summarize and bring together several rather diverse reptile families and the endoparasites investigated on them.

MATERIAL AND METHODS

The authors have performed randomized post-mortem examinations on 85 different reptiles received at the Laboratory of the UNNE - Faculty of Veterinary Sciences for parasitological investigation over the 15-year period between 2006 and 2021, although the bulk of the accessions were performed during the last 7 years.

Different sources provided the carcasses: private owners (n = 2), a local serpentarium displaying reptiles in captivity (n = 5), farming (n = 25), and free-ranging individuals came from mostly urban or wild environments (n = 53) scattered throughout the vast Argentinean province of Corrientes (28°40′S 57°38′O). Specimens belong to 7 families: Alligatoridae (*Caiman latirostris* Daudin, 1802; Caiman yacare Muller & Hellmich 1936; *Caiman* sp.), Boidae (*Eunectes murinus* Linnaeus, 1758; *Eunectes notaeus* Cope, 1862), Viperidae (*Bothrops alternatus* Duméril, Bibron & Duméril, 1854; *Bothrops diporus* Cope, 1862), Colubridae (*Hydrodynastes gigas* Dumeril, Bibron & Dumeril, 1854; *Leptophis ahaetulla* Linnaeus, 1758), Chelidae (*Phrynops hilarii* Dumeril & Bibron, 1835; *Phrynops* sp.), Testudinidae (*Chelonoidis chilensis*, Gray 1870), and Teidae (*Salvator merianae* A.M.C. Duméril & Bibron, 1839).

These pelted and frozen carcasses were submitted to us for examination. Sex and estimated age were determined, and animals were then roughly categorized into two main groups each (male –M- and female –F-; juvenile –J- and adult –A-), having these total numbers: Alligatoridae (13 M: 7J & 6A, and 19 F: 8J & 11A, total = 32), Boidae (5 M: 2 J & 3 A, and 6 F: 3 J & 3 A, total = 11), Viperidae (6 M: 2 J & 4 A, and 4 F: 2 J & 2 A, total = 10), Colubridae (5 M: 3 J & 2 A, and 8 F: 4 J & 4 A, total = 13), Chelidae (11 M: 7 J & 4 A, and 6 F: 3 J & 3 A, total = 17), Testudinidae (1 J M and 1A M, total = 2), and Teiidae (2 A F, total = 2).

A general inspection at necropsy was performed, and isolation of helminths was attempted on the gastrointestinal tract (GI), and the major viscera. The stomach and intestines were opened separately in water, their contents removed and linings scraped. The larger helminths were collected directly by macroscopical examination. The helminth specimens isolated from the different viscera were preserved in 70% ethanol and glycerol (9:1) and subsequently counted using a stereoscope. Nematodes were cleared in lactophenol or clove oil. Trematodes and cestodes were stained in acetoalum carmine, cleared in lactophenol or clove oil and mounted in Canada balsam. Species identification was realized in accordance with literature[7,8,9].

Due to partial decomposition of the GI contents in some individuals, parasite identification could not always be made at species level. When possible, fecal samples (0.05 to 0.15 g) were obtained from the rectum and examined for the presence of Sporozoa. The formalin-ether concentration method was employed to make fecal smears; these were stained with the modified Ziehl-Neelsen method for the detection of *Cryptosporidium* spp. oocysts. A 5 g fecal sample from rectum was also preserved in vials of polyvinyl alcohol fixative for subsequent trichrome staining and then examined for 15 minutes at 500x magnification for the presence of *Giardia* spp. cysts and trophozoites. A centrifugal sugar flotation technique (sp. gr. 1.27) was used to demonstrate coccidian oocysts in a 1 g fecal sample.

Statistical analysis: Prevalence was calculated as the percentage of infected animal and mean intensity as the average number of parasites in the total number of infected animal[10] and data analysis was performed using the Chi-square tests using $p < 0.05$ as the level of significance, relative risk (odds ratio, OR), and the Taylor series 95% confidence limits (CFs) were calculated with the statistical software InfoStat and were used to evaluate differences on the prevalences and relationships to family reptile, age and gender.

ISSN 1852-771X

RESULTS AND DISCUSSION

It is likely that the sample of free ranging reptiles received here for examination was biased toward to wildlife traumatized cases because these animals, often found along roadsides or at private residences, were the most exposed to come to the attention of the public. The relative proportions of other groups within this survey, therefore, has probably been underestimated.

