Case Report

Fatal disseminated toxoplasmosis in captive red kangaroo (*Macropus rufus*) and Parma wallaby (*Macropus parma*): pathological and immunohistochemical investigations

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Abstract

Toxoplasmosis is a protozoan zoonosis caused by *Toxoplasma gondii*. Marsupials are considered a susceptible intermediate host. *T. gondii* is mainly transmitted to intermediate hosts via the fecal-oral route. A captive red kangaroo was diagnosed with disseminated toxoplasmosis based on clinicopathological and immunohistochemical (IHC) investigations. Two years later, a five-year-old Parma wallaby was kept in the same area as the red kangaroo and showed clinical signs of depression, withdrawal from the troop, and weakness. The wallaby died within two days after showing the clinical signs. Pathological findings in this wallaby were compatible with disseminated toxoplasmosis. Tachyzoites and tissue cysts of *T. gondii* were demonstrated in the brain, striated and myocardial muscles, and lungs of these marsupials using periodic acid Schiff (PAS) staining and IHC. Stray cats were considered the most likely source of infectious oocysts for these marsupials. Taken together, we report fatal disseminated toxoplasmosis in a captive red kangaroo and Parma wallaby in Thailand. Controlling the population of stray cats should be implemented to reduce the exposure rate of *T. gondii* to zoo staff, animals, and visitors.

Keywords: disseminated, immunohistochemistry, red kangaroo, toxoplasmosis, wallaby

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Received: October 9, 2023
Accepted: December 10, 2023

Introduction

Toxoplasmosis is a zoonotic protozoan disease caused by *Toxoplasma gondii*, an obligate intracellular protozoan (Elmore *et al.* 2010). A wide variety of warm-blood animals, particularly marsupials and New World primates, have been reported as susceptible intermediate hosts (Epiphanio *et al.* 2003; Dubey and Lappin 2012). Domestic and wild felids act as definite hosts that can shed oocysts into the environment (Jittipalapong *et al.* 2010; Dubey and Lappin 2012). Stray cats have been reported as a source of toxoplasmosis in humans and animals (Basso *et al.* 2007; Jittapalpong *et al.* 2006). Herbivorous intermediate hosts most commonly receive infectious oocysts via the fecal-oral route to complete the transmission cycle of *T. gondii* (Elmore *et al.* 2010). Intermediate hosts may be asymptomatic or develop variable clinical signs, including diarrhea, respiratory distress, neurological signs, and sudden death after ingesting the infectious oocysts (Canfield *et al.* 1992; Epiphanio *et al.* 2003; Elmore *et al.* 2010). However, marsupials are susceptible intermediate hosts that frequently develop fatality without any preliminary signs (Basso *et al.* 2007; Bermudez *et al.* 2009; More *et al.* 2010). In Thailand, toxoplasmosis has been described in piglets that showed signs of convulsion, dyspnea, fever, and death (Thiptara *et al.* 2006). However, toxoplasmosis in marsupials has not been described in Thailand, although stray cats with seropositivity to *T. gondii* is common in Bangkok (Jittapalpong *et al.* 2006). In the present study, we report fatal disseminated toxoplasmosis in a captive red kangaroo and Parma wallaby in Thailand, where stray cats are considered a source of infectious oocysts.

Case description

Case No. 1: An adult male captive red kangaroo (*Macropus rufus*) weighing 35 kg was kept at the Dusit Zoo in Bangkok. The kangaroo had shown clinical signs of transient diarrhea and responded to antibiotic treatment two months earlier. After that, the kangaroo developed respiratory distress, muscular weakness, ataxia, blindness, and lateral recumbency. Physical examination revealed an emaciated condition with swelling of both carpal joints. The clinical signs progressively worsened, and the animal died within one month after respiratory distress was recognized. Since the red kangaroo died due to disseminated toxoplasmosis, serum from the cage mate, including three red kangaroos and three wallabies, was subjected to *T. gondii* serological test using a commercially available latex agglutination test kit (Toxocheck-MT, Eiken Chemical Co., Ltd. Tokyo, Japan). The cutoff was set at the titer of 1 ≥ 64.

