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DEWORMING PROTOCOLS OF ISLAND SNAKES KEPT IN CAPITIVITY AT THE BUTANTAN INSTITUTE

(Protocolos de vermifugação de serpentes de ilha mantidas em cativeiro no Instituto Butantan)

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ABSTRACT: *Bothrops insularis* and *B. alcatraz* are critically endangered species inhabiting two different coastal islands of the state of São Paulo. Although they are closely related species, they show distinct morphological characteristics due to their isolation from the continent. There is a general lack of studies on conservation and captive husbandry of these species, including their parasites. Our aim was to evaluate the deworming protocols used in *B. insularis* and *B. alcatraz* in captivity. We evaluated three deworming protocols through fecal examination: (1) adult *B. insularis* were treated with ivermectin and praziquantel, (2) adult *B. alcatraz* were treated with ivermectin, and (3) juvenile *B. insularis* and *B. alcatraz* were treated with ivermectin. The snakes are kept in the Laboratory of Ecology and Evolution at the Instituto Butantan (São Paulo, Brazil) for conservation purposes. To evaluate the deworming protocols, we performed the Willis-Mollay (1921) technique. After treating with the first protocol, 19% of the animals showed only eggs of a pseudoparasite (*Syphacia obvelata*). After the second protocol, 75% of the animals showed cestodes (*Ophiotaenia* sp.). After the third protocol, 32% of the animals showed only the pseudoparasite, *S. obvelata*. The pseudoparasite was found only in *B. insularis* individuals that ate adult mice in captivity. However, cestode eggs were found only in those adult snakes of the second protocol that did not receive praziquantel. Based on the negative results for pathogenic parasites, we conclude that the first protocol was effective for adult snakes whereas the third protocol was effective for juveniles.
Keywords: *Bothrops alcatraz*; *Bothrops insularis*; helminths; nematodes; tapeworms
RESUMO: *Bothrops insularis e B. alcatraz* são espécies criticamente ameaçadas de extinção que habitam duas ilhas diferentes da costa do estado de São Paulo. Embora elas sejam espécies intimamente relacionadas, apresentam características morfológicas distintas devido ao seu isolamento do continente. Há uma falta geral de estudos sobre conservação e criação em cativeiro dessas espécies, incluindo seus parasitas. O objetivo desse trabalho foi avaliar os protocolos de vermifugação utilizados em *B. insularis* e *B. alcatraz* em cativeiro. Foram avaliados três protocolos de vermifugação através do exame coproparasitológico: (1) *B. insularis* adultas foram tratadas com ivermectina e praziquantel, (2) *B. alcatraz* adultas foram tratadas com ivermectina, e (3) *B. insularis* e *B. alcatraz* juvenis foram tratadas com ivermectina. As serpentes são mantidas no Laboratório de Ecologia e Evolução do Instituto Butantan (São Paulo, Brasil) para projetos de conservação. Para avaliar os protocolos de vermifugação, foi realizado a técnica de Willis-Mollay (1921). Depois de tratado com o primeiro protocolo, 19% dos animais apresentaram somente ovos de pseudoparasitas, (*Syphacia obvelata*). Depois do segundo protocolo, 75% dos animais apresentaram cestóides (*Ophiotaenia* sp.). Depois do terceiro protocolo,

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32% dos animais apresentaram somente pseudoparasitas, *S. obvelata.* O pseudoparasita foi encontrado somente em indivíduos de *B. insularis* que comeram camundongos adultos em cativeiro. Contudo, ovos de cestóides foram encontrados somente nas serpentes adultas do segundo protocolo que não receberam praziquantel. Com base nos resultados negativos para parasitas patogênicos, concluímos que o primeiro protocolo foi eficaz para serpentes adultas, enquanto que o terceiro protocolo foi efetivo para juvenis.
Palavras-chave: *Bothrops alcatraz*; *Bothrops insularis*; helmintos; nematóides; tênias

