

Absence of Hemoparasites in Wildlife Snakes, Located in the Ecological Reserves Cota 70, Cotacachi-Cayapas and Sumaco-Napo-Galeras in Ecuador

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Abstract Ecuador is one of the most mega diverse countries in the world, covering a large amount of flora and fauna in all its geographical regions. The aim of this study was to determine the presence or absence of blood parasites in wildlife snakes in the ecological reserves Cota 70, Cotacachi - Cayapas and Sumaco - Napo - Galeras, located in the continental Ecuador. A total of 14 individuals were captured: 9 Colubrids (*Dipsa elegans*, *Atractus dunni*, *Sibonne bulata*, *Mastigodryas heathii*, *Oxyrhopus petola digitalis*, *Atractus major*, *Oxyrhopus petolarius*, *Phrynonax polylepis*, *Mastigodryas pulchriceps*), 3 boids (*Corallus hortolanus*) 2 *Boa constrictor*, 1 viperid (*Bothrops asper*) and 1 Elapid (*Micrurus surinamensis surinamensis*). Samples of blood were collected from the caudal vein or by cardiac puncture depending on the size of the snake. The blood smears were dried, fixed with methanol and stained with Giemsa solution. Samples were examined by light microscopy using a 100 × magnification lens in search for hemoparasites. The present study shows that in the snakes analyzed there is no evidence of the presence of hematic parasites showing that the areas where the ecological reserves are located could be free of blood parasites. No ectoparasites were found in the captured animals.

Keywords Snakes, Haemoparasites, White blood cells, Giemsa

1. Introduction

Medicine in wild species is relatively new in Ecuador, however, the interest in its practice and learning has been growing in the country, so it is necessary to know more about these animals, their characteristics, the threats to which they are exposed and their development with the environment [1]. According to Carrillo et al. (2005) [1] in his book " *Lista Roja de Reptiles del Ecuador* ", presents 16 species of snakes considered endangered in the country, 21 vulnerable species and 4 in almost critical situation. There are several causes for the increase of species at risk of extinction, among which we can mention diseases, many of which have gained space due to human intervention [2, 3]. In recent years important findings have been made regarding the life cycles of the parasites. This has provided information about the biosecurity that must be had to prevent disease transmission [4]. In reptiles, due to morphological variability and high diversity of hemoparasites, there are not as many reports as there are in mammals and birds, probably related to the large

size of reptile erythrocytes and to the presence of a prominent nucleus within them, Which can strongly influence the appearance of the parasite within the cell and also because the diversity of hemoparasites is greater in reptiles, both in genus and in species [5].

Within the hemoparasites, in the genus *Hepatozoon*, family *Hepatozoidea* we find more than 50 species of intracellular hemoparasites which occur frequently in groups of vertebrates and some invertebrates; Representatives of this genus can parasite leukocytes of birds and mammals (including dogs and cats), also red blood cells in reptiles, amphibians and fish; All vertebrate animals infected with hemoparasites are considered intermediate hosts [6].

Hemoparasites in snakes and reptiles in general affect the organism in different ways, either by forming cysts in various organs such as liver, spleen, kidney and brain, which may contain pigment deposits or may be surrounded by inflammatory cells, causing variation in the Hemoglobin, alterations in serum or plasma proteins [3]. In cases of generalized infection or immunosuppression, hemolytic anemia may occur; in natural hosts these changes mentioned are rare or absent. In young animals, the presence of hemoparasites, added to stress situations or other pathologies, can lead to the death of the animal [3].

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Table 1. Location and characteristics of the studied reserves. Source: Ministry of the Environment - Ecuador, 2016

Province	Canton	City	Ecological Reserve	Altitude	Latitude	Length
Manabí	Portoviejo	Portoviejo	Cota 70	53 msnm (average)	N 1° 2' 45.149	W 80° 27' 36.081
Imbabura	Cotacachi	Cotacachi	Cotacachi - Cayapas	1601 - 4939 msnm	N 0° 33' 9.046"	W 78° 36' 41.399"
Napo – Orellana	Cotacachi	Cotacachi	Sumaco – Napo - Galeras	500 - 3732 msnm	S 0° 21' 57.27"	W 77° 28' 12.709"

The data obtained in the present research are of importance for their originality and refer to a population not studied, which sets precedents for further research. It is expected to continue the study of wildlife snakes in the country, as the populations of these animals have declined considerably in recent years, and more attention is required from the scientific community to address such a worrying situation. The main objective of this study was to determine the presence or absence of hemoparasites in wildlife snakes in Ecuador's natural reserves: Cota 70, Cotacachi - Cayapas and Sumaco - Napo - Galeras.

