

## ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

### PARASITIC NEMATODE INFECTION IN THE SOUTH AMERICAN RED-FOOTED TORTOISE *GEOCHELONE CARBONARIA* FROM UPATA, BOLIVAR, VENEZUELA

#### PARASITISMO POR NEMATODES EN LA TORTUGA SURAMERICANA *GEOCHELONE* *CARBONARIA* DE UPATA, BOLÍVAR, VENEZUELA

Arlett Pérez Mata<sup>1</sup>, Aixa Aguirre<sup>1</sup>, Jessica Quijada Piñango<sup>1</sup>, Jesmil Lecuna Olivares<sup>1</sup>, Angélica Bethencourt Camacho<sup>1</sup>,  
Nataly Bautista<sup>2</sup> & Otto Núñez<sup>3</sup>.

<sup>1</sup>Veterinary Parasitology Department, Faculty of Veterinary Sciences (FCV), Central University of Venezuela (UCV),  
Campus Maracay, Maracay 2101, Aragua, Venezuela

<sup>2</sup>Undergraduated student. FCV-UCV <sup>3</sup>Veterinary Surgeon, private practice.  
E-mail: arletperez@gmail.com; Tel:00-58-243-8710125.

Suggested citation Pérez Mata, A, Aguirre, A, Quijada Piñango, J, Jesmil Lecuna Olivares, J, Bethencourt Camacho, A, Bautista, N & Núñez, O. 2014. *Parasitic nematode infection in the South American Red-footed tortoise Geochelone carbonaria from Upata, Bolivar, Venezuela*. Neotropical Helminthology, vol. 8, n°2, jul-dec, pp. 251-257.

#### Abstract

*Geochelone carbonaria* is one of the two species of tortoises that have been described in Venezuela. We report herein a severe parasitic infection with strongylid and pinworm nematodes in 15 red-footed tortoises, *G. carbonaria* from Upata, State of Bolivar, Venezuela. Animals were obtained from the owner of a cattle ranch in the savannah and allocated in a restricted area. Two months later, animals became ill, displaying anorexia, lethargy and diarrhea. Faeces were fluid, mucoid and haemorrhagic. Five animals were found dead. At necropsy, caecum and colon were enlarged due to the presence of hundreds of small nematode parasites in their lumen; mucosal haemorrhage and fibrin tracts in the liver were also observed. Faecal samples and part of the intestinal content were analyzed in the laboratory. Faecal examination showed a severe strongylid and pinworm infection. Intestinal content was abundantly infected by pinworm nematodes identified, at genus level, as *Atractis* (Order Ascaridida: Cosmoceroidea), and *Thelandros* (Order Oxyurida: Pharyngodonidae). This represents the first report of both genera in *G. carbonaria* in Venezuela. Animals were orally treated with Albendazole (10 mg·kg<sup>-1</sup>), once a day for three days. Nematodes were expelled and health condition of the animals improved dramatically. Stress is a common cause of outbreak of parasitism in recently captured reptiles.

**Keywords:** albendazole - nematodes - tortoise - Venezuela.

## Resumen

*Geochelone carbonaria* es una de las dos especies de Tortugas terrestres que han sido descritas en Venezuela. Aquí reportamos un caso de infección parasitaria severa con nemátodos strongilidos y oxyuridos en 15 tortugas de la localidad de Upata, Estado Bolívar, Venezuela. Los animales fueron capturados en la sabana por el propietario de una finca ganadera en la zona y encerrados en un área restringida con cerca. Dos meses más tarde, los animales se mostraron enfermos, presentando anorexia, letargia y diarrea. Las heces eran fluidas, mucoides y hemorrágicas. Cinco animales fueron encontrados muertos. A la necropsia se observó un aumento de tamaño del colon y ciego con presencia de cientos de pequeños nematodos en la luz de estos órganos. Muestras fecales y parte del contenido intestinal fueron enviados y analizados en el laboratorio. El examen coprológico demostró infección parasitaria severa con gran cantidad de huevos strongilidos y oxyuridos. El contenido intestinal presentó abundante cantidad de nematodos, los cuales fueron identificados a nivel de género como *Atractis* (Orden Ascaridida: Cosmocercoidea), and *Thelandros* (Orden Oxyurida: Pharyngodonidae). Este representa el primer reporte de ambos géneros para *G. carbonaria* en Venezuela. Los animales enfermos fueron tratados con Albendazol vía oral (10 mg·kg<sup>-1</sup>), una vez al día por tres días. Grandes cantidades de nematodos fueron expulsados y la condición de los animales mejoró drásticamente. El estrés es una causa común de brotes de enfermedad parasitaria en reptiles recientemente capturados.

