Rev. Ciencias Veterinarias, Vol. 34, N° 1, [51-54], ISSN: 2215-4507, enero-junio, 2016
DOI: http://dx.doi.org/10.15359/rcv.34-1.4
URL: http://www.revistas.una.ac.cr/index.php/veterinaria/index

Revista de Ciencias Veterinarias

First Report of *Hepatozoon procyonis* in Raccoons from Costa Rica

Primer Reporte de *Hepatozoon procyonis* en Mapaches de Costa Rica

Ana Meneses[1], Gilbert Alvarado[2], María Runnebaum[3], Marcela Herrera[4], Gustavo Gutiérrez-Espeleta[5], Andrea Chaves[6]✉

[1] Clinical Analysis Laboratory, School of Veterinary Medicine, Universidad Nacional, Benjamín Núñez Campus, Heredia, Costa Rica; anag.meneses@gmail.com
[2] Department of Pathology, School of Veterinary Medicine, Universidad Nacional, Benjamín Núñez Campus, Heredia, Costa Rica; Microscopic Structures Research Center (*Centro de Investigación en Estructuras Microscópicas*), Universidad de Costa Rica, Ciudad de la Investigación, San José, Costa Rica; School of Biology, Universidad de Costa Rica, Rodrigo Facio Campus, San José, Costa Rica; gilbert.alba@gmail.com
[3] School of Biology, Universidad de Costa Rica, Rodrigo Facio Campus, San José, Costa Rica; mariaruj@gmail.com
[4] School of Biology, Universidad de Costa Rica, Rodrigo Facio Campus, San José, Costa Rica; marce.sarrias@gmail.com
[5] School of Biology, Universidad de Costa Rica, Rodrigo Facio Campus, San José, Costa Rica; gustavo.gutierrez@ucr.ac.cr
[6] School of Biology, Universidad de Costa Rica, Rodrigo Facio Campus, San José, Costa Rica; andreachaves.biol@gmail.com

Received: 11 November 2015. **Corrected:** 2 March 2016. **Accepted:** 10 March 2016.

Abstract: This study reports for the first time the detection of *Hepatozoon procyonis* in raccoons, *Procyon lotor* from Costa Rica. Blood smears of naturally infected raccoons were taken to characterize the gametocytes. Gametocyts consistent with *Hepatozoon procyonis* were observed in the cytoplasm of neutrophils, and their morphology, which is similar to the one reported in raccoons throughout the Americas, is described.

Keywords: Apicomplexan, Central American, Costa Rica, *Hepatozoon procyonis*, *Procyon lotor*.

Resumen: El estudio muestra, por primera vez, la detección de *Hepatozoon procyonis* en prociónidos, *Procyon lotor* de Costa Rica. Para caracterizar los gametocitos, se tomó muestras de sangre de mapaches infectados naturalmente. Los gametocitos concuerdan con *Hepatozoon procyonis*, los cuales fueron observados en neutrófilos del citoplasma, resultando su morfología similar a la reportada en mapaches de todas las Américas.

Palabras clave: Apicomplexa, Centroamérica, Costa Rica, *Hepatozoon procyonis, Procyon lotor.*

✉ * Corresponding author: andreachaves.biol@gmail.com

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Introduction

The genus Hepatozoon consists of apicomplexan blood parasites that have been described in a wide range of mammals and other vertebrates (Smith 1996). Hepatozoon in procyonids has been reported in several areas of the Americas. The genus was reported for the first time in North American raccoons, *Procyon lotor,* describing the new species *Hepatozoon procyonis* in Georgia, United States (Richards 1961). Other reports have included *P. lotor* from the United States (Clark *et al.* 1973; Schaffer *et al.* 1978; Hanion *et al.* 1989). Additionally, *H. Procyonis* in *Procyonis cancrivorous* was reported in Panama (Schneider 1968) and Brazil (Massard & Massard 1978; Rodrigues *et al.* 2006).

Raccoons are widely distributed in the Americas. The species *P. lotor* and *P. cancrivorus* are both found in Costa Rica. *P. lotor* occurs from southern Canada to Panama, while *P. cancrivorus* is found from Costa Rica and Panama to as far as Brazil (Nowak and Paradise 1983). Although there have been no studies on the subject, large groups of raccoons (*P. lotor*) are increasingly reported in the metropolitan areas of Costa Rica.

The objective of this study is to provide an initial report of *H. procyonis* in raccoons (*P. lotor*) of Costa Rica.

Materials and Methods

The twelve raccoons analyzed in this survey were captured in an urban forest, *Reserva Biológica Leonel Oviedo* at the Rodrigo Facio Campus (Universidad de Costa Rica), San Pedro de Montes de Oca - San José (9° 56' 15.8994" N, 84° 2' 55.572" W), with a live animal cage trap (Havahart®, Woodstream Corporation, 69 N. Locust St. Lititz, PA. 17543, USA) baited with fruits. Trapping was conducted during the first semester of 2010 (March to June). The captured animals were anesthetized with intramuscular Tiletamina-Zolazepam (Zoletil 50®, Virbac Laboratories, S.A. 06511 Carros, France), using doses of 10mg/kg of body weight. A blood sample was taken with EDTA as anticoagulant, kept at 4°C for 4 days, until it was transferred to the Clinical Analysis Laboratory at the School of Veterinary Medicine of Universidad Nacional, Heredia, Costa Rica. All subjects captured were monitored and released after recovering from the effects of the anesthesia.