It seems that the presence of hemoparasites in reptiles is due to their infestation with ectoparasites (like ticks) as it happens in green iguanas in the Guayaquil city [7]. The transmission of *Haemoproteus* in snakes require the presence of a natural vector (*Crhysops* fly), for the transmission of *Haemoproteus metchnikovi* [2].

2. Materials and Methods

2.1. Study Area

The study, of a descriptive nature, was carried out in three natural reserves of Ecuador, located in the coast, east and mountain region: Reserve Cota 70, Portoviejo - province of Manabí, Reserve Sumaco Napo Galeras, Napo - province of Tena [8], Reserve Cotacachi Cayapas, Cotacachi - Province of Imbabura [9] (Table 1).

2.2. Size and Target Population

The target population for this study was all snakes that were located within the natural reserves. The search for snakes was by means of sampling by transects (technique of observation and recording of data along a line that crosses the zones), in anthropic and non-anthropic areas, with emphasis on sectors with dry leaves, fallen trees, hollow logs, holes in the ground, branches of trees, and every place a snake could hide, besides to paths to which they come out to warm themselves. The size of the sample was based on the availability of individuals in wildlife throughout the project, for four months.

In total, 14 snakes were collected, distributed as follows: 6 in the Sumaco - Napo - Galeras reserve, 5 in the Cotacachi - Cayapas reserve and 3 in the Cota 70 reserve. The individuals were captured in situ, by means of search and direct capture. It was also shown if there was presence or absence of ectoparasites on snakes.

2.3. Collection of Blood Samples

The method of manipulation and collection of blood samples were performed according to the standards of preservation and animal ethics established by the Ministry of the Environment in the "Codification of forest law and conservation of natural areas and wildlife (Law No. 2004-017, Official Register No. S-418 of September 10, 2004).

Data on weight (gr), size (cm), sex and species were taken from all individuals captured. Heparinized syringes were used to control the blood clotting process. Approximately 1 mL of blood sample was collected by puncture of the caudal vein, in case of boids and large viperids, and by cardiac puncture in colubrids and small elapids. The samples obtained were stored in properly identified heparinized microtubes for the subsequent blood smear. When the blood sample was insufficient, the blood smear was performed immediately using a drop of blood.

2.4. Examination of Blood Smears

The blood smears of the captured individuals were withdrawn for a period of 10 minutes and then fixed for 4 minutes in 95% methanol. Once the blood smears were fixed, they were stained with Giemsa solution (belonging and prepared by the Laboratory of Parasitology of the Faculty of Veterinary Medicine and Animal Science of the Central University of Ecuador) for four minutes, according to protocol already established [10]. Examination of the smears was performed by light microscopy with a 100X magnification, using 10 quadrants in search of haemoparasites.

3. Results and Discussion

A total of 14 individuals were captured: 9 Colubrids (*Dipsa elegans*, *Atractus dunni*, *Sibonne bulata*, *Mastigodryas heathii*, *Oxyrhopus petola digitalis*, *Atractus major*, *Oxyrhopus petolarius*, *Phrynonax polylepis*, *Mastigodryas pulchriceps*), 3 boids (*Corallus hortolanus*) 2 *Boa constrictor*, 1 viperid (*Bothrops asper*) and 1 Elapid (*Micrurus surinamensis surinamensis*). Of the 14 snakes, 6 belong to the reserve Sumaco - Napo - Galeras (%), 5 in the Cotacachi - Cayapas (%) and three in reserve Cota 70 (%). Of the total captured snakes, 28.57% were males, while the remaining 71.43% were females. The 14 samples taken, showed negative results, did not evidence the presence of

hemoparasites in the blood cells of the captured individuals. Neither vector (ticks, mosquitoes) were found on the individuals found in the sample (Table 2). Most of the specimens found in the field belong to the *Colubridae* family, a fact that coincides with the research carried out by Moreno and Bolaños in 1977, in this study, none of the 28 specimens of colubrids were found to be parasitized. In the same study, it was also evidenced that in the snakes where ticks were found, after a histopathological analysis, all of these animals were found to be positive for Haemogregarinidae hemoparasites [11].

In a study carried out in Gecos of the genus *Tarentola*, from African and European countries, parasites of at least 4 different lineages of *Hepatozoon* spp were detected in 572 animals of 6 species [12]. Likewise in snakes of species *Elaphecarinata*, in Shanghai - China, were found parasites of *Hepatozoon* for the first time, with levels of parasitemia among 4-43 infected erythrocytes by 1,000 examined [13]. *Hepatozoon* spp was also identified for the first time in amphibians Order *Gymnophiona*, habitants of the Seychelles Island of Silhouette [14]; while in Iran, in a study of 87 snakes of 8 different species, 2 different genera of endoparasites were determined in 64 (73.56%) of 87 snakes examined, 47.12% had gastrointestinal parasites and 23 (26.43%) had at least one hemoparasite [15].