**Palabras clave:** albendazole- nematodos - tortugas - Venezuela.

## INTRODUCTION

*Geochelone* (*syn. Chelonoidis*) *carbonaria* Spix, 1824 (Testudines: Testudinidae) is one of the two species of tortoises that have been reported in Venezuela, where is commonly named “Morrocoy”. This tortoise inhabits a wide range of habitats in Venezuelan territory, with preference to the lowlands, open savannahs and gallery forest (Fernández, 1991). There are no reports of its presence in the Andes. The other species, *G. denticulata*, has a more restricted distribution, and its presence has been reported only in Amazonas and Bolivar States, preferring tropical forest habitats. Some of the zoological characteristic features of *G. carbonaria* include: Carapace relatively flat top, typically black, interrupted only by a small yellow area around the areola of each scute. The limbs are black with bright red tips on many of the scales, contrasting sharply with the yellow head markings (Pritchard & Trebbau, 1985). *G. carbonaria* is a very popular pet and its meat has also been used in several regions of Venezuela to cook local dishes. According with the CBC (2007), *G. carbonaria* is an endangered species. Terrestrial

tortoises, as *G. carbonaria* can be infected by several species of nematodes, but their pathogenicity in wild animals is controversial, even though parasitism is a real concern in captive animals (Mader, 1996; Messonnier, 1996).

Oxyurids, ascarids and strongylid nematodes are the most common nematodes in tortoises and wild specimens usually can carry these parasites, especially pinworms (oxyurids). In Spain sixteen oxyurid species were found in *Testudo graeca* Linnaeus 1758, including *Tachygonetria* and *Pharyngodon* genus (Chávarri *et al.*, 2012). *Atractis marquezii* was described from *Geochelone nigrita* Quoy & Gaimard, 1824 by Bursay & Flanngan (2002), and Rideout *et al.* (1987) reported mortality in *G. carbonaria* and *G. pardalis* from an US zoo, due to Oxyurid nematodes (*Protractis* genus). In Venezuela, the nematode species *Chapiniella larensis* (different from *Chapiniella variabilis* Chapin, 1924), and two nematodes of Kathlanidae: *Labiduris zschokkei* Linstow, 1899 and *Labiduris gulosa* (Rudolphi, 1819) Schneider, 1866 were described by Díaz-Ungría & Gallardo

(1967) and Díaz-Ungría (1973) in *Testudo* (sic) *denticulata*.

The aim of this report was to describe a severe parasitic infection with pinworms (Oxyurids), ascarids (Atractidae) and strongylid nematodes in 15 wild *G. carbonaria* specimens from Upata, Bolivar, Venezuela, and to establish the first report for *Atractis*, and *Thelandros* genus in Venezuelan *G. carbonaria*.

## MATERIAL AND METHODS

Fifteen adult specimens of *G. carbonaria* (nine females and six males) with carapace length ranging between 23 and 34 cm (media 26.91 cm, S: 3.25 cm) were captured for local people in the savannah near a cattle ranch (ordered by the owner of that ranch) located in Upata, Bolivar, Venezuela and allocated in a little yard confined with a fence. Faeces and food wastes were not frequently removed from that allocation. Two months later, animals became ill, showing initially anorexia and lethargy, and later mucoid diarrhea. No treatment was applied and five tortoises (three males, two females) were found dead three weeks later. A local veterinarian was called and took samples from faeces and

performs necropsies of the dead animals. Necropsy showed hundreds of tiny parasites into the intestinal content of caecum and colon, mucoid diarrhoea, haemorrhagy of the mucosal surface and the liver of one of the animals was slightly pale.

