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Journal of Threatened Taxa

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www.threatenedtaxa.org

ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)

SHORT COMMUNICATION

HISTOPATHOLOGICAL FINDINGS OF INFECTIONS CAUSED BY CANINE DISTEMPER VIRUS, *TRYPANOSOMA CRUZI*, AND OTHER PARASITES IN TWO FREE-RANGING WHITE-NOSED COATIS *NASUA NARICA* (CARNIVORA: PROCYONIDAE) FROM COSTA RICA

Jorge Rojas-Jiménez, Juan A. Morales-Acuña, Milena Argüello-Sáenz, Silvia E. Aceve-do-González, Michael J. Yabsley & Andrea Urbina-Villalobos

26 January 2021 | Vol. 13 | No. 1 | Pages: 17521–17528
DOI: 10.11609/jott.5907.13.1.17521-17528

Journal of Threatened Taxa

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Journal of Threatened Taxa | www.threatenedtaxa.org | 26 January 2021 | 13(1): 17521–17528
ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)
https://doi.org/10.11609/jott.5907.13.1.17521-17528
#5907 | Received 25 March 2020 | Final received 27 November 2020 | Finally accepted 18 January 2021
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Histopathological findings of infections caused by canine distemper virus, *Trypanosoma cruzi*, and other parasites in two free-ranging White-nosed Coatis *Nasua narica* (Carnivora: Procyonidae) from Costa Rica

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Abstract: Canine distemper virus (CDV) causes systemic infections and immunosuppression in carnivores, which subsequently makes animals highly susceptible to opportunistic infections. Although *Trypanosoma cruzi* infects procyonids, chagasic myocarditis in Coatis has not been reported in Central America. The aim of this study was to report the histopathological findings caused by canine distemper virus, *T. cruzi*, and other parasites in two free-ranging White-nosed Coatis *Nasua narica* found dead in a national park on the Pacific coast of Costa Rica. Heart, lung, tongue, liver, brain and spleen samples were subjected to macroscopic and microscopic examination. A mononuclear meningoencephalitis associated with intra-nuclear eosinophilic inclusion bodies consistent with canine distemper virus was observed in nervous tissue. Myocarditis and associated nests of amastigotes of *T. cruzi* were observed during microscopic examination in cardiac tissue, and in muscle from the tongue of both animals. Molecular analysis confirmed *T. cruzi* in formalin-fixed paraffin embedded cardiac tissues. The myocardial damage caused by the opportunistic infection due to *T. cruzi* in these individuals could be the result of a severe compromised immunological status associated to the CDV infection, and subsequent opportunistic polyparasitism described herein. To the authors knowledge this is the first report of chagasic myocarditis in free-ranging coatis from Central America.

Keywords: CDV, myocarditis, *Nasua narica*, PCR, polyparasitism, *Trypanosoma cruzi.*

Editor: Carolyn L. Hodo, University of Texas MD Anderson Cancer Center, Texas, USA. **Date of publication:** 26 January 2021 (online & print)

Citation: Rojas-Jiménez, J., J.A. Morales-Acuña, M. Argüello-Sáenz, S.E. Acevedo-González, M.J. Yabsley & A. Urbina-Villalobos (2021). Histopathological findings of infections caused by canine distemper virus, *Trypanosoma cruzi*, and other parasites in two free-ranging White-nosed Coatis *Nasua narica* (Carnivora: Procyonidae) from Costa Rica. *Journal of Threatened Taxa* 13(1): 17521–17528. https://doi.org/10.11609/jott.5907.13.1.17521-17528

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Funding: This project was partially funded by Project ID#0494-17 from the Diagnóstico de Micosis Veterinarias y de Enfermedades Zoonoóticas Parasitarias, Escuela de Medicina Veterinaria, Universidad Nacional de Costa Rica, Heredia, Costa Rica.

Competing interests: The authors declare no competing interests.

Ethical approval: The procedure was performed under the approval of the National Environment Ministry (MINAE), and the National System of Conservation Areas (SINAC), Área de Conservación Pacífico Central (ACOPAC) (No permits found due to poor records). No animals were specifically harmed for the purpose of this study but were part of a mortality investigation; in addition, the necropsy protocols were performed at the Pathology Service of the School of Veterinary Medicine of the National University of Heredia, Costa Rica, (EMV-UNA), within the cases identification ND 107-2010 (female) and ND 112-2010 (male), respectively.