INTRODUCTION

The golden lancehead (*Bothrops insularis*) and the Alcatrazes lancehead (*B. alcatraz*) are critically endangered snakes (IUCN, 2018) that have attracted much scientific interest (Marques et al., 2002a, 2002b; Furtado, 2005; Marques et al., 2012; Moraes et al., 2012). These species are endemic to two different coastal islands of the state of São Paulo, Brazil. *Bothrops insularis* is endemic to the Queimada Grande Island and *B. alcatraz* to the Alcatrazes Island. Both species are close relatives to the lancehead *B. jararaca*, which occurs in mainland Atlantic forest areas (Marques et al. 2002a, 2002b). The island lanceheads likely diverged from populations of a *B. jararaca*-like ancestor through isolation caused by sea level oscillations during the Pleistocene (Marques et al., 2002a). Island lanceheads differ from their mainland relative in several attributes as morphology, venom, diet, behavior, and physiology, reflecting adaptations to their current environment (Marques et al., 2002a, 2002b; Furtado, 2005; Marques et al., 2012; Moraes et al., 2012). For instance, because there are no mammals on Queimada Grande and Alcatrazes islands, *B. insularis* and *B. alcatraz* have evolved different feeding habits. *Bothrops insularis* has evolved arboreal and diurnal habits and a modified venom to prey on migratory birds (Zelanis et al., 2008; Marques et al., 2012), and *B. alcatraz* feeds on ectothermic animals such as centipedes, amphibians, and lizards (Marques et al., 2002a; Furtado, 2005).

Wild reptiles are frequently infected with various endoparasites, many of which are minimally pathogenic. However, once in captivity, stress, improper husbandry, and poor sanitation can lead to serious diseases and eventually to death in heavily parasitized individuals (Wilson and Carpenter, 1996). The severity of the disease depends on the host's immunity and the number of parasites in their bodies and the environment (Luz et al., 2012). The most common pathogenic parasites found in viperid snakes are the nematodes *Kalicephalus* (found in the intestine), *Ophidascaris* (found in the stomach or esophagus), *Rhabdias* (found in the lungs), and *Strongyloides*. These parasites cause anorexia, diarrhea, and weight loss (Sousa et al., 2014). Cestodes such as *Acanthotaenia*, *Bothridium, Ophiotaenia*, and *Spirometra* are also common and cause malnutrition, enteritis, and imbalance of the intestinal microbiota. Several anthelmintics can be used for treatment such as ivermectin, thiabendazole, fenbendazole, pyrantel pamoate, praziquantel, and levamisole. Ivermectin is one of the less toxic antiparasitics used to treat nematodes in snakes (Luppi et al., 2007).

Our aim was to evaluate three deworming protocols in island lancehead snakes, *B. insularis* and *B. alcatraz*, kept in captivity.

MATERIAL AND METHODS

We evaluated three deworming protocols in *B. insularis* and *B. alcatraz* individuals kept in the Laboratory of Ecology and Evolution (Butantan Institute, São Paulo, Brazil). In the first protocol, we administered ivermectin and praziquantel followed by a second dose two weeks later and evaluated 26 adults of *B. insularis* (12 males and 14 females; Table 1). These snakes had been in captivity for eight years. In the second protocol, we administered ivermectin followed by a second dose two weeks later and evaluated four adults of *B. alcatraz* (one male and three females; Table 1). These snakes had been in captivity for four years. In the third protocol, we administered ivermectin followed by a second dose two weeks later. We evaluated 16 juveniles of *B. insularis* (10 males and six females) and five juvenile *B. alcatraz* (two males and three females), totaling 21 individuals (Table 1). These snakes were three years old and were born in captivity.

Table 1 – Sample size, biometric traits, and time in captivity of *B. insularis* and *B. alcatraz* per deworming protocol.

Variables	Protocol 1		Protocol 2		Protocol 3			
	B. insularis (N=26)		*B. alcatraz* (N=4)		*B. insularis* (N=16)/*B. alcatraz* (N =5)			
	Adults		Adults		Juveniles			
	M	F	M	F	M	F	M	F
	(N=12)	(N=14)	(N=1)	(N=3)	(N=10)	(N=6)	(N=2)	(N=3)
Body mass (g)	157.33± 30.51	514.71± 76.08	52.00± 0	174.00± 17.96	127.00± 25.36	271.83± 13.63	61.50± 6.57	174.00± 1.69
Snout-vent length (mm)	739.16± 32.71	912.50± 74.94	435.00± 0	591.66± 51.69	684.00± 47.15	748.33± 11.34	492.50± 17.50	516.66± 16.99
Tail length (mm)	120.00± 40.35	137.14± 13.59	70.00± 0	80.00± 4.08	113.00± 12.68	117.50± 18.65	82.50± 2.50	78.33± 8.49
Time in captivity (years)	8		4		3			

The snakes were housed individually in plastic boxes (46 × 30 cm) containing corrugated cardboard and water *ad libitum,* in an air-conditioned room with average temperature of approximately 22ºC, relative humidity around 70%, and natural photoperiod. Snakes were fed approximately 10% of their body mass in mice monthly. *Bothrops insularis* individuals were fed mice weighing around 25 g, and *B. alcatraz* individuals were fed mice weighing around 5 g. Mice were obtained from a breeding colony established at the Butantan Institute.