Case No. 2: An approximately five-year-old male Parma wallaby (*Macropus parma*) weighing 3.5 kg was kept in the same area as case No. 1. The wallaby had been imported from the Czech Republic 5 years before developing clinical signs of depression and withdrawal from cage mates, and weakness. Despite antibiotic and supportive treatments, the wallaby died within two days after the first signs were noticed. The wallaby died within two years after the red kangaroo. The remaining cage mates appeared normal.

A necropsy was performed. The selected organs were fixed in 10% buffer formalin, embedded in paraffin wax, cut into 4 µm thickness, and stained with hematoxylin and eosin (H&E) and periodic acid Schiff (PAS). Sections of the brain, striated muscle, heart, and lung were subjected to immunohistochemistry (IHC) using Autoimmunohistochemistry (Bond™ Polymer Refine Detection, Leica Biosystems, United Kingdom). The polyclonal rabbit anti-*T. gondii* antibody (DAKO, USA) was used as a primary antibody.

Result and Discussion

The serological result revealed one seropositive to *T. gondii* antibody in red kangaroo at the titer 1:256. The remaining five marsupials were seronegative.

Macroscopically, the dead red kangaroo was emaciated with low subcutaneous and abdominal fat tissues. Multiple ulcerations were observed on bony prominences. Muscular atrophy was presented in the temporal, masseter, and limb muscles. Multiple petechial hemorrhages were noted in the neck muscle and testes. The heart and lungs were pale. The lymph nodes were enlarged. The meningeal vessels were congested. The remaining organs were grossly normal. The Parma wallaby was emaciated. The lung was a diffuse dark red color with a firm consistency and whitish nodules measuring 1–2 mm (Fig. 1). The tracheobronchial and mesenteric lymph nodes were enlarged. The meningeal vessels were congested with multifocal hemorrhages. The other organs were normal in appearance.

Microscopically, the pathological findings in the red kangaroo and the Parma wallaby were presented almost similarly. Interstitial and granulomatous pneumonia characterized by thickening of the alveolar wall by infiltration of lymphocytes and plasma cells and accumulation of macrophages and multinucleated giant cells in the alveolar lumen (Fig. 2) contributed to the respiratory distress (Canfield *et al.* 1990; Epiphanio *et al.* 2003; Thiptara *et al.* 2006). The cerebrum of the red kangaroo revealed multifocal necrosis and infiltration of predominate neutrophils and occasional granulomas (Fig. 3a). In addition, multifocal hemorrhages were also observed in the wallaby (Fig. 3c). Tachyzoites were commonly seen in the necrotic areas surrounded by neutrophils, lymphocytes, plasma cells, and macrophages (Fig. 3a). The tissue cysts (measuring 25 – 45 µm) containing numerous bradyzoites and surrounded by a thin cyst wall were observed in the neuropil (Fig. 3b), myocardial and muscle cells (Fig. 5), and lung without an inflammatory cell response. Myocarditis and myositis were characterized by vacuolated cytoplasm, loss of striation, fragmentation of muscle fibers, and infiltration by predominately mononuclear cells accompanied by tachyzoites and tissue cysts (Figs. 4 and 5). The remaining muscle fibers were atrophied. Encephalitis and myositis corresponded to muscular weakness and neurological signs as previously described in Australian marsupials and piglets, respectively (Canfield *et al.* 1992; Thiptara *et al.* 2006). Lymphoid necrosis was seen in the germinal centers of