Acknowledgements: We thank Dr. Fernando Riera, (DVM, Costa Ballena Veterinary, Uvita, Puntarenas) for submitting the individuals to the Pathology Service of the School of Veterinary Medicine of the National University of Heredia, Costa Rica, (EMV-UNA) for evaluation.

In Latin America, *Nasua* spp. host for several zoonotic parasites including *Trypanosoma cruzi*, and viruses such as canine distemper virus (Chinchilla 1966; Herrera 2010; Santoro et al. 2016; Duarte Moraes et al. 2017; Michelazzo et al. 2020). In Costa Rica, the White-nosed Coati *Nasua narica* is a procyonid that is widely distributed in protected areas and is a frequent inhabitant of semi-urban or peridomiciliary zones (Wainwright 2007; Cuarón et al. 2016). An epidemic of sudden death in coatis with previous neurological signs occurred in a conservation area in the southern Pacific coast of Costa Rica in 2010. This report provides details on the diagnostic investigation of two individuals.

In 2010, a troop of 15–20 White-nosed Coatis from the Bahia Ballena Marine National Park (BBMNP) in Uvita, Dominical, Puntarenas (9.157N, -83.746W) presented with neurological signs and incoordination followed by sudden death. Due to decomposition and limited logistics, only two adult coatis (one male and one female) were collected and transported to a local small animal veterinary clinic by local authorities from the National System of Conservation Areas, Costa Rica (SINAC). In addition, the outbreak occurred only once and no more events of this type have been reported subsequently in the region (Dr. Fernando Riera pers. comm. January 2020). The veterinarian in charge submitted the carcasses to the Pathology Service of the School of Veterinary Medicine of the National University of Heredia, Costa Rica, (EMV-UNA) for necropsy and diagnostic evaluation. Selected tissues from heart, lung, tongue, liver, brain, and spleen were routinely processed, embedded in paraffin, and 4–5 mm thick sections were stained with hematoxylin and eosin (H & E), toluidine blue or Giemsa for light microscopy.

For detection of *T. cruzi* DNA, a conventional PCR using formalin-fixed paraffin-embedded (FFPE) tissues was performed on selected tissues from heart, lung, tongue, liver, brain and spleen. About 25mg sections were cut from each block and placed in a 1.5ml sterile microcentrifuge tube. The microtome and blade were cleaned with xylene between each block. The sections were de-waxed by cutting off paraffin edges and then mixed with 500µl xylene for eight minutes followed by washing with 95% ethanol and with 70% ethanol for five minutes each. Pellets were dried at 80°C for eight minutes on a heat block. As positive controls, FFPE cardiac tissue sections of mice experimentally infected with *T. cruzi* were used. The *T. cruzi* strain used in the mice experimentally infected corresponded to *T. cruzi* I (TcI) and was isolated from a naturally infected dog from Costa Rica (Mena-Marín et al. 2012). FFPE cardiac tissue sections of healthy mice were used as negative controls. Total DNA extraction from the paraffin material was performed using a commercial kit (QIAmp DNA Mini Kit ® QIAGEN). The primers used for PCR were S35 (5'AAATAATGTACGGGTGGAGATGCATGA-3') and S36 (5'-GGGTTCGATTGGGGTTGGTGT-3') (Ferrer 2015). This set of primers amplifies a 330bp fragment derived from the variable region of *T. cruzi* minicircles (Ferrer 2015). Although this set of primers amplifies the majority of strains of *T. cruzi,* and it is considered specific to *T. cruzi*, two fragments of *Trypanosoma rangeli* (300bp and 450bp) can be also amplified with this set (Vallejo et al. 1999). This is, however, a non-pathogenic parasite and it is not observed in mammalian tissue (Vallejo et al. 2015). PCR reaction conditions were carried out as previously described with the S35–S36 set with some modifications (Barrera et al. 2008). Briefly, PCR was carried out using a DNA thermal cycler (Gene Amp PCR System. Perkin Elmer), the amplification reaction was performed in a final volume of 20µl with 10µl of 2X Dream Taq™ PCR Master Mix (Thermo Fisher Scientific™, Whatman, MA), 4µl of nuclease free water (Thermo Fisher Scientific™ Whatman, MA), 1.5µl of 99.99% DMSO, 2µl of sample DNA, 1µl of each of the primers at 10µM and 0.5µl of bovine serum albumin (BSA) at a concentration of 20mg/ml, one cycle of initial denaturation for 5min at 95°C followed by 35 cycles of denaturation for 20sec at 95°C, alignment for 30sec at 63°C, elongation for 30sec at 72°C, and a final cycle of elongation for 10min at 72°C. The amplified DNA was visualized with 2% agarose gel using a UV transilluminator.