In a study in green iguanas [7], in the Guayaquil city, yields positive results that associates to the strong presence

of ticks in the body of the reptiles sampled; In the mentioned study, two species of ticks *Amblyomma dissimile* y *Amblyomma scutatum* were found, both species identified can parasitize wild and domestic animals, human being, and are important because they are hematophagous parasites and vectors of enzootic and zoonotic diseases. This study demonstrates the existence of hemoparasites in this species, and shows that their appearance was linked to the presence of ectoparasites.

The transmission of *Haemoproteus* (a species of hemoparasite that infects snakes) is believed to require the presence of a natural vector, such as the reported case of the *Crhysops* fly, for the transmission of *Haemoproteusmetchnikovi*, without the presence of a vector, transmission would not be possible [2].

In the study "Blood profile and presence of hemoparasites in reptiles of the National Zoological Park, El Salvador", was able to observe eight individuals with ectoparasites, however, with respect to the appearance of hemoparasites, only four individuals showed positive results to the presence of *Hepatozoon* sp [16]. Thus, it is evident that, although not all snakes with ectoparasites tested positive for hemoparasites, the four individuals affected with hemoparasites also showed the presence of ectoparasites. This is confirmed by Jacobson (2007) [2], that protozoan hemoparasites require the presence of invertebrates as vectors for their transmission, including arthropods and annelids.

Table 2. Results of research

Place	Zone	Data of individuals					Results	
		Species	Sex	Weight (g)	Size (cm)	Microhabitat	Vector / ectoparasite	Haemoparasite
Reserve Cotacachi Cayapas	Anthropized	<i>Dipsa elegans</i>	F	32	73,5	Path	Negative	Negative
		<i>Atractus dunni</i>	M	6	22,1	Decomposing trunk	Negative	Negative
		<i>Sibonne bulata</i>	F	23	60	Path	Negative	Negative
		<i>Mastigodryas pulchriceps</i>	F	12	46,4	Path	Negative	Negative
	Non Anthropized	<i>Mastigodryas heathii</i>	M	94	103	Dry leaves	Negative	Negative
Reserve Sumaco Napo Galeras	Anthropized	<i>Oxyrhopus petola digitalis</i>	F	52	81	Path	Negative	Negative
		<i>Atractus major</i>	M	19	40,2	Path	Negative	Negative
		<i>Oxyrhopus petolarius</i>	F	36	76,5	Path	Negative	Negative
	Non Anthropized	<i>Micrurus surinamensis surinamensis</i>	M	11	31	Litter	Negative	Negative
		<i>Phrynonax polylepis</i>	F	21	58,8	Path	Negative	Negative
		<i>Corallus hortulanus</i>	F	533	141	Dry leaves	Negative	Negative
Reserve Cota 70	Non Anthropized	<i>Boa constrictor</i>	F	790	157,5	Branches	Negative	Negative
		<i>Boa constrictor</i>	F	1685	182,5	Soil	Negative	Negative
		<i>Bothrops asper</i>	F	735	148	Soil	Negative	Negative

In a study by experimental parasitisation of snakes *Phytonregius*, *Boa constrictor* and *Lamprophis fuliginosus* with mosquitoes, positive results for the presence of hemoparasites were 78.2% [17]. In the individuals subjected to the study, infestation with the tick *Aponomma latum* was observed in 90.9% of the snakes of the *Phyton regius* species [17]. In this investigation, two ectoparasites were the cause of infection with hemoparasites: mosquitoes and ticks. In the present study there were no external parasites or vectors in all the animals analyzed, which could explain the absence of hemoparasites in these animals. These data are showing that in areas where ecological reserves are located they may be free of hemoparasites. However, further studies should be carried out to continue collecting data on snake health [17].

After the capture of 14 snakes in the reserves Cota 70, Cotacachi - Cayapas and Sumaco - Napo - Galeras, by the application of Giemsa staining to search for hemoparasites, the results were negative. No hemoparasites were found on the stained plaques. After the direct observation exam, were no found possible hemoparasitic transmitting vectors in the captured individuals, possibly because most of the individuals belong to the *Colubridae* family, which seems to influence the absence of vectors due to the scarce space between the scales of the colubrid, where the ectoparasites are usually located.

4. Conclusions

The present study shows that there are no hemoparasites present in the ophidians analyzed, suggesting that the ecological reserves at Ecuador where the animals were captured could be free of hemoparasites, however more studies should be performed in order to corroborate these results.