the lymph nodes and spleen. The testes of the red kangaroo revealed multifocal necrosis and mineralization of the seminiferous tubules with infiltration of lymphocytes, plasma cells, and macrophages in the interstitial areas. The remaining seminiferous tubules were degenerated and lined by a single layer of Sertoli cells. Testicular lesions found in the red kangaroo also support the involvement of reproductive organs in *T. gondii*-infected animals, as previously mentioned that male reproductive performance in humans and animals was affected by toxoplasmosis (Epiphanio *et al.* 2003; Dalini and Abdoli 2013). Tachyzoites and tissue cysts were demonstrated by PAS staining in the brain (Figs. 3d, 3e), striated muscle (Fig. 5), and heart, indicating that PAS stain is useful to illustrate *T. gondii* in the tissues as previously reported (Canfield *et al.* 1990; Epiphanio *et al.* 2003). Immunohistochemically, strong positive signals were detected in tachyzoites (Figs 6a, 6c) and tissue cysts (Figs.6b, 6d) in the brain, myocardial and muscle cells, and lung. IHC was used to confirm the presence of *T. gondii* (Canfield *et al.* 1990; Portas 2010; Waree *et al.* 2007). However, tissue cysts of *T. gondii* were occasionally not detected by IHC (Canfield *et al.* 1990).

Disseminated toxoplasmosis was diagnosed in a captive red kangaroo, and a Parma wallaby kept in the same area according to clinical signs, pathological findings, and the presence of *T. gondii* in various organs as demonstrated by PAS staining and IHC. *T. gondii* complete their entero-epithelial life cycle and excrete oocysts strictly in domestic and wild felids into the environment (Elmore *et al.* 2010). Previously, stray cats have been considered an important source of toxoplasmosis in Bennett's wallabies (Basso *et al.* 2007; Bermudez *et al.* 2009) and piglets (Thiptara *et al.* 2006). Jittapalapong and colleagues (2010) reported that stray cats in Bangkok were generally seropositive to *T. gondii*. The kangaroo and wallaby were possibly infected via the fecal-oral route from oocyst-contaminated soil and water (Dubey 2006; Basso *et al.* 2007). After infection by *T. gondii*, intermediate hosts show various clinical signs ranging from asymptomatic infection to sudden death without any precautionary signs (Basso *et al.* 2007; Dubey and Lappin 2012). The marsupials in the present study demonstrated obvious signs and succumbed, which might be correlated with a high infective dose, host factors, and the severity and distribution of tissue destruction in the affected organs (Epiphanio *et al.* 2003; Dubey and Lappin 2012). However, their cage mates possibly harbored subclinical infections (Basso *et al.* 2007). In the present study, tachyzoites and tissue cysts accompanied by an inflammatory cell response in affected organs indicated that the marsupials had reactivated chronic toxoplasmosis. Tissue cysts are usually noted in chronic infection (Dubey 2006; Waree *et al.* 2007), which can be asymptomatic and persist throughout life in intermediate hosts (Dubey and Lappin 2012). Immunosuppression is the key contributing factor for the breakdown of tissue cysts and induction of inflammatory cell response, leading to the predominant signs in this study (Dubey and Lappin 2012).

In conclusion, we have described disseminated toxoplasmosis in a captive red kangaroo and Parma wallaby. Stray cats were considered to be a source of infection. These findings indicate that the zoo environment was contaminated with oocysts of *T. gondii*. The personal hygiene of zoo staff and visitors should be emphasized to diminish the exposure rate. In addition, control of the population of stray cats should be implemented to eliminate the shedding of *T. gondii* oocysts in the zoo environment.