At gross examination, both coatis were in poor body condition, with dermatological lesions including alopecia, exudative dermatitis, abscesses, scabs, and pustules. Other significant findings were conjunctivitis and mass muscle atrophy of limbs. Both individuals had enlarged spleens. The female coati had several nematodes compatible with *Dirofilaria* sp. in the right ventricle and pulmonary artery (Image 1a). The lungs were congested, edematous, had multifocal hemorrhages, and a 0.5cm hemorrhagic nodule with eosinophilic infiltrate associated with the migration of immature trematodes compatible with *Paragonimus* spp. and microfilariae were in the right diaphragmatic lobule. Small numbers of cestodes, acanthocephalans and nematodes were found in the small intestine. In the male coati, the lungs were congested and edematous. Additionally, the male coati had a dilated esophagus and small numbers of acanthocephalans and nematodes were present.

Histologic evaluation revealed a mixed exudative

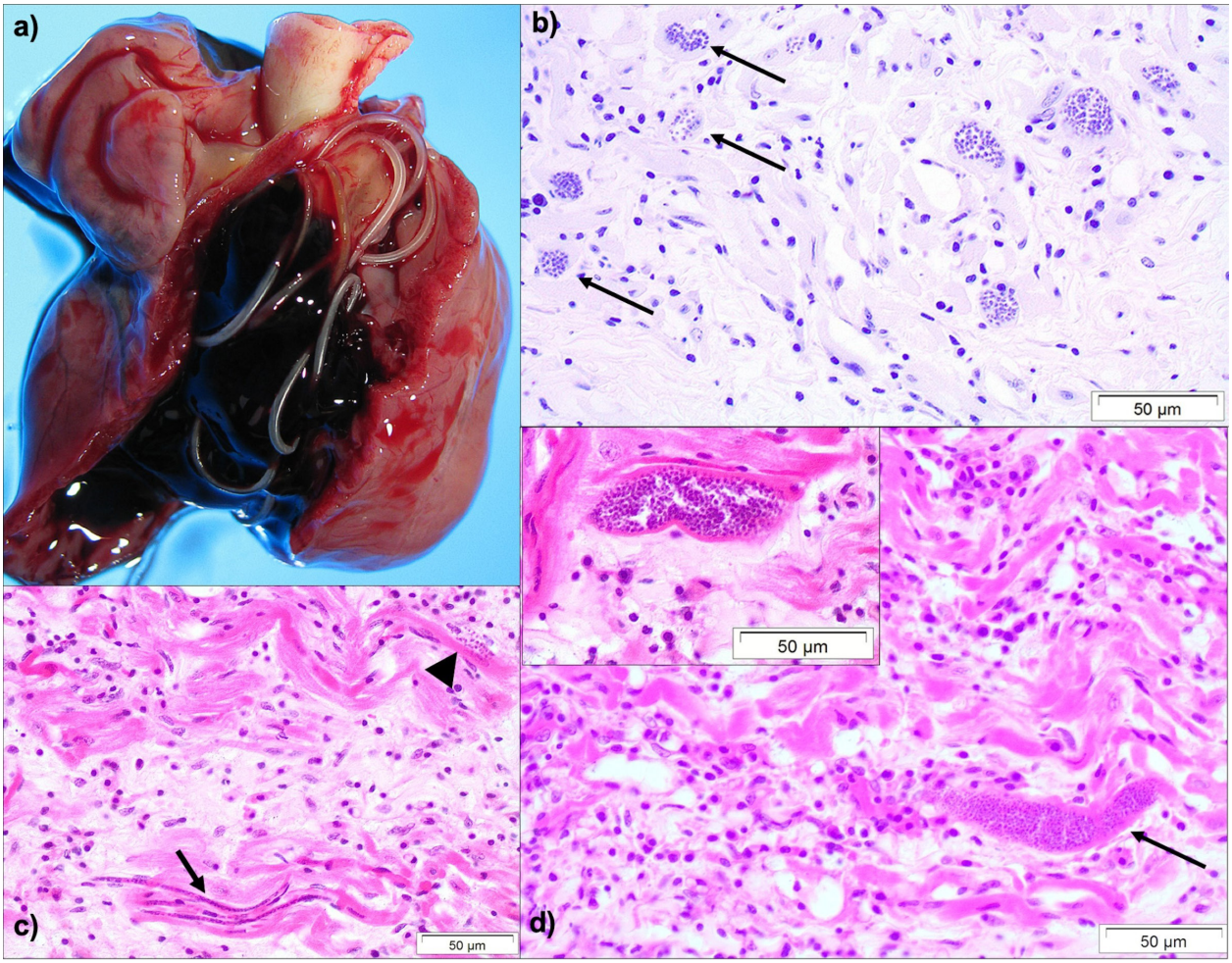

Image 1. a—Nematodes morphologically similar to *Dirofilaria* spp. in the heart of a White-nosed Coati *Nasua narica* | b—mononuclear inflammatory infiltrate associated with *Trypanosoma cruzi* amastigote nests in cardiac tissue (arrows). Giemsa stain. 40X | c—microfilaria (arrow) and mononuclear inflammatory infiltrate associated with *T. cruzi* amastigote nests in cardiac tissue (arrowhead). Hematoxylin-eosin stain. 40X | d—*T. cruzi* amastigote nest in cardiac tissue (arrow). Hematoxylin-eosin stain. 40X. © Morales-Acuña & Departamento de Patología, Escuela de Medicina Veterinaria, Universidad Nacional de Costa Rica.