All the study subjects had been previously treated with antiparasitics in 2016, receiving two doses at a two-week interval. No fecal examination was performed before the treatment in 2016. All fecal examinations were conducted one year after dewormed, only in 2017.

Ivermectin was administered at a dose of 0.02 mL/100 g diluted in propylene glycol solution (1:10), (one-part of ivermectin to nine parts of propylene glycol), and praziquantel was administered at 0.02 mL/100 g (Greiner and Mader, 2006; Carpenter, 2010; Grego et al., 2014). Both drugs were from renowned brands, within the expiry date, and were administered by intramuscular injection.

Feces (~2 g) were collected after defecation and stored in a refrigerator at 6 ºC until examinations. Each sample was qualitatively examined using the Willis-Mollay (1921) (flotation method) technique. For each individual, two slides of technique were evaluated. The presence of parasite eggs and larvae was determined by examination of microscopic slides using an optical microscope at ×40 and ×100 magnification. The eggs were observed, analyzed, and qualitatively diagnosed. The mouse feces were analyzed by the Willis-Mollay (1921) technique.

RESULTS

After treating with the first protocol, we found that 19% of the snakes showed the pseudoparasite *Syphacia obvelata* (Figure 1A). After the second protocol, we observed that 75% of the snakes showed proglottids (Figure 1B) and eggs (Figure 1C) of the cestode *Ophiotaenia* sp. Only females had this

parasite. After treating using the third protocol, we found that only *B. insularis* showed pseudoparasites (*S. obvelata*; Figure 1A), corresponding to 32% of the snakes examined (Table 2).

Table 2 - Results of fecal examinations of *Bothrops insularis* and *Bothrops alcatraz.*

Parasite species	Protocol 1 Ivermectin + Praziquantel		Protocol 2 Ivermectin		Protocol 3 Ivermectin			
	B. insularis (N=26)		*B. alcatraz* (N=4)		*B. insularis* (N=16)/*B. alcatraz* (N =5)			
	Adults		Adults		Juveniles			
	M (N=12)	F (N=14)	M (N=1)	F (N=3)	M (N=10)	F (N=6)	M (N=2)	F (N=3)
Ophiotaenia sp	-	-	-	3	-	-	-	-
Pseudoparasite *Syphacia obvelata*	3	2	-	-	3	4	-	-

F = female; M = male and N= individuals number

The egg of *S. obvelata* is a flattened ellipsoid an undifferentiated embryo in utero (Figure 1A). The egg-shell consists of five layers: an external uterine layer, an internal uterine layer, an outer vitelline layer, a middle chitinous layer, and an inner lipid layer. An operculum is present at one pole of the egg (Wharton, 1979).

We did not determine the internal structures of the proglottids (Figure 1B) of *Ophiotaenia* sp. Eggs of *Ophiotaenia* sp. (Figure 1C) were spherical, with a thin, hyaline, and collapsed outer envelope and an inner envelope consisting of two-layered embryophore with three pairs of embryonic hooks (Patra et al., 2017).

Figure 1- Parasites and eggs found in the feces of captive snakes by the Willis- Mollay (1921) technique. A) Pseudoparasite: *Syphacia obvelata* (egg); B) *Ophiotaenia* sp. (proglottids); and C) *Ophiotaenia* sp. (egg). The arrow black indicates the two-layered embryophore.

Examination of mouse feces indicated the presence of pseudoparasites (*Syphacia obvelata*).

DISCUSSION

The island snakes *B. insularis* and *B. alcatraz* are closely related species endemic to islands in southeastern Brazil differing in several traits including feeding habits. Adults of *B. insularis* from the Queimada Grande Island feed mostly on migratory birds, such as *Turdus flavipes* and *Elaenia chilensis* (Montanhini, 2010). However, juveniles prey mostly on ectothermic preys as centipedes (*Otostigmus scabricauda*), lizards (*Colobodactylus launayi*), and frogs (Martins et al., 2002; Andrade et al., 2010; Marques et al., 2012).

In the first protocol, *Bothrops insularis* adults showed no parasitic infection. These individuals certainly ate a variety of prey in nature, but in captivity, they were fed mice only. Consequently, they did not show any parasite but only pseudoparasites. No cestode was found in *B. insularis*. This result was expected since *B. insularis* individuals were treated with praziquantel, and this species preys mostly on birds in nature (Marques et al., 2012), which are not potential cestode hosts (Klingenberg, 1997). Nevertheless, on the island, lizards and amphibians can be potential food items and cestode hosts for *B. insularis* (Leão, 1950).