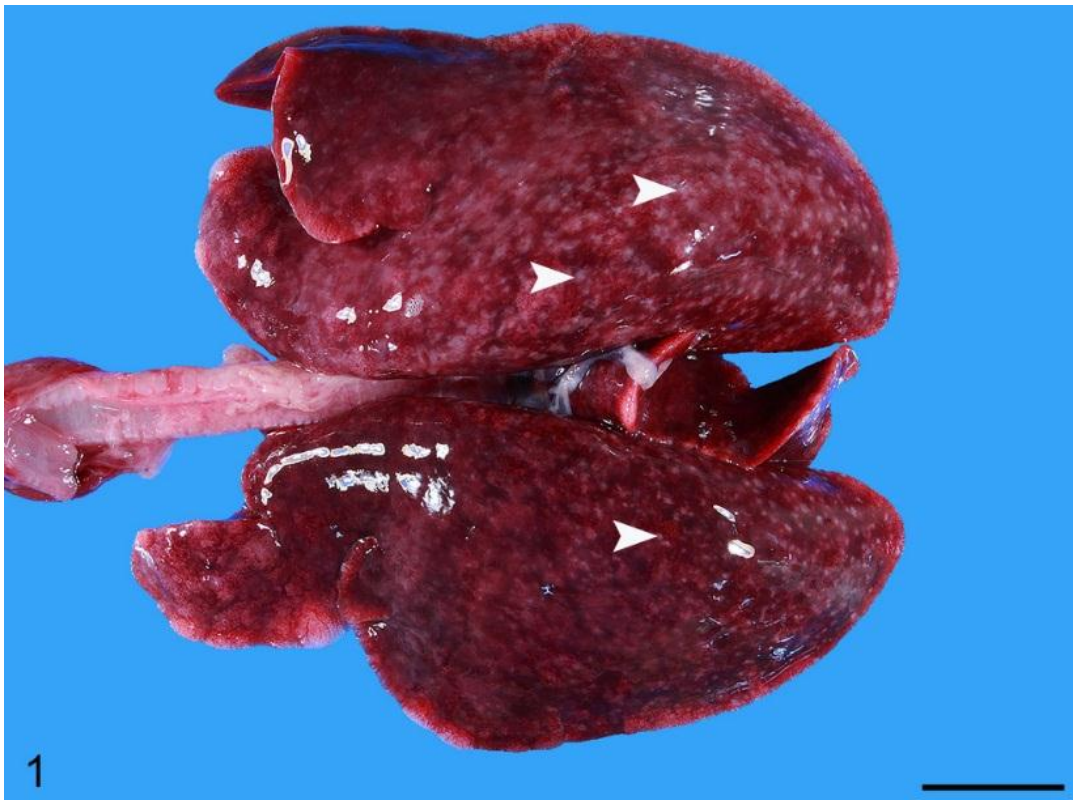

Figure 1 The lung of the wallaby revealed a diffusely dark red color, firm consistency, failure to collapse, and whitish nodules measuring 1–2 mm (arrowhead). Bar = 2 cm.