Fecal samples from dead and alive animals and part of the intestinal content was sent to the Helminthology Laboratory, at the Faculty of Veterinary Sciences, Central University of Venezuela (FCV-UCV, Maracay, Aragua, located 900 Km to the west of the farm). Faecal samples were examined using direct smears and fecal flotation techniques with a sugar solution. Intestinal content was diluted 50:50 with 70% alcohol and examined under a stereomicroscope (Karl Zeiss Stemi 2000®). All nematodes recovered were first stored at 70% alcohol and then examined without clarification and/or thereafter clarified with lactophenol and observed under binocular microscope (Nikon Eclipse E200®). Microphotographs of specimens were made with a digital camera (Canon Cibershot®) attached to the microscope (Nikon Eclipse E200®) using 10x, 25x and 40x objectives. Parasites were identified using proper keys (Yamaguti, 1961; Chabaud, 1978). After identification nematodes were deposited in the parasitological collection of CIPV-MARA

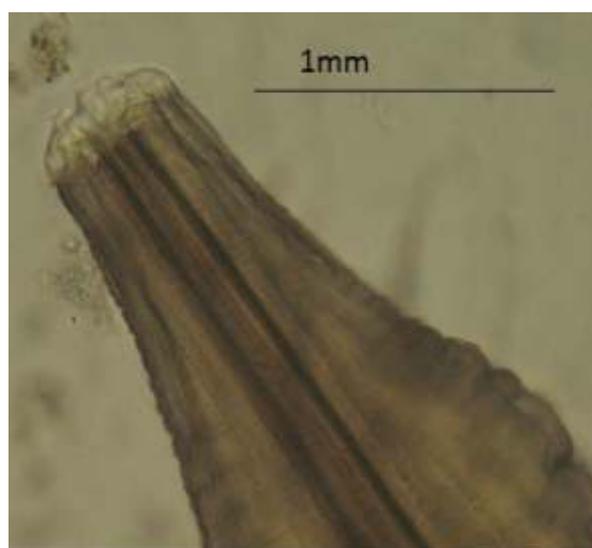


Figura 1. *Atractis* sp., Head. 250X.

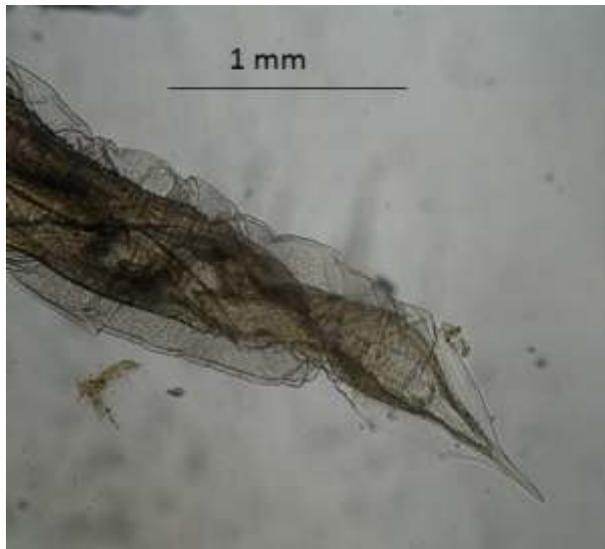


Figura 2. *Thelandros* sp. 125X.