Praziquantel is clinically effective against a wide range of cestode and trematode infections. At low concentrations, this drug increases muscle activity due to the passage of calcium into the parasite, followed by contraction and spastic paralysis stimulated by sodium intake and potassium inhibition. The drug acts on the parasite and is reversibly absorbed, resulting in the vacuolization and disintegration of the helminth integument. The drug also acts on the carbohydrate metabolism of the parasite, causing its death by starvation (Almeida and Ayres, 2006).

Food influences the presence of parasites and pseudoparasites. Some o

the *B. insularis* that ate mice above 20 g showed pseudoparasites (*Syphacia obvelata*) (Rudolphi, 1802). The mice used to feed the snakes were bred in controlled laboratory conditions. *Syphacia obvelata* is normally apathogenic and asymptomatic to rodents (Doyle et al., 2006). Therefore, treating these parasites in captive snakes is not necessary. Controlling this parasite in the laboratory is difficult because of the ease of egg dispersion and the ability of retroinfection (Sousa et al., 2016). In reptiles, *S. obvelata* is considered as pseudoparasite, a parasite originating from the prey (mice) and found during fecal examinations of snakes. Pseudoparasites are common and can easily be misclassified as true parasites. This distinction is important because pseudoparasites do not need treatment (Greiner and Mader, 2006).

In the second protocol, we found that adult *B. alcatraz* had cestodes but no pseudoparasite (*S. obvelata*). Adult mice are hosts of *S. obvelata*. In captivity, *B. alcatraz* individuals are fed neonate mice, which likely explains the lack of the pseudoparasite in this snake species. *Bothrops alcatraz* individuals were not dewormed with praziquantel because this species usually presents adverse reactions to this protocol. So, the individuals were wormed only with ivermectin that does not act on the cestodes. The absence of praziquantel was the cause of the presence of cestodes. Morphological and morphometric data allowed us to speculate that the tapeworm parasitizing *B. alcatraz* was a species of the genus *Ophiotaenia* (Patra et al., 2017).

All individuals of *B. alcatraz* received the same dose of vermifuge and the examinations were performed at the same time. Adult females are larger than adult males (Marques et al., 2002a); consequently, sexual differences in dietary habits are likely to occur in nature. In captivity, however, *B. alcatraz* individuals are fed 5 g mice. Eggs and proglottids of *Ophiotaenia* sp. were found only in females. This finding suggests that, in nature, females ingest the host infected with parasites, bringing them into the captivity. Wild-caught individuals of *B. alcatraz* certainly had previous contact with their natural prey, which includes centipedes, lizards, and amphibians (Marques et al., 2002a).

The tapeworms of the order Proteocephalidea parasitize widely freshwater fish, amphibians, and reptiles (Chambrier et al., 2010) and need an intermediate host to complete their life cycle. Therefore, we expect that the amphibians and lizards from the Alcatrazes Island (Marques et al., 2002a; Brasileiro, 2008) are potential intermediate hosts of *Ophiotaenia*. These cestodes are stenoxenous parasites, infecting only one definitive host species with generally restricted geographic distribution (Chambrier et al., 2010).

Cestodes cause severe infections and may lead to anorexia, emaciation, intestinal obstruction, and death (Frank, 1981). Thus, the animals of the second protocol should be wormed with antiparasitics for cestodes parasitic control.

In the third protocol, we examined juvenile *B. insularis* and *B. alcatraz*. The juveniles were not dewormed with praziquantel because in captivity they do not eat prey that can be carriers of cestodes. Although the wild-caught individuals of *B. alcatraz* certainly ate a variety of prey in nature, they are fed only mice in captivity.

Some individuals of *B. insularis* showed pseudoparasites due to the ingestion of adult mice (≥ 20 g). However, *B. alcatraz* individuals only ate unparasitized mice (≤ 5 g).

CONCLUSION

This is the first study to examine parasites of island snakes in Brazil. Parasitic disease is a major problem for captive maintenance and breeding. Therefore, establishing deworming protocols and appropriate treatments is important for successful maintenance. We conclude that the first (ivermectin and praziquantel) and third (ivermectin only) deworming protocols were effective for adult and juvenile snakes, respectively, since they showed negative results for pathogenic parasites. In the third deworming protocol, the absence of praziquantel was the cause of the presence of cestode (*Ophiotaenia* sp). Further studies on captive maintenance of island snakes are needed, to reduce disease and mortality.

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Informative Notes

This study was conducted at the Laboratory of Ecology and Evolution, Butantan Institute, São Paulo, Brazil, under the approval of the institute's Animal Ethics Committee (approval number 5440031017).

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