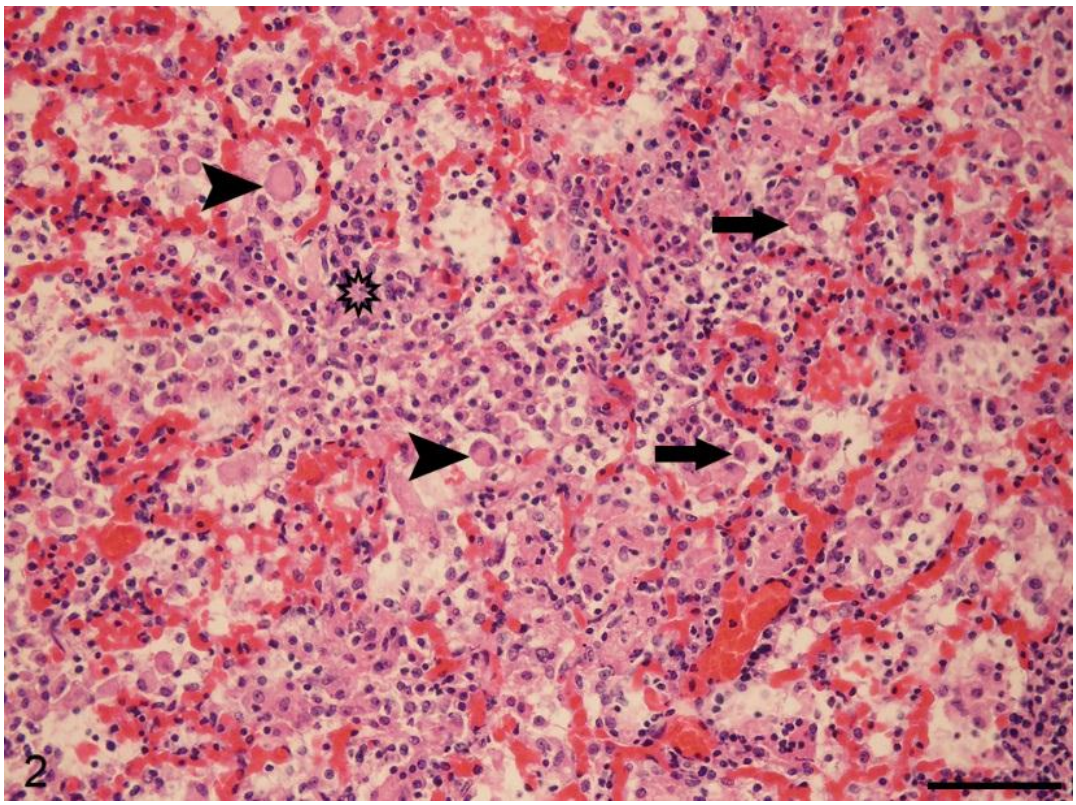

Figure 2 Interstitial (asterisk) and granulomatous pneumonia characterized by thickening of the alveolar wall by infiltration of mononuclear cells and accumulation of macrophages (arrow) and binucleated giant cells (arrowhead) with necrotic debris. H&E. Bar = 50 µm.

