

Infection with *Toxoplasma gondii* in a red kangaroo (*Macropus rufus*) and a Patagonian mara (*Dolichotis patagonum*) in captivity

Infecção por *Toxoplasma gondii* em um canguru vermelho (*Macropus rufus*) e em uma mara (*Dolichotis patagonum*) em cativeiro

Nataly Díaz-Ayala¹; Ezequiel Hidalgo-Hermoso²; Constanza Cabello-Araya¹; Francisco Carvallo-Chaigneau^{1,3*}

¹ Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Santiago, Chile

² Departamento de Conservación e Investigación, Parque Zoológico Buin Zoo, Buin, Chile

³ California Animal Health and Food Safety Laboratory, San Bernardino, CA, USA

Received May 11, 2016

Accepted October 19, 2016

Abstract

Toxoplasmosis is an infectious, zoonotic and parasitic disease, caused by *Toxoplasma gondii*. In this manuscript, two cases of infection with *T. gondii* in captive animals from a zoological park in the central region of Chile are described. One case was a red kangaroo (*Macropus rufus*), which is highly susceptible to the infection, and the other was a Patagonian mara (*Dolichotis patagonum*), a rodent in which there is no previous report of the infection. Both animals had myocarditis, with the presence of intralesional tachyzoites and cysts suggestive of infection with *T. gondii*. This infection was confirmed by immunohistochemistry in both animals. The origin of the infection is unknown, but it is likely that free ranging domestic felines were associated with the dissemination of the parasites. This highlights the importance of controlling the domestic animal populations in zoological parks. To the best of our knowledge, this is the first time that *T. gondii* infection is described in a Patagonian mara, adding a new host for this infectious agent.

Keywords: *Toxoplasma gondii*, domestic feline, myocarditis, encephalitis.

Resumo

Toxoplasmose é uma doença parasitária infecciosa e zoonótica causada por *Toxoplasma gondii*. São descritos dois animais com toxoplasmose, mantidos em cativeiro, em um zoológico na região central do Chile. Um destes foi um canguru vermelho (*Macropus rufus*), o qual é altamente suscetível à infecção; o outro foi uma mara (*Dolichotis patagonum*), um roedor sem relato prévio de infecção por *T. gondii*. Ambos os animais tinham miocardite associada a organismos protozoários intralesionais, sugestivos de infecção por *Toxoplasma gondii*. Esta infecção foi confirmada pela imuno-histoquímica, nos dois animais. A origem da infecção é desconhecida, mas é provável que felinos domésticos errantes estejam associados com a disseminação do protozoário. Isso destaca a importância do controle populacional de animais domésticos em zoológicos. Até onde se sabe, esta é a primeira vez que a infecção por *T. gondii* é descrita em uma mara, adicionando um novo hospedeiro para este agente infeccioso.

Palavras-chave: *Toxoplasma gondii*, felino doméstico, miocardite, encefalite.

Introduction

Toxoplasmosis is a globally distributed parasitic disease caused by the protozoan *Toxoplasma gondii*. The definitive hosts of the parasite are members of the Felidae family. A wide variety of warm-blooded animals can act as intermediate hosts, including human beings (DUBEY & BEATTIE, 1988). This disease has been reported in numerous wild animals in zoological parks, and it is believed that

the domestic cat plays a role in the dissemination of the parasite (HARRISON et al., 2007; DUBEY, 2010). Toxoplasmosis is mainly transmitted through the fecal-oral route, but it can also be transmitted by carnivorousism and through the placenta (FREDES, 2010). Diagnosis can be achieved through serology and confirmed with molecular techniques, histopathology, immunohistochemistry and isolation (DUBEY, 2010)

Despite the vast amount of literature on the impact of *T. gondii* in wild animals, to the best of the authors' knowledge there are no reports of this disease in animals from zoological parks in the

*Corresponding author: Francisco Carvallo-Chaigneau, California Animal Health and Food Safety Laboratory, UC Davis, 105 W Central Ave, 92408, San Bernardino, CA, USA. e-mail: fcarvallo@ucdavis.edu

scientific literature in Chile, nor of the infection or disease in Patagonian maras (*Dolichotis patagonum*). Two cases of disseminated infection with *T. gondii* in animals from a zoological park of the Metropolitan area are described.