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REFERENCES

- [1] E. Carrillo, S. Aldás, M. Altamirano, F. Ayala, D. Cisneros, A. Endara, C. Márquez, M. Morales, F. Nogales, P. Salvador, M. Torres, J. Valencia, F. Villamarin, M. Yáñez, and P. Zarate, *Lista roja de los Reptiles del Ecuador*. Quito-Ecuador: Fundación Novun Milenium, 2005.
- [2] E. Jacobson, *Infectious Diseases and Pathology of Reptiles*, Pimera. Boca Ratón-USA, 2007.
- [3] N. Zamudio and M. Ramírez, "Presencia de Hepatozoon spp. en serpientes del Centro de Atención y Valoración de Fauna silvestre (CAV) del Área Metropolitana del Valle de Aburrá, Barbosa – Antioquia," *Rev. CES*, vol. 2, no. 2, p. 34, 2007.
- [4] G. S. Erzinger, *Parasites: Ecology, diseases and management*, Primera. Joinville-Brazil, 2013.
- [5] S. Telford, *Hemoparasites of the reptilian: color atlas and text*, First. Boca Ratón-USA, 2008.
- [6] A. Korzh and V. Zadorozhnyaya, "The biological characteristics of representatives of the genus Hepatozoon (Apicomplexa, Adeleorina) in the marsh frog (Pelophylax ridibundus) separate populations of Zaporizhzhya region," *Vestn. Zool.*, vol. 47, no. 2, pp. 34–39, 2013.
- [7] J. Balón and L. Placecio, "Detección del género Haemogregarina y Hepatozoon en las especies Iguana iguana en el Parque Histórico y en el Parque Seminario de la ciudad de Guayaquil," Universidad de Guayaquil, 2011.
- [8] M. del A. del Ecuador, "Parque Nacional Sumaco," 2016. [Online]. Available: <http://www.ambiente.gob.ec/parque-nacional-sumaco/>.
- [9] M. del A. del Ecuador, "Reserva Ecológica Cotacachi-Cayapas," 2016. [Online]. Available: <http://www.ambiente.gob.ec/reserva-ecologica-cotacachi-cayapas/>.
- [10] L. H. O'Dwyer, T. C. Moço, T. H. Barrella, F. C. Vilela, and R. J. Silva, "Prevalence of Hepatozoon spp. (Apicomplexa, Hepatozoidae) among recently captured Brazilian snakes," *Arch. Bras. Med. Veterinária e Zootec.*, vol. 55, no. 3, pp. 309–314, 2003.
- [11] E. Moreno and R. Bolaños, "Hemogregarinas en serpientes de Costa Rica," *Rev. Biol. Trop.*, vol. 25, no. 1, pp. 47–57, 1977.
- [12] B. Tomé, C. Rato, D. Harris, and A. Perera, "High Diversity of Hepatozoon spp. in Geckos of the Genus Tarentola," *J. Parasitol.*, vol. 102, no. 4, pp. 176–480, 2016.
- [13] H. Han, Y. Wu, H. Dong, S. Zhu, L. Li, Q. Zhao, D. Wu, E. Pei, Y. Wang, and B. Huang, "First report of Hepatozoon (Apicomplexa: Adeleorina) from king ratsnakes (Elaphe carinata) in Shanghai, with description of a new species," *Acta Parasitol.*, vol. 60, no. 2, pp. 266–274, 2015.
- [14] J. Harris, I. Damas-Moreira, J. Maia, and A. Perera, "First Report of Hepatozoon (Apicomplexa: Adeleorina) in Caecilians, with Description of a New Species," *J. Parasitol.*, vol. 100, no. 1, pp. 117–120, 2014.
- [15] V. Nasiria, I. Mobedic, A. Dalimib, A. Z. Mirakabadid, F. Ghaffarifarb, and H. P. Teymurzadehd, Shohreh Gholamreza Karimia, Amir Abdolib, "A description of parasites from Iranian snakes," *Exp. Parasitol.*, vol. 147, pp. 7–15, 2014.
- [16] M. A. Cortez Martinez, "Perfil hemático y presencia de hemoparásitos en reptiles del Parque Zoológico Nacional, El Salvador," Universidad de El Salvador, 2015.
- [17] M. Sloboda, M. Kamler, J. Bulantová, J. Votýpka, and D. Modrý, "A New Species of Hepatozoon (Apicomplexa: Adeleorina) from Python regius (Serpentes: Pythonidae) and Its Experimental Transmission by a Mosquito Vector," *J. Parasitol.*, vol. 93, no. 5, pp. 1189–1198, 2007.