dermatitis consistent with *Dermatophilus congolensis* and unidentified mites in both animals. In cardiac tissue of both coatis, a mild mononuclear infiltrate with lymphocytes, plasma cells, histiocytes, and few eosinophils were observed in both the myocardium and pericardium, from mainly the left ventricle and the atria. In addition, myocardial muscle fibers were edematous and exhibiting degeneration and necrosis. The inflammation was associated with multifocal amastigote nests in myocardial muscle fibers, and nematode larvae morphologically similar to *Dirofilaria* spp. (Images 1b, 1c, 1d). Moreover, cardiac tissue from both animals was positive for the expected 330bp amplicon product for *Trypanosoma cruzi*.

Both individuals had eosinophilic infiltrates in the lungs associated with the migration of immature trematodes compatible with *Paragonimus* sp. and microfilariae compatible with *Dirofilaria* sp. (Images 2a, 2b). In muscle tissue from the tongue of both coatis, an infiltrate with lymphocytic cells, macrophages and giant cells were observed in the muscle fibers. This inflammation was associated with protozoal cysts consistent with *Sarcocystis* sp. and with amastigote nests of *T. cruzi* (Image 2c). A multifocal mild infiltrate of neutrophils, eosinophils and macrophages was observed in the mucosa and submucosa of the ileum and colon. This inflammation was associated with acanthocephalans. In addition, unidentified adult nematodes consistent with spirurids were detected in the pancreatic ducts from both animals (Image 2d). In nervous tissue of both animals, the medulla oblongata and pons were edematous and congested, with gliosis, satellite glial cells, demyelination, and multifocal areas of encephalomalacia. Moreover, the meninges were