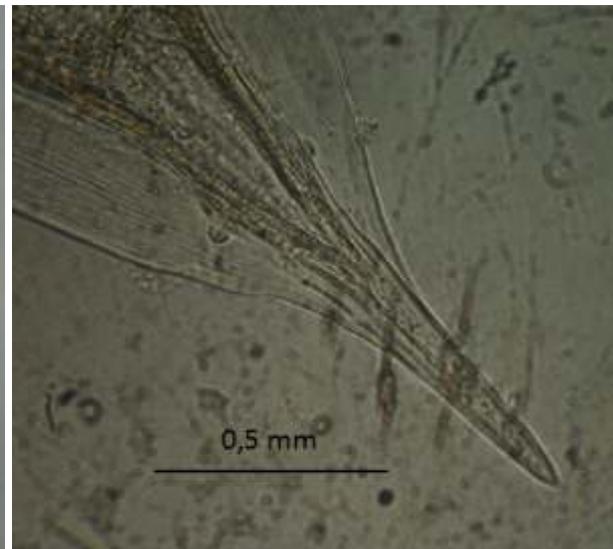


Figura 3. *Thelandros* sp. detail of Tail, 400X.

FCV-UCV under n° CIPVMARA-N8900100-2013 (*Atractis*), and CIPVMARA-N9000101-2013 (*Thelandros*), Sauricolinae specimen were too damaged to be conserved.

## RESULTS

Two strongylid nematodes (only male) recovered from faecal samples were identified at subfamily level as Sauricolinae (Nematoda: Strongylidae). This identification was based on: the presence of cuticular striae widely spaced, slender spicules, and the host. Nevertheless, specimens were in bad condition and it was not possible to establish neither genus nor species.

Another group of nematodes were identified at genus level as *Atractis* (Order Ascaridida, Superfamily Cosmoceroidea) and presenting the following characteristic features: esophagus

composed of a cylindrical corpus, anteriorly differentiated distinct pharyngeal part, a subspherical or elongated isthmus and a valved bulb (Figure 1). Male with a coiled tail, constriction after anus, forming a large conical process without caudal alae. Female with vulva apart from anus, pointed tail. Ten Oxyurid nematodes were identified as genus *Thelandros*. This identification was based on: the presence of the genital cone as well as the quitinized structure V-shaped at the posterior end (Figures 2, 3).

While parasitological procedures were performed, animals were treated orally by the local veterinarian with a bovine formulation of albendazole (Valbazen®, 10 mg·Kg<sup>-1</sup> once a day for three days). All animals successfully recovered and start to feed after treatment, and diarrhea stopped. Hundreds of nematodes were expelled after treatment.

## DISCUSSION

Several species of oxyurid, and cosmocercoid nematodes (formerly included in Oxyurida) have been described from turtles, most of them described in *T. graeca* and other species of *Testudo* (Buckley, 1970; Johnson, 1973; Bursay & Flannagan, 2002; Traversa *et al.*, 2005; Chávarry *et al.*, 2012). There are two genus for oxyurid nematodes found in *Podocnemys* (a freshwater turtle genus) from Colombia (Gibbons *et al.*, 1995). None of the nematodes we found in this report fulfill the characteristics of the description of nematodes formerly described in *G. denticulata* from Venezuela by Díaz-Ungría & Gallardo (1968) and Díaz-Ungría (1973). Unfortunately, the two strongylid nematodes that were recovered from faeces were not well preserved during transportation and therefore, it was impossible to establish the genus. Nevertheless, it is important to highlight that this is the first report of clinical illness in *G. carbonaria* due to oxyurid nematode infection and the first record of *Thelandros* and *Atractis* genera, infecting *G. carbonaria* in our country. The pathogenicity of these helminths in tortoises is unclear, and most of the authors suggest that animals might carry great amount of helminths without showing any clinical sign (Frye, 1973; Holt *et al.*, 1979; Mader, 1996). In contrast, there is a report of mortality due to a heavy infection with *Atractis* sp nematodes (Rideout *et al.*, 1987). We believe that although tortoises can carry a high burden of nematodes without displaying clinical signs when living in nature, the situation might change in captivity, and factors such as: changes in feeding habits, crowding effect, wrong allocation and stress of captivity; probably might exert an important impact on the composition and abundance of helminth fauna in tortoises, as have been discussed by other authors (Rideout *et al.*, 1987; Rataj *et al.*, 2011; Chávarry *et al.*, 2012).