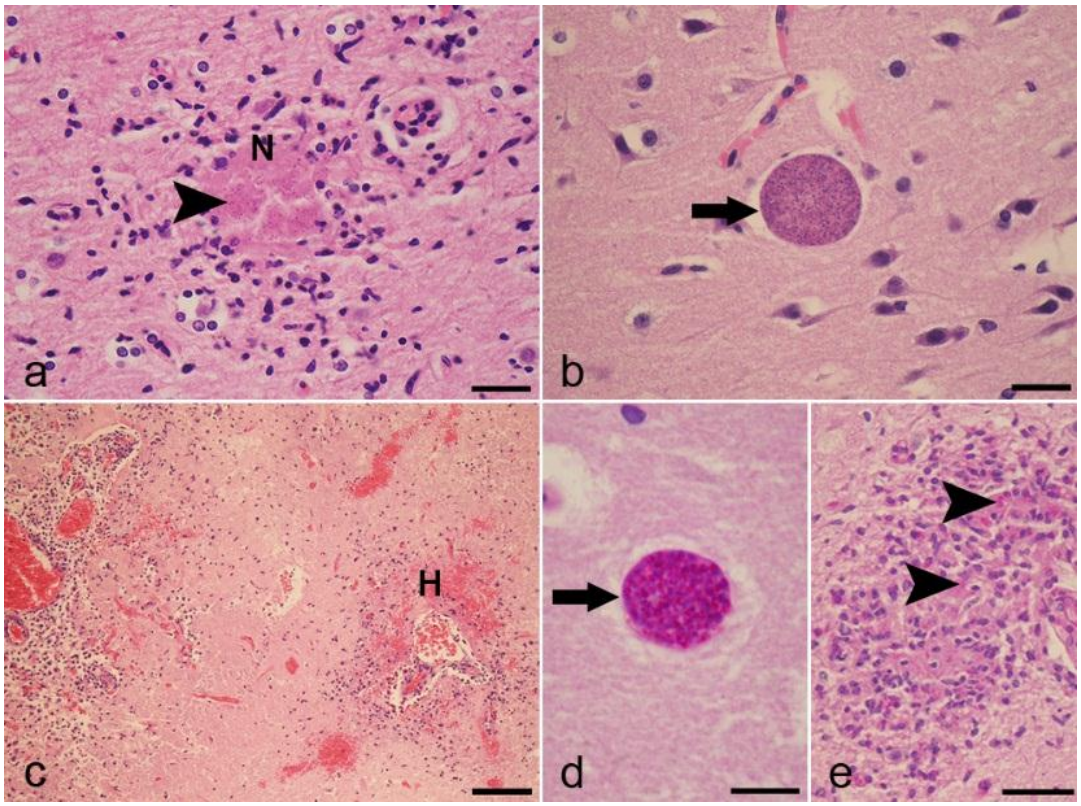

Figure 3 a) Free tachyzoites (arrowhead) were seen in the necrotic area (N) with infiltration of predominately neutrophils in the cerebral cortex of the red kangaroo. H&E. Bar = 25 µm. b) The tissue cyst (arrow) containing bradyzoites was occupied in the neuropil. H&E. Bar = 25 µm. c) The cerebral cortex of the wallaby revealed multifocal hemorrhages (H) and necrosis accompanied by infiltration of inflammatory cells. H&E. Bar = 100 µm. d) A tissue cyst (arrow) is positive for PAS staining. PAS. Bar = 25 µm. e) Free tachyzoites (arrowhead) in the necrotic area of the red kangaroo were positive for PAS staining. PAS. Bar = 25 µm.

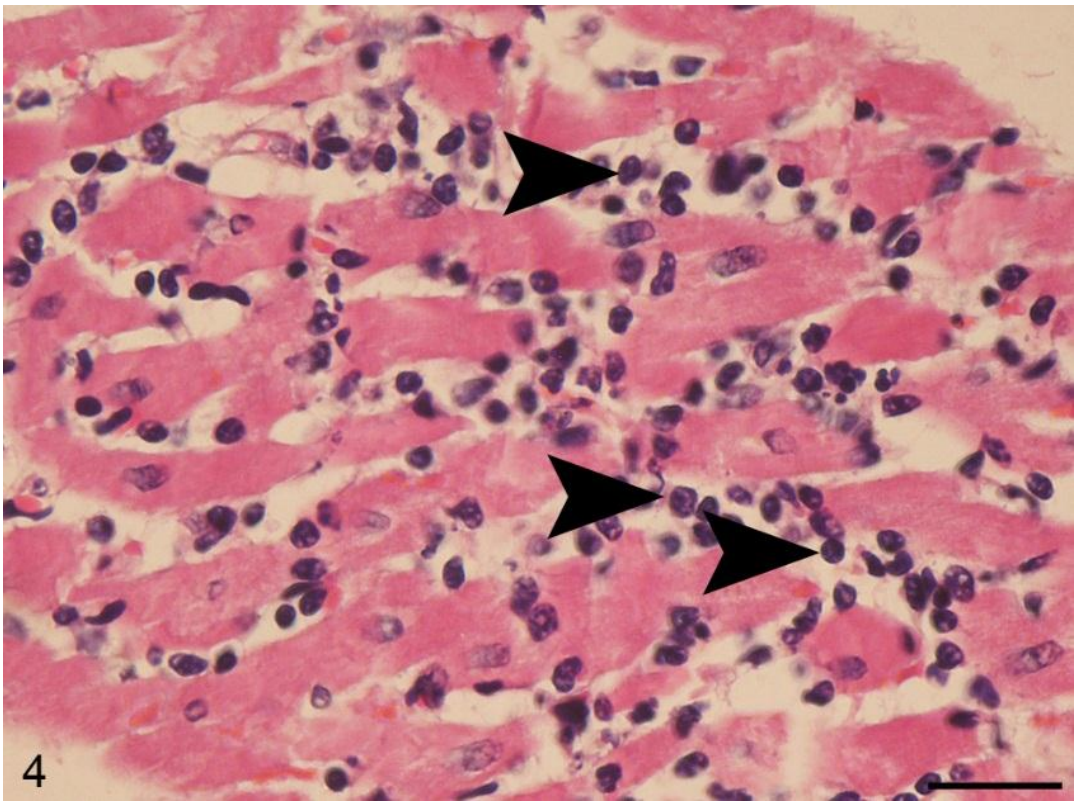

Figure 4 Myocarditis characterized by infiltration by mononuclear cells (arrowhead). H&E. Bar = 25 µm.