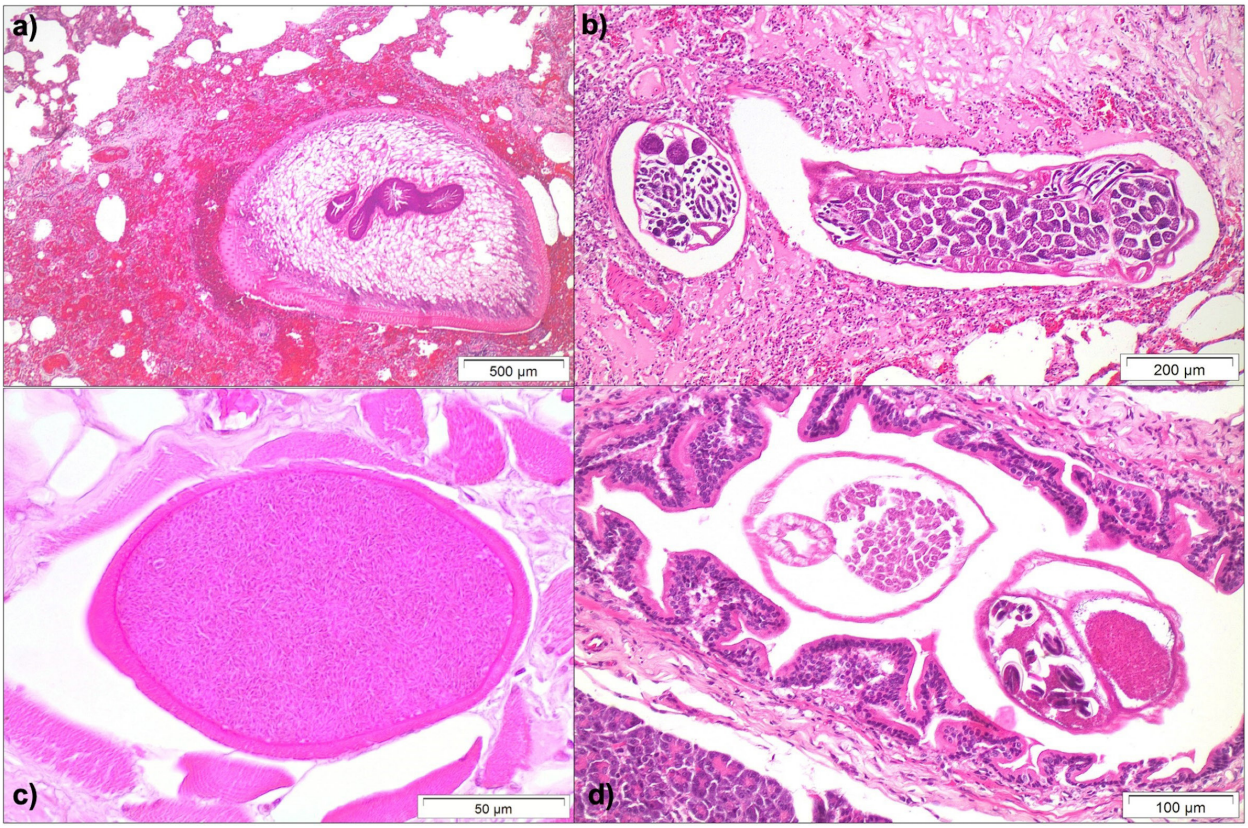

Image 2. a—Eosinophilic inflammatory infiltrate associated to *Paragonimus* spp. in lung tissue. Hematoxylin-eosin stain. 4X | b—unidentified nematodes in lung tissue (arrow). Hematoxylin-eosin stain. 10X | c—*Sarcocystis* sp. cyst in skeletal muscle tissue of the tongue. Hematoxylin-eosin stain. 60X | d—unidentified spirurid nematodes in excretory ducts in pancreatic tissue. Hematoxilin-eosin stain. 20X. © Morales-Acuña & Departamento de Patología, Escuela de Medicina Veterinaria, Universidad Nacional de Costa Rica.

edematous and congested, and a mild perivascular mononuclear infiltrate with neuronal necrosis, and syncytial cells containing intranuclear eosinophilic inclusions consistent with canine distemper virus (CDV) were observed in the hippocampus (Images 3a, 3b). Also, unidentified microfilaria were found in blood vessels of the cerebral cortex and nervous tissue.

