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IMAGING DIAGNOSIS

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# Imaging Diagnosis: Thoracic radiographic features of toxoplasmosis in a 14-month-old Red Kangaroo (*Macropus rufus*)

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## Abstract

A privately owned 14-month-old intact female red kangaroo (*Macropus rufus*) was presented for acute onset respiratory distress and lethargy. On presentation, the kangaroo was laterally recumbent, tachypneic, dyspneic, lethargic, and obtunded. Thoracic radiographs revealed a severe diffuse mixed pulmonary pattern (alveolar pattern superimposed on a bronchial pattern) and subjective mild generalized cardiomegaly. Due to the severity of clinical signs and grave prognosis, euthanasia was elected. Postmortem examination was consistent with systemic toxoplasmosis. Histopathology and immunohistochemistry staining on infected tissues confirmed *Toxoplasma gondii*. This is the first published report of radiographic findings for confirmed toxoplasmosis in a red kangaroo or marsupial.

KEYWORDS
Apicomplexan, Macropodidae, Marsupial

## 1 | SIGNALMENT, HISTORY, AND CLINICAL FINDINGS

A privately owned 14-month-old intact female red kangaroo was presented to the emergency service for evaluation of acute onset of respiratory distress and lethargy. The owner reported that the previous night, the patient seemed to sputter while taking her evening bottle, but after this event, these signs did not persist. The following morning, she was lethargic and developed a progressively increasing respiratory effort.

On presentation, the patient was hypothermic (temperature too low to read), obtunded, and laterally recumbent, with tacky and muddy mucous membranes (estimated 8% dehydration). The patient was tachypneic (60 breaths/min), dyspneic, and had decreased lung sounds in all quadrants. Heart rate was 90 beats/min, with no murmurs or arrhythmias. Her pupillary light reflex was delayed but present and her withdrawal reflex in all limbs were decreased. She had a < 4 week old joey present in her pouch. The remainder of the physical exam was normal. Point-of-care blood work revealed severe hypoglycemia (54 mg/dL, reference interval; 59–229 mg/dL), acidemia (pH < 6.8 (reference not available), hypercarbemia ($PCO_2$ too high to read, reference interval; 11.2-35 mEq/L) and hyperlactatemia (> 15 mmol/L, reference not available).

## 2 | IMAGING, DIAGNOSIS, AND OUTCOME

Following initial stabilization with heat support, intravenous fluid administration, and dextrose supplementation, thoracic and abdominal radiographs (right and left lateral, ventrodorsal, 121 kVp and

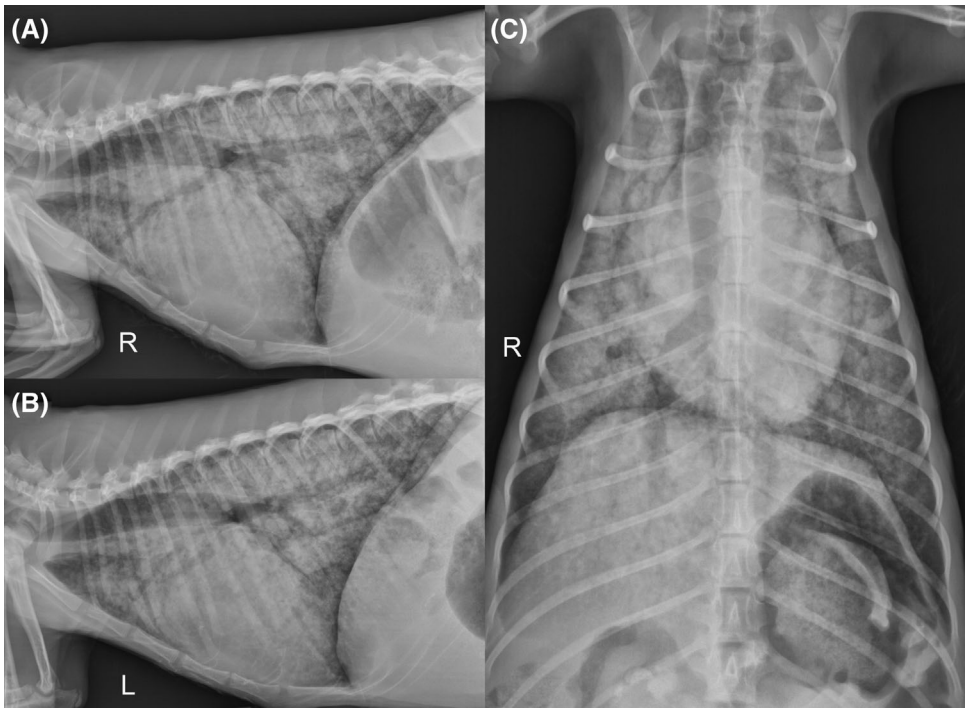


**FIGURE 1** Right lateral (A), left lateral (B), and dorsoventral (C) thoracic radiographs of a 14-month-old red kangaroo (*Macropus rufus*). The cardiac silhouette is subjectively mildly generally enlarged. The lung has a severe diffuse mixed pattern, characterized by a diffuse alveolar pattern superimposed on a bronchial pattern. Technique 121 kVp and 5.6 mAs, CXDI-50G, Canon Medical Systems USA, Inc., Tustin, CA