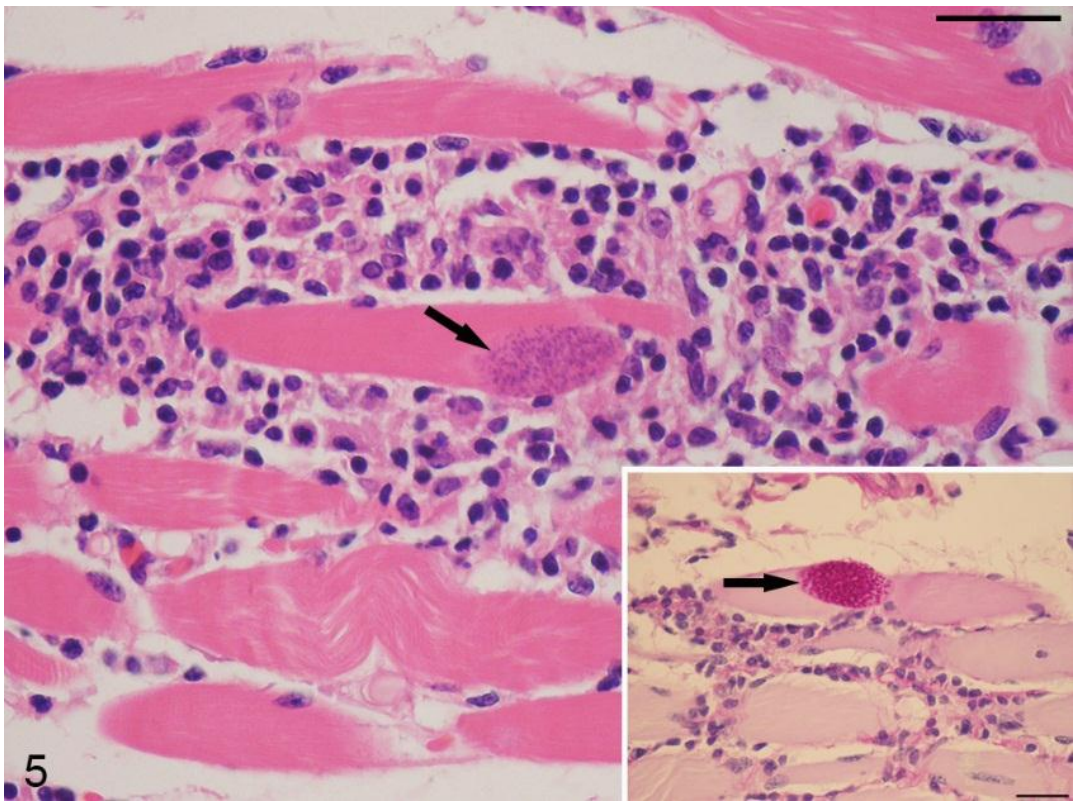

Figure 5 A tissue cyst (arrow) is seen in a muscle cell surrounded by lymphocytes, plasma cells, and macrophages. H&E. Bar = 25 µm. The tissue cyst (arrow) was positive for PAS staining (inset, PAS. Bar = 20 µm).