Of the 12 samples analyzed, the gametocytes from the blood smears of naturally infected raccoons were determined in two individuals. The blood smears were air-dried and stained with May Grünwald-Giemsa. Gametocytes were observed using a light microscope with an ocular lens with a magnification of ×1,000 (Olympus model BH-2, Olympus optical Co, LTD, Tokio, Japan).

Results and Discussion

According to Richards (1961), regarding the morphology of gametocytes, they are in a capsule with a curved extremity, typical of the species, and show a small structure at the tip of the extremity, which is stained red with May Grünwald Giemsa. The gametocytes

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that Richards (1961) reported in *P. lotor* had the nucleus in the center of the cell. Similar morphology was found in *P. lotor* in Costa Rica (Figure1) in two of 12 subjects analyzed. The nuclei cannot be observed in the photograph, as the samples were not fresh after being kept for four days at 4°C, in addition to the parasite being very labile.

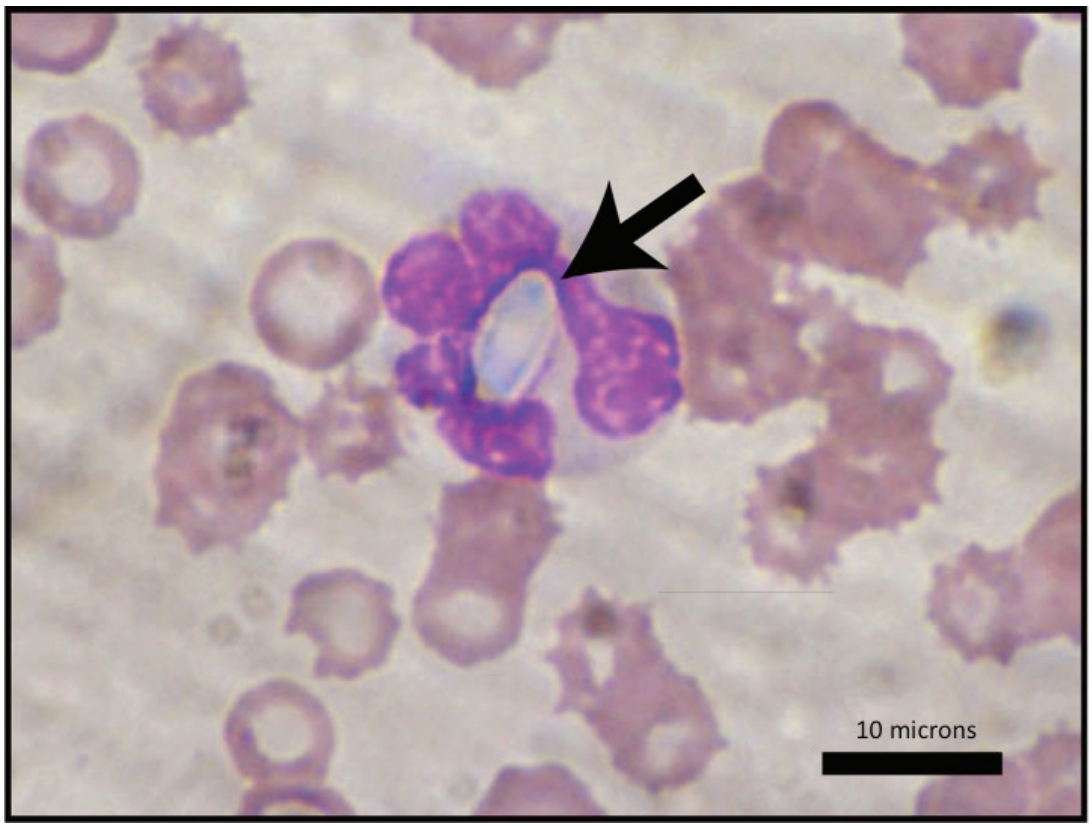

Figure 1. Morphology of gametocyte *Hepatozoon procyonis* observed in *Procyon lotor* from Costa Rica.

The gametocyte observed in *P. lotor* was infecting a segmented neutrophils, in accordance to what was reported by Rodrigues *et al.* (2006), as observed in *P. cancrivorus* and *N. nasua,* with the parasite infecting the neutrophils and rarely in monocytes. On the contrary, in the cases of Richards (1961) and Schneider (1968), the gametocytes of *H. procyonis* were reported to infect monocytes, and in the case of Clark *et al.* (1973) the gametocyte infected monocytes and rarely neutrophils.

The hepatozoon observed in our study is consistent with the hepatozoon species reported in Panama (Schneider 1968), in the United States (Richards 1961; Clark *et al.* 1973; Schaffer *et al.* 1978 and Hanion *et al.* 1989) and in Brazil (Massard & Massard 1978; Rodrigues *et al.* 2006). We recommend the use of molecular diagnostics to corroborate results whenever possible for future investigations.

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Acknowledgments

We are grateful to all the contributors who assisted us in sampling. This research was funded by the University of Costa Rica and the School of Veterinary Medicine of the National University of Costa Rica.

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