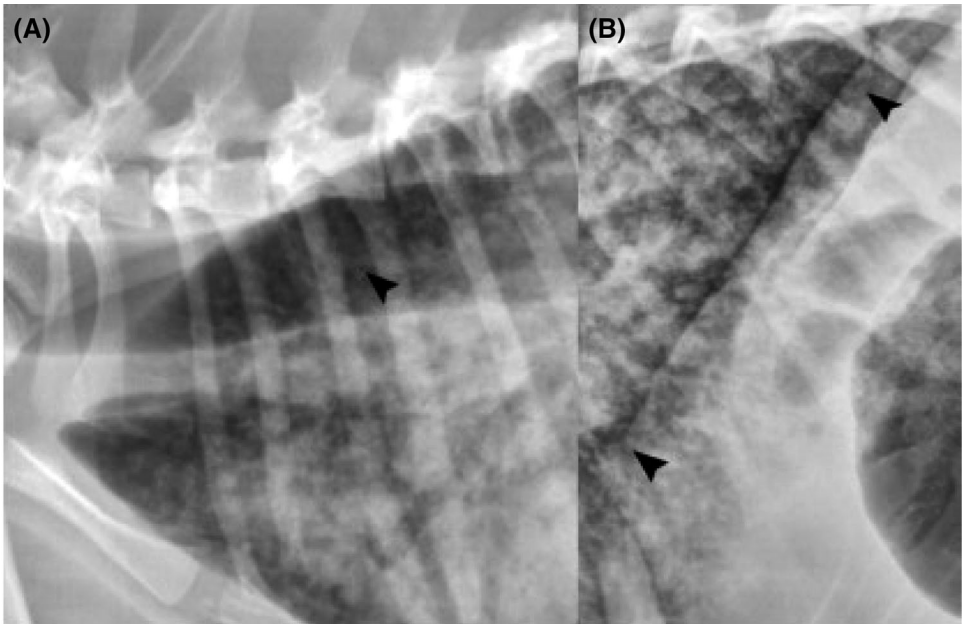


**FIGURE 2** Zoomed right lateral views of the lung of a 14-month-old red kangaroo (*Macropus rufus*) demonstrated the underlying bronchial pattern. Arrowheads indicate thick rings/bronchial markings. Technique 121 kVp and 5.6 mAs, CXDI-50G, Canon Medical Systems USA, Inc., Tustin, CA

5.6 mAs, CXDI-50G, Canon Medical Systems USA, Inc., Tustin, CA) were performed without sedation or anesthesia. On thoracic radiographs, the cardiac silhouette was subjectively mildly generally enlarged. The lungs had severe, diffuse increased soft tissue opacity, primarily characterized by effacement of pulmonary vascular markings and air bronchograms, with thick lines and rings in regions of more aerated lung indicating a severe alveolar pattern superimposed upon a bronchial pattern (Figures 1 and 2). On abdominal radiographs, the gastrointestinal tract was moderately gas distended, and a joey was visible within the pouch (not provided). Given patient signalment