All reptiles obtained were dead and the mean physical condition ratings of the investigated animals were mostly good, except for those specimens crushed by vehicles at the road or attacked by predators. None of them showed signs of gastrointestinal disease and some parasites, either trematodes, cestodes or nematodes, were unidentifiable because of the poor condition of the specimens.

There were no statistically significant differences between parasite infestation and age or sex by reptile families: Alligatoridae (age: $p>0.05$, OR = 0.7, 95% CF = 0.5–1.2 ; sex: $p>0.05$, OR = 0.6, 95% CF = 0.5–1.2), Boidae (age: $p>0.05$, OR = 1.5, 95% CF = 1.4–1.9 ; sex: $p>0.05$, OR = 0.6, 95% CF = 0.3–1.2), Viperidae (age $p>0.05$, OR = 0.5, 95% CF = 0.5–0.8; sex: $p>0.05$, OR = 0.3, 95% CF = 0.2–5.2), Colubridae (age: $p>0.05$, OR = 1.1, 95% CF = 0.8–1.3; sex: $p>0.05$, OR = 0.3, 95% CF = 0.1–0.9), Chelidae (age: $p>0.05$, OR = 1.0, 95% CF = 0.5–1.2; sex: $p>0.05$, OR = 0.5, 95% CF = 0.4–0.9), Testudininae (age: $p>0.05$, OR = 0.4, 95% CF = 0.4–6.1; sex: $p>0.05$, OR = 0.5, 95% CF = 0.3–0.9), and Teiidae (age: $p>0.05$, OR = 0.6, 95% CF = 0.5–1.7; sex: $p>0.05$, OR = 0.4, 95% CF = 0.1–0.6).

Herein, most of the infected reptiles (79%) harbored ≤ 2 species of parasites, meanwhile 9% harbored three to 4 species, although differences weren´t significant ($p>0.05$, OR = 0.3, 95% CF = 0.2–7.2). Except for *Telorchis clava* and *Eunectes notaeus* or Sporozoa sp. on *Phrynops* sp., the rest of the accessions apparently showed low to moderate mean intensity infection and commonly without significant differences, according to the author own interpretation. In general, wild and captive reptiles are infected with a wide range of parasites including protists, helminths and arthropods[8,11]. Recent studies from different countries have demonstrated that endoparasites in reptiles are quite common and often at a high prevalence; thus, reported prevalences of endoparasite infections ranged from 47.3 to 88.5% of animals depending on the reptile species surveyed[3].

Herein, Trematoda (72.8%), accounted significantly for the most prevalent parasite class found, following by Cestoda (6.8%) and Sporozoa (12.6%) ($p<0,05$, OR = 0.4, 95% CF = 0.2–1.1). The results are summarized in Tables 1 to 6 by host (reptile families), parasite spp., infection indices and source.

Table 1. Prevalence and mean intensity of endoparasites collected from 32 specimens of the Alligatoridae family, 2009-2010

Alligatoridae (32)				
Host Family/species (No. collected)	Parasite (location)	Infection indices		Source
		P (%)	MI ± SE	
***Caiman* latirostris** (19)	*Proterodiplostomum* sp. (I)	42.1 (8/19)	2.0	farming (17) wildlife (2)
	Eimeria sp. (I)	31.5 (6/19)	3.6	
Caiman yacare (11)	*Proterodiplostomum sp.* (I)	45.4 (5/11)	9.0	farming (8) wildlife (3)
	Dujardinascaris sp. (ST,I)	27.2 3/11	0.2	
	Eimeria sp. (I)	18.1 (2/11)	ND	
Caiman sp. (11)	*Proterodiplostomum sp.* (I)	50.0 (1/2)	1.0	wildlife (2)
	Dujardinascaris sp. (I)	100 (2/2)	2.0	

P: prevalence (number of animals infested / number examined expressed as a percentage); MI: mean intensity (the mean number of parasites per infested animal); ST: stomach; I: intestine. ND: not determined. Own elaboration

Table 2. Prevalence and mean intensity of endoparasites collected from 11 specimens of the Boidae family, 2009-2010