Materials and Methods

The carcasses of a red kangaroo (*Macropus rufus*) and a Patagonian mara (*Dolichotis patagonum*) were submitted to the Department of Pathology, School of Veterinary Medicine, University of Chile for post mortem examination. A full necropsy was performed and in both animals, samples from the brain, trachea, lung, heart, liver, kidneys, spleen, stomach, small intestine, large intestine, adrenal gland and thyroid gland were collected. These samples were fixed in 10% buffered formalin, routinely processed for histopathology and stained with hematoxylin and eosin (H&E). Liver sections of the mara were also stained with Brown and Brenn Gram stain (CHURUKIAN & SCHENK, 1982).

Immunohistochemistry for *T. gondii* detection, using samples of heart of both animals, was performed at the California Animal Health and Food Safety Laboratory, San Bernardino branch. Briefly, after antigen retrieval with pepsin (Sigma P7000; 0.4% HCl), rabbit anti-*T. gondii* polyclonal antibody (UCDavisRbt#58; 1:1500) was used, followed by anti-rabbit polymer (Dako K4003, Carpinteria, CA). The reaction was visualized with Novared for peroxidase following the manufacturer's instructions (VectorLab

SK4800, Burlingame, CA). Slides were counterstained with Mayer hematoxylin, air dried and cover slipped. As a positive control, heart from a cat with a confirmed infection with *T. gondii* was used.

Results

Case 1: Red kangaroo (*Macropus rufus*)

An adult female red kangaroo was found dead without premonitory signs. At necropsy, the carcass displayed good body condition, bilateral serosanguinous nasal secretion, pulmonary edema, rib imprints in the visceral pleura and small amounts of a red tinged fluid in the thoracic cavity, pericardium and abdomen. Microscopically, discrete infiltrates of lymphocytes, plasma cells and macrophages were identified in the myocardium, associated with segmental degeneration and necrosis of myofibers and the presence of small numbers of tissue cysts in the sarcoplasm. These cysts contained round to oval, basophilic bradizoites, which measured 2-3 μm in diameter (Figure 1a). In the lung, there was interstitial pneumonia, with abundant fibrin in alveolar spaces, thickening of alveolar septa with mononuclear cells, type II pneumocyte hyperplasia and occasional parasitic structures. In the brain, there were multiple perivascular infiltrates of mononuclear cells, with occasional areas of gliosis, presence of neutrophils and macrophages and intralesional tissue cysts.