White-nosed Coatis are widespread in Central America and Mexico and occur in parts of the southwestern United States. Their conservation status is Least Concern (Cuarón et al. 2016) and they do not have any protection in Costa Rica. They are distributed throughout the country including the mangrove and the beach of the BBMNP where this mortality event occurred (Wainwright 2007). This species is exposed to a great diversity of pathogens due to its diverse diet, long life expectancy, ability to disperse over long distances, and the use of both arboreal and terrestrial habits (de Lima et al. 2015; Alves et al. 2016). Moreover, living in social groups may increase the probability of transmission of parasites and other pathogens (Hass & Valenzuela 2002).

The interaction between several parasites and other infectious agents should be further investigated in order to explain the immune response in coatis. For example, the finding of mononuclear meningoencephalitis and intranuclear eosinophilic inclusions as seen in CDV deserves more study. CDV is one of the most important infectious agents of carnivores worldwide including procyonids (Deem et al. 2000). To date, the only published study on CDV in Costa Rican wildlife was on wild cats in the Osa Peninsula region which is located ~145km from the BBMNP (Avendaño et al. 2016). There are only a few previous published reports of CDV in coatis (Martinez-Gutierrez & Ruiz-Saenz 2016), and some studies have failed to detect CDV antibodies in wild or captive coatis (Furtado et al. 2016; Taques et al. 2018). The social nature of coatis likely makes them particularly at risk as this virus can quickly spread among different individuals belonging to the same troop, as has been reported in raccoons (Hass & Valenzuela 2002; Kapil & Yeary 2011; Rentería-Solís et al. 2014; Dr. Catao-Dias pers. comm. July 2019). In addition, due to the mass mortality event we suggest that CDV was the primary cause of death, with polyparasitism likely contributing to an impaired