Boidae (11)				
HostFamily /species (No collected)	Parasite (location)	Infection indices		Source
		P(%)	MI ± SE	
Eunectes murinus (7)	*Telorchis* sp. (I)	42.8 (3/7	0.3	wildlife (6) serpentarium (1)
	Cestoda* (ST,I)	28.5 (2/7)	1.1	
Eunectes notaeus (4)	*Telorchis clava* (I)	75.0 (3/4)	46	wildlife (2) serpentarium (2)
	Crepidobothrium sp. (I)	75.0 (3/4)	2.1	
	Nematoda* (I)	25.0 (1/4)	ND	
	Cestoda* (ST,I)	25.0 (1/4)	0.3	

P: prevalence (number of animals infested / number examined expressed as a percentage); MI: mean intensity (the mean number of parasites per infested animal); ST: stomach; I: intestine. ND: not determined; * Unidentifiable. Own elaboration.

Table 3. Prevalence and mean intensity of endoparasites collected from 10 specimens of the Viperidae family, 2009-2010

Viperidae (10)				
Host Family /species (No collected)	Parasite (location)	Infection indices		Source
		P (%)	MI ± SE	
Bothrops alternatus (3)	*Telorchis clava* (I)	60.0 (6/10)	2.2	wildlife (2) serpentarium (1)
	Opisthogonimus lecithonothus (ST)	66.6 (2/3)	3.2	
	Trematoda* (I)	30.0 (3/10)	0.6	
	Eimeria sp. (I)	20.0 (2/10)	ND	
Bothrops diporus (7)	*Opisthogonimus lecithonotus* (ST)	57.1 (4/7)	2.2	wildlife (7)
	Nematoda* (I)	28.5 (2/7	3.0	

P: prevalence (number of animals infested / number examined expressed as a percentage); ST: stomach; I: intestine; ND: not determined; * Unidentifiable.
Own elaboration

Table 4. Prevalence and mean intensity of endoparasites collected from 13 specimens of the Colubridae family, 2009-2010

Colubridae (13)				
Host Family /species (No collected)	Parasite (location)	Infection indices		Source
		P (%)	MI ± SE	
Hydrodynastes gigas (10)	*Opisthogonimus lecithonotus* (ST)	60.0 (6/10)	2.8	wildlife (9) serpentarium (1)
	Glossidiella ornata (L)	30.0 (3/10)	2.5	
	Glossidioides loossi (L)	10.0 (1/10)	ND	
	Telorchis clava (I)	50.0 (5/10)	6.0	
Leptophis ahaetulla (3)	*Renifer heterocoelium* (M,I)	66.6 (2/3)	2.4	wildlife (3)

P: prevalence (number of animals infested / number examined expressed as a percentage); MI: mean intensity (the mean number of parasites per infested animal); M: mouth; ST: stomach; I: intestine; L: liver. ND: not determined. Own elaboration.

Table 5. Prevalence and mean intensity of endoparasites collected from 17 specimens of the Chelidae family, 2009-2010

Chelidae (17)				
Host Family /species (No collected)	Parasite (location)	Infection indices		Source
		P (%)	MI ± SE	
Phrynops hilarii (11)	*Prionosomoides scalaris* (I)	27.2 (3/11)	2.0	wildlife (11)
	Nematophila grandis (ST,I)	18.1 (2/11)	0.7	
	Cheloniodiplostomum testudinis (I)	9.09 (1/11)	ND	
	Camallanus spp. (I)	9.09 (1/11)	ND	
Phrynops sp. (6)	*Prionosomoides scalaris* (I)	66.6 4/6	0.6	wildlife (6)
	Nematophila grandis (ST,I)	33.3 (2/6)	0.9	
	Eimeria& Isospora spp.* (I)	16.6 (1/6)	11.7	

P: prevalence (number of animals infested / number examined expressed as a percentage); MI: mean intensity (the mean number of parasites per infested animal); ST: stomach; I: intestine; ND: not determined; * Unidentifiable. Own elaboration.