Although no anthelmintic have been formulated for tortoises and studies on their efficacy or toxicity are limited (Claussen & Forstner, 1981; Teare & Bush, 1983; Gianetto *et al.*, 2007), and most reports of benzimidazole drugs used in

reptiles refers mainly to Oxfendazole and Fenbendazole (Frye, 1973, Gianetto *et al.*, 2007) and in a lesser extent, Albendazole as Valbazen® at the dosage of 50mg/kg PO, once (Mader, 1996), we reported herein a good efficacy for the bovine presentation of Albendazole at the lower dosage of 10mg/kg repeated for three days to treat this kind of multiple helminthic infection in tortoise. Because the use of ivermectin is not recommended in turtles (Teare & Bush, 1983), it is important to remark the successful treatment of the animals with Albendazole at this lower dose. Authors found several limitations on the development of this work. First of all, this is a clinical case produced 800 km away from university. Authors were not implied in capture and storage of animals. The Veterinarian (Otto Núñez, coauthor) who served as contact with parasitology laboratory did not know the conditions under which animals were captured. He worked in extreme conditions far away from any pharmacy or drugstore, and made the necropsies on the field under very rough conditions.

Treatment of the animals with albendazole did not represent an injury for the tortoise and was an emergency treatment which ends successfully. Regarding with the identification of nematodes we found, taxonomical keys for identification of tortoise oxyurids at species level were not available. So, we could only establish the genera of specimens. Nevertheless is remarkable that before the present work there had not been in our country any report of these genera in Venezuelan tortoises exception made for those species previously mentioned by Díaz-Ungría & Gallardo (1968) and Díaz-Ungría (1973). So, even the establishment of genera is especially important for Venezuelan records. With all limitations this study represented a unique opportunity to study the helminth fauna of this endangered species of tortoise in Venezuela. As a final conclusions we can say that Venezuelan tortoise *G. carbonaria* can be naturally parasitized for *Thelandros* sp and *Atractis* sp, and Albendazole could be a good choice for treat this helminth infection on this host. Additional studies, including the use of molecular tools are needed to identify nematode founds at species level.

## ACKNOWLEDGEMENTS

Authors are fully grateful to Héctor Zerpa (Physiology Department, FCV-UCV), for revision of English manuscript and Gisela García (Histology Dept, FCV-UCV) for her gently aid with microphotographies. Conflict of interest: Authors declare we have no interest conflict for this paper.