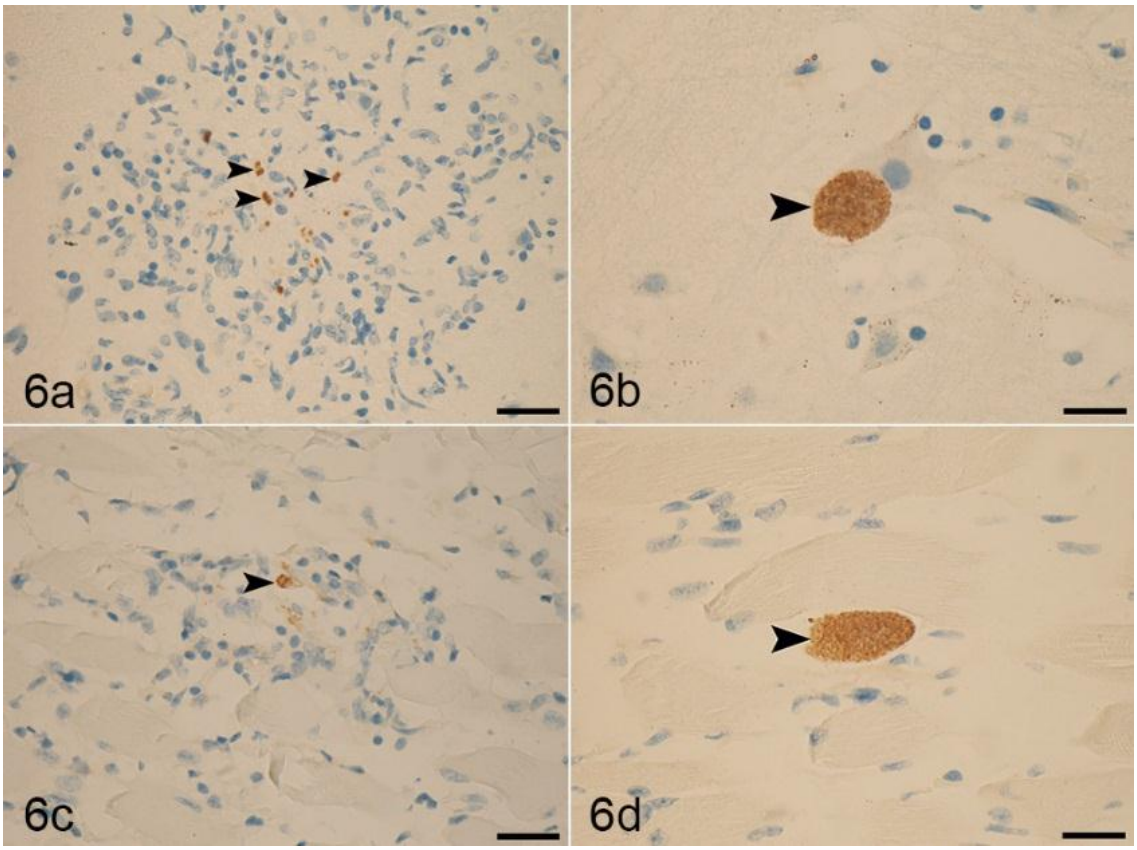

Figure 6 Immunoreactivity to tachyzoites and tissue cysts in the brain (a and b) and muscle (c and d). IHC. Bond™ Polymer Refined Detection, DAB, Mayer's hematoxylin counterstain. Bar = 25 µm.

Conflicts of interest: The author (s) declare that no conflicts of interest exist in this article's research, authorship, and publication.

Acknowledgments

We would like to thank Mr. Sittichoke Lacharoje for his technical assistance.

Ethical approval: No ethical approval was obtained because this study did not involve laboratory animals and only involved non-invasive procedures.

Authors' contribution: S. Kesdangsakonwut contributed to the design, performed clinical studies pathological and immunohistochemical investigations, analyzed the data, and prepared the manuscript. K. Teankum and W. Banlunara contributed to pathological and immunohistochemical investigations, and Y. Utara and S. Sanannu contributed to collected clinical data and animal care.

Funding: This report was supported financially by grants from the Chulalongkorn University-Veterinary Science Research Fund (RG15/2558).

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