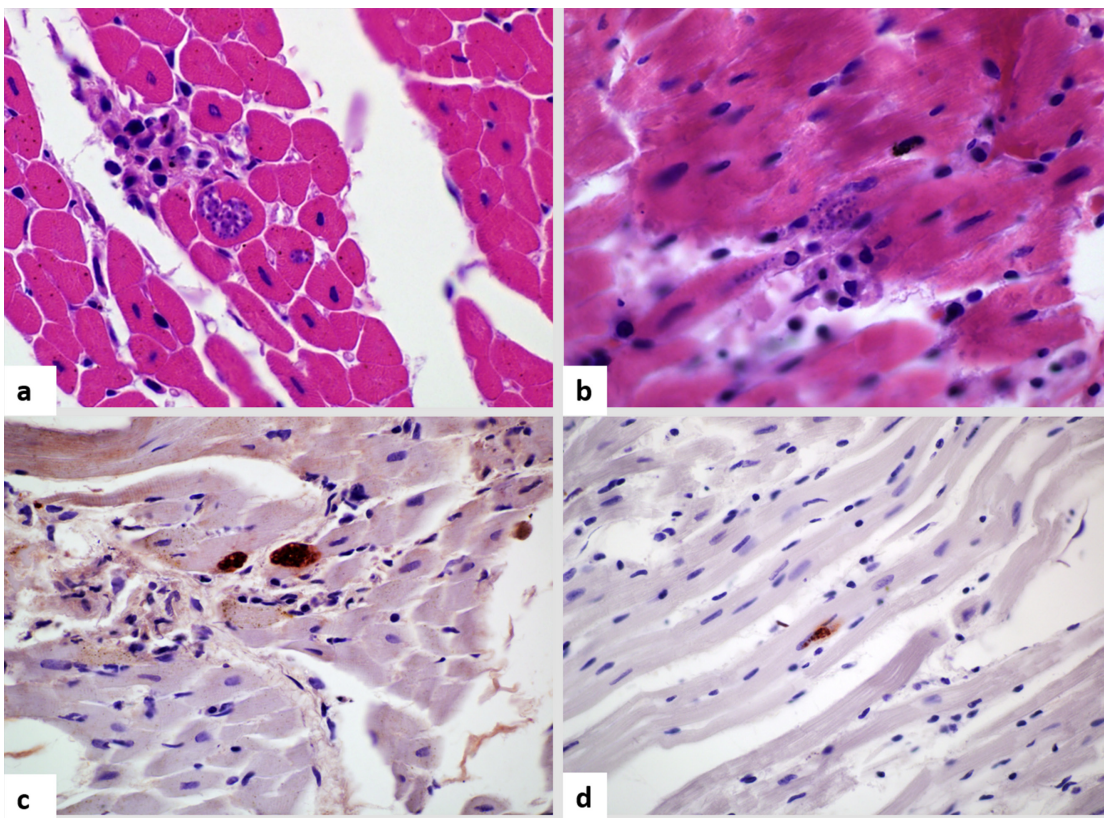


Figure 1. Heart, (a) red kangaroo (*Macropus rufus*) and (b) Patagonian mara (*Dolichotis patagonum*): A few *T. gondii* in the transverse section of a myofiber. Hematoxylin & eosin, 600X. Heart, (c) red kangaroo (*Macropus rufus*) and (d) Patagonian mara (*Dolichotis patagonum*): Immunohistochemical detection of *T. gondii*, Mayers hematoxylin counterstain, 600X.

Case 2: Patagonian mara (*Dolichotis patagonum*)

An adult male mara with a history of anorexia, progressive weight loss and lethargy was presented for post mortem examination. The necropsy revealed poor body condition. The lungs were edematous and displayed ecchymotic hemorrhages in all lobes, and serous atrophy of fat was detected in the heart. In the abdominal cavity, there was abundant serosanguinous fluid and fibrin, and there were numerous abscesses in the liver and small intestine. Microscopically, there was multifocal degeneration and necrosis of the myocardium, with intrasarcoplasmic tissue cysts, associated with mild lympho-histiocytic infiltrates (Figure 1b). The abscess of the liver and intestine had numerous bacterial colonies composed of Gram negative bacilli were identified with Brown and Brenn stain.

In both cases, *T. gondii* was identified in the heart with immunohistochemistry (Figure 1c, d).

Discussion

To the best of the author's knowledge, this is the first descriptive study of the infection with *T. gondii* in captive wild animals in Chile, and new host record for *T. gondii* in a Patagonian mara. Previously, Gorman (1993) reported seropositivity in different wild animals in a zoological park, with a prevalence of 27.5%.

The origin of *T. gondii* in zoological parks is unknown, although the presence of domestic cats prowling in the parks has been linked to contamination of water and food with oocysts (HARRISON et al., 2007; PATRONEK, 1998). On the other hand, it is less likely that oocysts were excreted from other animals in the collection due to no connection of the habitat of different species.