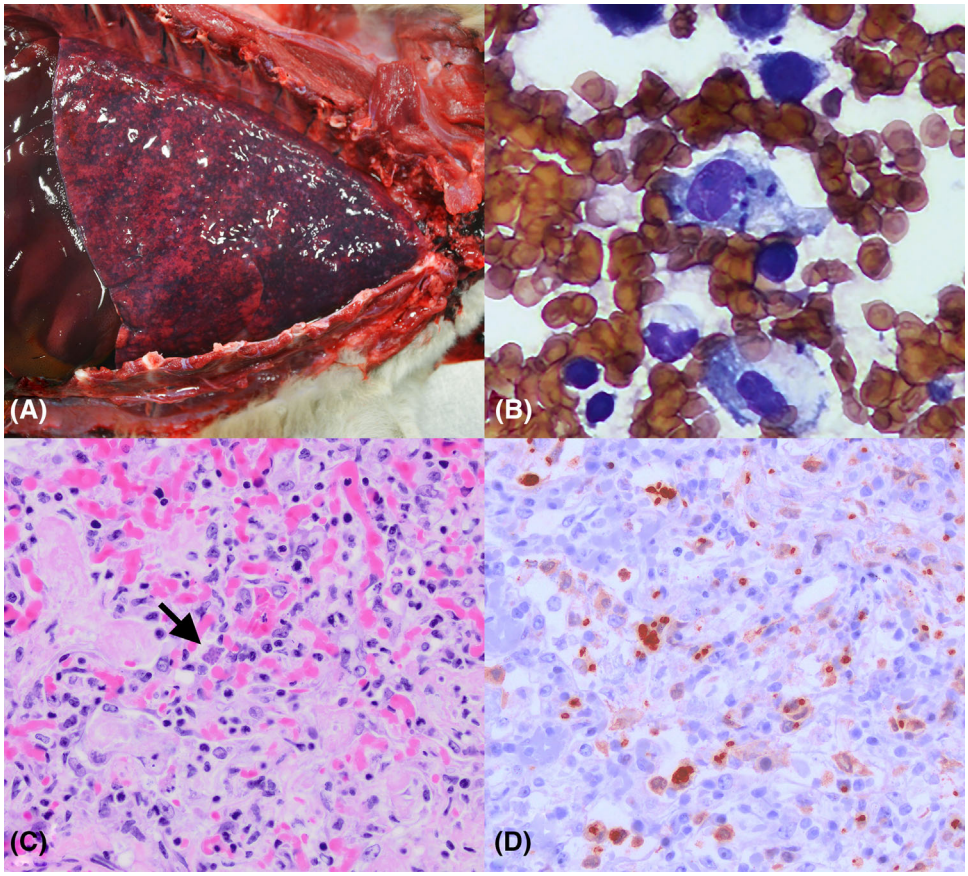


**FIGURE 3** Gross and microscopic post-mortem findings in a 14-month-old Red Kangaroo with systemic toxoplasmosis. A, Gross post-mortem findings. The lungs fail to collapse and contain numerous pale tan and dark red foci, corresponding to areas of inflammation and necrosis with hemorrhage, respectively. B, Cytologic touch impression of the lung reveals multiple intracytoplasmic tachyzoites, both within pneumocytes and macropahges. Diff-Quik 100×. C, Histopathology of the lung identifies protozoal cysts within pneumocytes (black arrow), which contain multiple tachyzoites. Alveolar spaces are flooded with fibrin and cellular debris. H&E. 40×. D, Immunohistochemistry of lung identifies numerous intercellular and extracellular tachyzoites that exhibit strong immunolabeling (red-orange color) with *Toxoplasma gondii*. antibody (60×). [Color figure can be viewed at wileyonlinelibrary.com]

and history, toxoplasmosis (interstitial pneumonitis) with concurrent non-cardiogenic pulmonary edema (acute respiratory distress syndrome) was prioritized. Other differential diagnoses for the pulmonary pattern included hemorrhage, toxin exposure, and cardiogenic pulmonary edema. Although species variation was not excluded; it is documented that macropods hearts can be up to one-third larger than those of similarly sized Eutherian mammals.[6] Myocarditis secondary to toxoplasmosis was prioritized as the most likely cause of the subjective cardiomegaly given the concurrent findings. The gas within the gastrointestinal tract was presumed secondary to aerophagia and/or species variation in the absence of any reported gastrointestinal signs.

Given the patient's clinical condition and poor prognosis, humane euthanasia of both the doe and the joey was elected by the owners. Necropsy was performed on the doe, but the joey was not submitted for necropsy. Necropsy of the doe revealed a small volume of serosanguinous effusion within both the pleural and peritoneal cavities. The lungs were diffusely firm and failed to collapse. Multiple foci of pallor, ranging in size from pinpoint to 0.6 cm in maximum dimension, were scattered throughout the pulmonary parenchyma, myocardium, and renal parenchyma (Figure 3A). Histologically, greater than 90% of the pulmonary parenchyma was effaced by severe histiocytic and neutrophilic interstitial inflammation accompanied by necrosis and hemorrhage. Scattered throughout the lung, but primarily in regions of necrosis, alveolar macrophages and type II pneumocytes were expanded by intracytoplasmic protozoal cysts containing 2–4 $\mu$m basophilic tachyzoites, consistent with *Toxoplasma gondii* (Figure 3B). Additionally, moderate expansion of the bronchial interstitium by edema was noted. Free extra-cellular tachyzoites were also identified within foci of necrosis amongst cellular debris (Figure 3C). Within the brain, there were multiple scattered foci of necrosis, gliosis, lymphoplasmacytic, and histiocytic inflammation with rare protozoal cysts. The heart was similarly affected with foci of necrosis and inflammation scattered throughout the myocardium. Myocardiocytes were occasionally distended by protozoal cysts. Other affected organs included both kidneys and adrenal glands as well as the gastric mucosa and tracheobronchial lymph nodes. Histopathologic findings in this case were consistent with that of disseminated toxoplasmosis with associated necrotizing pneumonia, encephalitis, myocarditis, nephritis, adrenalitis,

and lymphadenitis. Immunohistochemistry performed on a section of lung containing tachyzoites which exhibit strong positive immunolabeling for *Toxoplasma gondii* (Figure 3D). Material from paraffin-embedded tissues were submitted to The Animal Disease Diagnostic Laboratory at Purdue University for PCR testing that confirmed the presence of *Toxoplasma gondii* DNA.