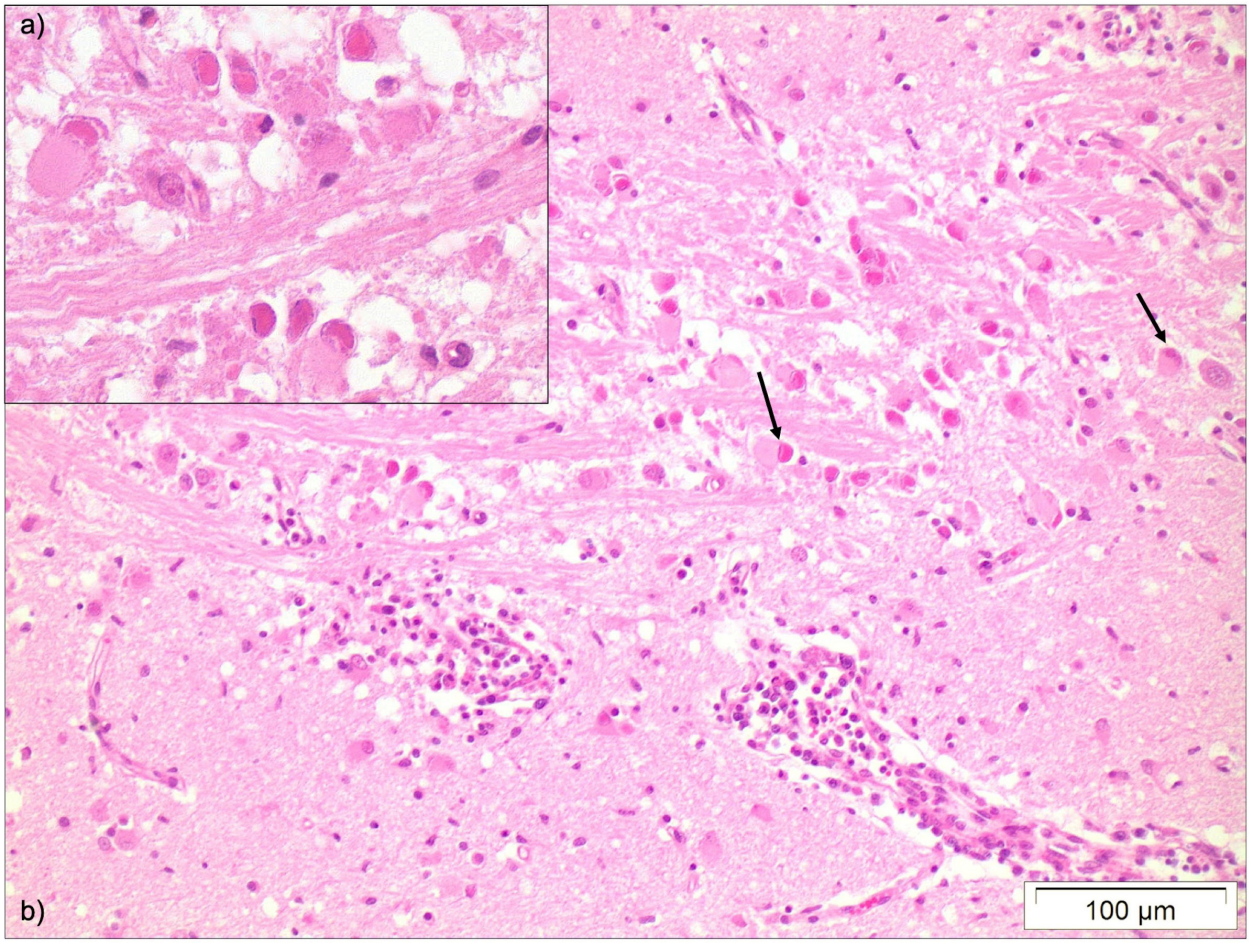

Image 3. a—Mild peri-vascular mononuclear infiltrate associated with intra-nuclear eosinophilic inclusion bodies consistent with canine distemper virus in syncytial cells from the hippocampus. Hematoxilin-eosin stain. 40X | b—intra-nuclear eosinophilic inclusion bodies compatible with canine distemper virus (arrows). Hematoxilin-eosin stain. 20X. © Morales-Acuña & Departamento de Patología, Escuela de Medicina Veterinaria, Universidad Nacional de Costa Rica.

immune status (Origgi et al. 2012; Kubiski et al. 2016). An initial differential diagnosis for CDV considered by local authorities was rabies, which is present in the region (Hutter et al. 2016). Compatible clinical signs, however, were not observed in the animals and Negri bodies were not detected in nervous tissue. Other differential diagnoses such as feline panleukopenia, toxoplasmosis and canine parvovirus were considered (Deem et al. 2000). Due to diagnostic limitations it was not possible to definitively rule these out, but the inclusion bodies observed were supportive of our diagnosis of CDV.

There have been numerous reports of *T. cruzi* infecting several species of coatis, including White-nosed Coatis in Costa Rica (Mehrkens et al. 2013), Mexico (Martínez-Hernández et al. 2016), and Honduras (Lainson 1965), and Ring-tailed Coati or Southern Coati *N. nasua* in Brazil and Peru (e.g., Alves et al. 2016; Morales et al. 2017). Coatis use of arboreal nests increases the risk of exposure to triatomine vectors, and these nests may be shared among multiple members of the social group (de Lima et al. 2015; Alves et al. 2016). Generally, the prevalence of *T. cruzi* can be high in coatis and they appear to have long-lasting parasitemias (Alves et al. 2011). Also, the attenuated pathogenicity of *T. cruzi* in neotropical mammals could be explained by its long co-evolution with this parasite (Schofield 2000). Moreover, experimental infection of another procyonid (i.e., *Procyon lotor*) with a strain of *T. cruzi* that normally does not infect that host led to severe clinical disease (hind limb paralysis and labored breathing) and severe myocarditis, suggesting that this corresponded to an acute phase of infection in these raccoons (Roellig et al. 2009). Alternatively, as reported in dogs by Barr et al. (1991) and also in raccoons by Curtis-Robles et al. (2016), the animals did not present cardiac lesions. In the latter, the authors mentioned that the high infection prevalence of *T. cruzi* detected in raccoons, could be explained since these animals were able to host infections