Australian marsupials are highly susceptible to infection with *T. gondii*, which frequently occurs with death without premonitory signs (DUBEY & CRUTCHLEY, 2008). Clinical signs have been described, characterized by diarrhea, lethargy, poor appetite, respiratory distress and neurological signs (CANFIELD et al., 1990). Gross lesions associated with this infection are usually minimal or absent, and consist mostly of congestion, edema and hemorrhages in the heart, lung and gastrointestinal tract. (BASSO et al., 2007; CANFIELD et al., 1990; OCHOA-AMAYA et al., 2012; PORTAS, 2010). Histopathology is a good diagnostic tool to diagnose the infection with *T. gondii*, because free or encysted bradizoites and tachizoites can be identified in the spleen, heart, lung, brain and liver, and are frequently associated with lymphoplasmacytic to granulomatous inflammation (STERN, 2010; OCHOA-AMAYA et al., 2012). In the brain, free or encysted parasites has been described, together with multifocal necrosis, mineralization and glial nodules. In the lung, it is common to find interstitial pneumonia associated with aggregates of macrophages in alveolar spaces and a fibrinonecrotic exudate (BOORMAN et al., 1977; CANFIELD et al., 1990; BASSO et al., 2007; BERMÚDEZ et al., 2009; PORTAS, 2010; OCHOA-AMAYA et al., 2012).

There are descriptions of sudden death and neurological signs in rodents with Toxoplasmosis. Grossly, it is common to find splenomegaly together with white mottled areas in the myocardium and liver (FORZÁN & FRASCA, 2004; JOKELAINEN & NYLUND,

2012). Histologic lesions in rodents consist of lymphoplasmacytic to granulomatous myocarditis and hepatitis, and the presence of parasitic cysts (PERCY & BARTHOLD, 2007). In rodents, the ingestion of water or vegetables contaminated with oocysts from members of the Felidae family has been suggested as the source of infection (PATRONEK, 1998; FORZÁN & FRASCA, 2004; PERCY & BARTHOLD, 2007; JOKELAINEN & NYLUND, 2012).

Although there are no reports of infection with *T. gondii* in Patagonian maras, findings in the heart are consistent with lesions observed in other hosts. Moreover, abscesses with intralesional Gram negative bacteria were identified in the liver and intestine. The cause of death of this animal is not fully clear and the infection with *T. gondii* may be an incidental finding. It is speculated that the presence of a bacterial infection with multiple abscesses is the most probable cause of death.

Conclusion

The inclusion of *T. gondii* as a differential diagnosis in captive wild animals in Chile is recommended. In addition, programs of domestic feline prevention and control should be implemented in zoological parks, in order to limit the impact of these animals on the health of animals kept in captivity. We highlight the presence of *T. gondii* in the heart of the Patagonian mara, adding a new host to the parasite and a new disease for the species.

Acknowledgements

The authors acknowledge Mrs. Sue Ellen Uzal and Dr. Francisco Uzal for the English review of this manuscript, and Mr. Miguel Sepulveda and the histopathology laboratory of CAHFS San Bernardino for the performance of histochemical and Immunohistochemical stains.

References

- Basso W, Venturini MC, Moré G, Quiroga A, Bacigalupe D, Unzaga JM, et al. Toxoplasmosis in captive Bennett's wallabies (*Macropus rufogriseus*) in Argentina. *Vet Parasitol* 2007; 144(1-2): 157-161. PMID:17056183. <http://dx.doi.org/10.1016/j.vetpar.2006.09.030>.
- Bermúdez R, Faílde LD, Losada AP, Nieto JM, Quiroga MI. Toxoplasmosis in Bennett's wallabies (*Macropus rufogriseus*) in Spain. *Vet Parasitol* 2009; 160(1-2): 155-158. PMID:19046816. <http://dx.doi.org/10.1016/j.vetpar.2008.10.082>.
- Boorman GA, Kollias GV, Taylor RE. An outbreak of toxoplasmosis in wallaroos (*Macropus robustus*) in a California Zoo. *J Wildl Dis* 1977; 13(1): 64-68. PMID:839626. <http://dx.doi.org/10.7589/0090-3558-13.1.64>.
- Canfield PJ, Hartley WJ, Dubey JP. Lesions of toxoplasmosis in Australian marsupials. *J Comp Pathol* 1990; 103(2): 159-167. PMID:2246391. [http://dx.doi.org/10.1016/S0021-9975\(08\)80172-7](http://dx.doi.org/10.1016/S0021-9975(08)80172-7).
- Churukian CJ, Schenk EA. A method for demonstrating Gram-positive and Gram-negative bacteria. *J Histotechnol* 1982; 5(3): 127-128. <http://dx.doi.org/10.1179/his.1982.5.3.127>.