## 3 | DISCUSSION

Radiography facilitated rapid identification of the cause of acute respiratory distress in the red kangaroo presented, and in conjunction with patient signalment allowed for creation of accurate differential diagnoses. *Toxoplasma gondii* is an obligate intracellular apicomplexan parasite that has been well-described in many veterinary species and humans, including multiple prior reports in Macropodidae. *Toxoplasma gondii* undergoes both asexual and sexual reproduction with the latter only occurring in the small intestinal gut epithelium of both domestic and wild felids, making them the sole definitive host. There are three infectious stages that can occur: tachyzoites, bradyzoites contained in tissue cysts, and sporozoites contained in oocysts. The first stage tachyzoite has the ability to rapidly multiply within the host's body. Once replicated, it will enter a host cell and conceal itself in a vacuole to evade the host's immune defense. Next, the tachyzoite will undergo repeated division to form thin-walled cysts that contain bradyzoites. These cysts can develop anywhere, but are most prevalent in muscular and neuronal tissues. In sexual reproduction, within epithelium of the gut, there *T. gondii* will differentiate and undergo sexual reproduction. This replication in the gut allows shedding of millions of oocytes into their feces. Unsporulated oocysts are passed in fresh feces of felids, which will become sporulated in the environment within 1 –5 days and can last for months to years in wet soil. Therefore, environmental contamination is widespread with cats shedding millions of oocysts after ingestion of a single infected tissue cyst.[7–8] Additionally, intermediate hosts like earthworms in toxoplasmosis, can be an important source of infection. It has been speculated that since Australian marsupials evolved in the absence of cats they therefore are thought to be more susceptible to toxoplasmosis.[2] Vertical transmission has also been proven to occur in marsupials, though infections in the joey may be subclinical.[3,9] It has also been shown that those that are immune compromised or stressed can have increased susceptibility to clinical manifestation of the disease. Therefore, environmental stressors should be minimized in captive macropods, which includes; reducing the need for restraint, confinement, and transportation.[7–8,10]

In both free-range and captive macropodoid species, toxoplasmosis causes significant morbidity and mortality.[8] Clinical signs vary from acute death, diarrhea, neurologic abnormalities, weight loss, respiratory signs, and anorexia.[4,8,10] Previous necropsy findings for pulmonary pathology have been described as failing to collapse upon opening of the thoracic cavity, pulmonary parenchyma being firm to touch or diffusely red, and variable numbers of raised tan foci.[8,11] Radiographic changes of the lung in domestic cats who develop respiratory signs following infection with toxoplasmosis has been described as a diffuse nodular interstitial pattern with areas of patchy alveolar infiltrates.[5] Correlating prior necropsy findings in kangaroos affected with toxoplasmosis, it was suspected that a thoracic radiographic study would likely demonstrate variable and mixed pulmonary pathology.

Based on the authors' review of the literature, this is the first confirmed systemic toxoplasmosis infection with thoracic radiographs in a kangaroo. Toxoplasmosis infection within various species of kangaroos have been described previously, but these reports lack correlation with diagnostic imaging.[2,4,8,10,11] This case report will serve as a reference resource for future Macropodidae patients with respiratory signs that have a high clinical suspicion for toxoplasmosis infection.

### LIST OF AUTHOR CONTRIBUTIONS

**Category 1**

(a) Conception and Design: Lehman, Cohen, Ozawa
(b) Acquisition of Data: Lehman, Cohen, Ozawa, Sommer, Hepps Keeney
(c) Analysis and Interpretation of Data: Lehman, Cohen, Ozawa, Sommer, Hepps Keeney

**Category 2**

(a) Drafting the Article: Lehman, Cohen, Ozawa, Sommer
(b) Revising Article for Intellectual Content: Lehman, Cohen, Ozawa, Sommer, Hepps Keeney

**Category 3**

(a) Final Approval of the Completed Article: Lehman, Cohen, Ozawa, Sommer, Hepps Keeney

**Category 4**

(a) Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved: Lehman, Cohen, Ozawa, Sommer, Hepps Keeney

### CONFLICT OF INTEREST

The authors declare no conflict of interest.

### DISCLOSURE

The authors have no relevant disclosures. This work has not been previously presented in any form.

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