Table 6. Prevalence and mean intensity of endoparasites collected from 4 specimens of the Testudinae & Teiidae families, 2009-2010

Testudinidae (2)				
Host Family /species (No collected)	Parasite (location)	Infection indices		Sources
		P (%)	MI ± SE	
Chelonoidis chilensis (2)	*Ascaridae* sp.* (I)	100 2/2	0.9	captivity (2)
	Taniae sp.* (I)	50.0 (1/2)	1.1	
	*Eimeria sp.** (I)	50.0 (1/2)	ND	

Teiidae (2)				
Host Family /species (No collected)	Parasite (location)	Infection indices		Source
		P (%)	MI ± SE	
Salvator merianae (2)	*Diaphanocephalus galeatus* (ST,I)	100 2/2	1.2	wildlife (2)
	Trematoda* (I)	50.0 (1/2)	0.6	
	Nematoda* (ST,I)	50.0 (1/2)	0.6	
	Eimeria sp. (I)	50.0 (1/2)	ND	

P: prevalence (number of animals infested / number examined expressed as a percentage); MI: mean intensity (the mean number of parasites per infested animal); ST: stomach; I: intestine; ND: not determined; * Unidentifiable. Own elaboration

Farming caimans showed here a low infection by trematodes like *Dujardinascaris* sp. and *Proterodiplostomum* sp. Both are well known described in *Caiman yacare* and *latirostris* from some Latino American countries like Bolivia, Brazil and Venezuela[12,13]. With declining wild populations in the past decades, modern crocodile farming began in South America. The late Professor Oscar Lombardero, historic chief of our laboratory, used to see these trematodes in both spp. of Caiman in the 60's and 70's (personal communication).

Among snakes, Trematoda accounted for the highest prevalence, mostly by *Telorchis clava* or *Opisthogonimus lecithonothus*. In fact, *Ophisthogonimus lecithonothus* was the most frequent in most of the snakes examined in a previous report from Argentina[14]. Although a number of parasites are known to cause significant disease in captive snakes, there are no reports of epizotics associated with parasitism in wild populations of snakes[4,7]. The cestode *Crepidobothrium* sp., found here in *Eunectes notaeus*, the popular green anaconda, has been previously described[15].

Phrynops hilarii, the most frequent wild turtle in the region, showed several intestinal trematodes, all vastly described in Latin America[12,16]. On the other hand, few endoparasites (and partially identified) were found on the only two specimens of *Chelonoidis chilensis*, the most common species in captivity among those tortoises inhabiting Argentina. A very well description of the occurrence of gastrointestinal parasitism in this tortoise has already been published[17].

Sporozoa accounted for low prevalences here in caimans, turtles and black & white tegus in this study, meanwhile *Cryptosporidium* or *Giardia* spp. were null. Again, the poor condition of the specimens might preclude their visualization. Most surveys on wild reptiles demonstrated that little is known about the diversity of *Cryptosporidium* species and reports of *Giardia* spp. are even very rare[1].

We have attempted to confront our results with published reports based on original records and some published in journals not easily available to scientists from other regions of the globe.

Some of our early collected data regarding age or sex, for example, were sometimes labored or even the freshness of the cadavers precluded a further deep statistical analysis including the ORs to assess exposure to risk factors or associations between other epidemiological variables and prevalence rates. Even though the limited number of reptile families and the small to moderate parasite intensities observed herein, the present study humbly contributes to the endoparasite knowledge of reptiles from the region and their discussion of the significance in their wild populations.

CONCLUSIONS

The present study showed that an ample range of endoparasites, particularly and significantly those belonged to Trematoda class, of reptiles are present in the covered indigenous area with relatively high prevalence and low to moderate mean intensity infection. There are some reports of parasitic diseases in the literature, but it has not been studied in such detail in this region. This is part of a continuing cooperative effort by the laboratories engaged to monitor the health status of these native reptiles, and it is a modest and general contribution to the knowledge on the occurrence, prevalence and the epidemiological dynamics of endoparasites in these wild populations.

Acknowledgements

The authors are grateful to the anonym individuals and groups of our community for referring dead reptiles for us to study. In memoriam of Professor Oscar Lombardero, *alma mater* of this laboratory, who died in 2001.

Conflict of interest

The authors of this study declare that there are no conflicts of interest related to the conduct of the survey and the preparation of the manuscript.

Ethical Statement

It is stated that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guides on the care and use of vertebrates.

All procedures were approved by the Animal Welfare Committee of the UNLP-Veterinary College (CICUAL protocol No.42-2-14E). No animal experimentation was conducted.

Funding

Funding for this study was provided by CIC (grant numbers 2410/12 and 833/14) and by the National Agency for Scientific and Technological Promotion FONCyT [grant number PICT 2007-Cat.I-01191].

Artificial Intelligence

No Artificial Intelligence assisted improvements, including wording and formatting changes to the texts or any autonomous content creation, were used.

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