## REFERENCIAS BIBLIOGRÁFICAS

- Buckley, J. 1970. *Two remarkable nematodes from a South American tortoise*. Transactions of Royal Society of Tropical Medicine and Hygiene, vol. 64, pp. 25.
- Bursary, C & Flannagan, J. 2002. *Atractis marquezii n.sp (Nematoda: Atractidae) and a revision of Atractis Dujardin, 1845, sensu Baker, 1987*. The Journal of Parasitology, vol. 88, pp. 320-324.
- Chávarri, M, Berriatua, E, Giménez, A, Gracia, E, Martínez-Carrasco, C, Ortiz, J & De Ybáñez, R. 2012. *Differences in helminth infections between captive and wild spurthighed tortoises Testudo graeca in southern Spain: a potencial risk of reintroduccion of this species*. Veterinary Parasitology, vol. 187, pp. 491-497.
- Claussen, J & Forstner, M. 1981. *Helminths of tortoises and deworming trials with drugs*. Berl Munch Tierarztl Wochenscher, vol. 94, pp. 411-414.
- Chabaud, AC. 1978 *Identification of Genera of the superfamilies Cosmocercoidea, Seuratoidea, Heterakoidea y Subuluroidea*. Anderson, R, Chabaud, A & Willmott, S. (Ed.) 1978. CIH keys to the nematodes of vertebrates, (n°6). CAB Int., Royal Bucks, England. 37p.
- Díaz-Ungria, C & Gallardo, MF. 1968. *Nematodes de reptiles Venezolanos, con descripción de varias especies nuevas*. Boletín de la Sociedad Venezolana de Ciencias Naturales, vol. 27, pp. 550-570.
- Díaz-Ungria, C. 1973. *Helminthos endoparásitos de Venezuela*. Ed. Centro Experimental de Estudios Superiores, Escuela de Ciencias Veterinarias, 126 p.
- Fernández, M. 1991. *Apuntes y observaciones sobre morrocayos venezolanos*. Manuscrito no publicado consultado en zoológico de Caricuao, Caracas, Distrito capital, Venezuela.
- Frye, F. 1973. *Husbandry, Medicine and Surgery of captive reptiles*. V M Publish., USA. 298 p.
- Catálogo de la Biodiversidad colombiana (CBC). 2007. *Geochelone carbonaria* S p i x , 1 8 2 4 . I n : <http://www.siac.net.co/sib/catalogoespecies/especie.do?idBuscar=457&method=displayAAT> Chamorro-Rengifo, J & Cubillos-Rodríguez, PA. Sistema de Información sobre Biodiversidad de Colombia SiB F. Actualizaciï½n: 25062007. Consulta: enero 2013.
- Giannetto, S, Brianti, E, Poglayen, G, Sorgi, C, Capelli, G, Pennisi, M & Coci, G. 2007. *Efficacy of oxfendazole and fenbendazole against tortoise (Testudo hermanni)*. Parasitology Research, vol. 100, pp. 1069-1073.
- Gibbons, L, Khalil, L & MarinKelle, C. 1995. *A new nematode genus Podocnematractis for Atractis orteplepi Thapar, 1925. (Cosmocercoidea: Atractidae) and the description of another new species, P. colombianaensis from turtles Podocnemis spp. in Colombia*. Systematic Parasitology, vol. 30, pp.47-56.
- Holt, P, Cooper, J & Needham, R. 1979. *Diseases of tortoises: a survey of seventy cases*. J Small Anim Pract, vol. 20, pp. 269-86.
- Johnson, S. 1973. *Some oxyurid nematodes of the genera Mehdiella and Thapararia from the tortoise Testudo hermanni*. Folia Parasitologica (Praha), vol.20, pp.141-148.
- Mader, D. 1996. *Reptile Medicine / Surgery*. W.B. Saunders Co., Phil, USA, 512 p.
- Messonier, S. 1996. *Common reptile diseases and treatment*. Blackwell Science Ed. USA. 178 p.
- Pritchard, R & Trebbau, P. 1985. *Geochelone (Chelonoidis) carbonaria*. In: *Turtles of Venezuela*. Ohio Society for the Study of

- Amphibians and Reptiles. pp. 207-229.
- Rataj AV, Lindtner-Knific R, Vlahović K, Mavri U & Dovč A. 2011. *Parasites in pet reptiles*. Acta Veterinaria Scandinavica, vol. 30, pp. 33.
- Rideout, B, Montall, J, Phillips, L & Gardner, C. 1987. *Mortality of captive tortoises due to viviparous nematodes of the genus Protractis (Family Atractidae)*. Journal of Wildlife Disease, vol. 23, pp.103-108.
- Teare, J & Bush, M. 1983. *Toxicity and efficacy of ivermectin in chelonians*. Journal of the American Veterinary Medical Association, vol. 183, pp. 1195-1197.
- Traversa, D, Capelli, G, Iorio, R, Bouamer, S, Cameli, A & Giangaspero, A. 2005. *Epidemiology and biology of nematodofauna affecting Testudo hermanni, Testudo graeca and Testudo marginata in Italy*. Parasitology Research, vol. 98, pp. 14-20.
- Yamaguty, S. 1961. *Sistema Helminthum*, Vol III. *The nematodes of vertebrates*. Interscience Publishers, INC., NY, USA. pp. 679.

Received November 13, 2013.  
Accepted June 6, 2014.