without serious chronic pathological implications (i.e., no cardiac lesions observed) (Curtis-Robles et al. 2016). Nonetheless, more research is needed to better understand the role of *T. cruzi* in co-infections with other agents in wild hosts, both experimentally and in wild conditions (Roellig et al. 2009; Curtis-Robles et al. 2016). In our case study, the myocardial damage caused by the opportunistic infection due to *T. cruzi* could be the result of a severe compromised immunological status associated to the CDV infection, and subsequent opportunistic polyparasitism described herein (Araujo Carreira et al. 1996; Herrera 2010; Origgi et al. 2012; Kubiski et al. 2016). The exact mechanisms underlying CDV infections and circulation of the virus among susceptible wild mesocarnivores, however, are relatively unknown, that is, several authors suggested that this could be related to CDV genotypes with different virulence and cell tropism (Origgi et al. 2012; Rentería-Solís et al. 2014; Kubiski et al. 2016). As for the discrete typing unit (DTU), TcI is the most common in Central America, and has been reported in coatis in Mexico, but unfortunately the DTU in these animals was not determined (Herrera 2010; Rocha et al. 2013; Martínez-Hernández et al. 2016; Dorn et al. 2017). More studies are necessary to elucidate if coatis may be competent reservoirs of *T. cruzi* and a source of infection for the triatomine bugs in the region (Roellig et al. 2009; Curtis-Robles et al. 2016).

Several other parasites were detected in these two individuals and though the worms found in the heart of the coatis were not saved so could not be identified, they were morphologically similar to *Dirofilaria* sp. These parasites are likely *Dirofilaria immitis* (i.e., canine heartworm), a parasite which is common in domestic dogs from coastal areas in Costa Rica (Montenegro et al. 2017). Additionally, *D. immitis* has been reported in Ring-tailed Coatis from Argentina and Brazil; however, infections in Brazil were based on morphology of microfilaria alone, and the parasites from Argentina were originally described as *D. nasuae* (now considered a synonym of *D. immitis*) (Vezzani et al. 2006; Duarte Moraes et al. 2017). Thus, given the extreme rarity of confirmed *D. immitis* infections in procyonids, careful morphologic and molecular characterization of heart-dwelling *Dirofilaria* species in coatis is warranted. Also, human and animal health authorities should draw attention to this parasite due to its potential for zoonotic transmission.

Paragonimus spp. have been reported in coatis previously (Calvopiña et al. 2014) and the parasites we detected could be *P. mexicanus* or *P. caliensis*, both of which have been reported in freshwater crabs in the same province (Hernández-Chea et al. 2017). Molecular and/or careful morphologic characterization are needed to determine the species. Similarly, the *Sarcosystis* sp. in these coatis is unknown but could be acquired from numerous carnivores or didelphid species in the region, the latter of which have a wide distribution in Costa Rica, including the Pacific coast (Wainwright 2007), and are known definitive hosts of several *Sarcocystis* spp. (Dubey et al. 2008). The unidentified spirurid nematodes in the pancreatic ducts may be related to the Thelazioidea which contains several interesting genera that inhabit the pancreatic ducts of non-human primates and rodents (*Trichospirura*), skates and sharks (*Pancreatonema*), marine teleosts (*Johnstonmawsonia*) and freshwater teleosts (*Prosungulonema*) (Anderson et al. 2009). Finally, the similarities in the histopathological findings in the two individuals included in this study may reflect life histories that lead to similar contact rates with vectors and parasites, since the animals apparently belonged to the same troop (Curtis-Robles et al. 2016).

To our knowledge this is the first report of chagasic myocarditis in free-ranging coatis from Central America. In addition, we recognize the diagnostic and logistic limitations of this report; however, we strongly consider the histopathological findings relevant because of the limited information on zoonotic infectious diseases that currently exists in wildlife populations such as coatis, in Costa Rica. Therefore, these findings may help local authorities to improve surveillance and conservation management strategies in protected areas such as keeping a safe distance from wild animals and discouraging supplemental feeding. Furthermore, we encourage local researchers and local authorities to carry out epidemiological surveys to assess the ecology of infectious diseases on coatis, and to better understand the current health status of *N. narica* populations that frequent protected areas and other regions of the country. A multidisciplinary approach, including education of local residents, park visitors and park rangers, is necessary to minimize cross-infections between wildlife, domestic animals and humans.

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ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)

January 2021 | Vol. 13 | No. 1 | Pages: 17455–17610
Date of Publication: 26 January 2021 (Online & Print)
DOI: 10.11609/jott.2021.13.1.17455-17610

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