- Dubey JP, Beattie CP. *Toxoplasmosis of animals and man*. Boca Raton: CRC Press; 1988.
- Dubey JP, Crutchley C. Toxoplasmosis in wallabies (*Macropus rufogriseus* and *Macropus eugenii*): blindness, treatment with atovaquone, and isolation of *Toxoplasma gondii*. *J Parasitol* 2008; 94(4): 929-933. PMID:18576797. <http://dx.doi.org/10.1645/GE-1448.1>.
- Dubey JP. *Toxoplasmosis of animals and humans*. 2th ed. New York: CRC Press; 2010.
- Forzán MJ, Frasca S Jr. Systemic toxoplasmosis in a five-month-old beaver, (*Castor canadensis*). *J Zoo Wildl Med* 2004; 35(1): 113-115. PMID:15193086. <http://dx.doi.org/10.1638/03-031>.
- Fredes F. Toxoplasmosis. In: Retamal P, Ábalos P, Fredes F. *Enfermedades animales producidas por agentes biológicos*. Santiago: Editorial Universitaria; 2010. p. 122-130.
- Gorman T. *Algunos antecedentes sobre Toxoplasma y Toxoplasmosis* [online]. Chile: Universidad de Chile; 1993 [cited 2016 Feb 22]. Available from: <http://www.monografiasveterinaria.uchile.cl/index.php/MMV/article/view/5003/4888>
- Harrison TM, Moorman JM, Bolin SR, Grosjean NL, Lim A, Fitzgerald SD. *Toxoplasma gondii* in an African crested porcupine (*Hystrix cristata*). *J Vet Diagn Invest* 2007; 19(2): 191-194. PMID:17402616. <http://dx.doi.org/10.1177/104063870701900210>.
- Jokelainen P, Nylund M. Acute fatal toxoplasmosis in three eurasian red squirrels (*Sciurus vulgaris*) caused by genotype II of *Toxoplasma gondii*. *J Wildl Dis* 2012; 48(2): 454-457. PMID:22493121. <http://dx.doi.org/10.7589/0090-3558-48.2.454>.
- Ochoa-Amaya JE, Ciuoderis-Aponte KA, Lim A, Bolin S, Langohr I. Systemic toxoplasmosis in a captive Red Kangaroo (*Macropus rufus*). *Int J Morphol* 2012; 30(1): 70-76. <http://dx.doi.org/10.4067/S0717-95022012000100012>.
- Patronek GJ. Free-roaming and feral cats: their impact on wildlife and human beings. *J Am Vet Med Assoc* 1998; 212(2): 218-226. PMID:9448827.
- Percy DH, Barthold SW. Guinea pig. In: Percy DH, Barthold SW. *Pathology of laboratory rodents and rabbits*. 3rd ed. Ames: Blackwell Publishing; 2007. p. 217-252.
- Portas TJ. Toxoplasmosis in macropodids: a review. *J Zoo Wildl Med* 2010; 41(1): 1-6. PMID:20722247. <http://dx.doi.org/10.1638/2009-0148.1>.
- Stern A. A retrospective study of disease in captive Kangaroos from Oklahoma State University, 1995-2009. *J Vet Diagn Invest* 2010; 22(2): 324-326. PMID:20224104. <http://dx.doi.org/10.1177/104063871002200232>.