

RH: TOXOPLASMOSIS IN RED PANDAS

CLINICOPATHOLOGICAL FEATURES OF TOXOPLASMOSIS IN FOUR RED PANDAS

(*AILURUS FULGENS*)

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Abstract: Two geriatric red pandas (*Ailurus fulgens*) over a four-year period presented with vague clinical signs including anorexia, lethargy, and difficulty ambulating. Treatment protocols using enrofloxacin, steroids, and clindamycin were unsuccessful. Necropsy examination confirmed disseminated toxoplasmosis infection in these cases, and a modified agglutination test had been positive for a prolonged period of time before showing signs of disease in one case. A review of the Red Panda Species Survival Plan pathology database revealed two additional cases of disseminated toxoplasmosis in geriatric red pandas. Many organ systems were affected, but dissemination to the brain, lungs, and liver predominated. Immunohistochemistry or polymerase chain reaction was required to confirm a diagnosis in serologically positive animals, as well as animals in which a histological diagnosis was suspected. This case series describes the clinical and pathological features of toxoplasmosis in geriatric red pandas.

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Key words: *Toxoplasma gondii*, toxoplasmosis, *Ailurus fulgens*, red panda, immunohistochemistry

INTRODUCTION

The apicomplexan protozoal parasite *Toxoplasma gondii* is found worldwide. Domestic cats and other Felidae are the definitive hosts which produce oocysts. Mammals, including Felidae, and birds act as intermediate hosts. Intermediate hosts harbor tissue cysts containing bradyzoites or rapidly-dividing tachyzoites. Infection occurs with ingestion of oocysts, bradyzoites, or tachyzoites and is presumed to be life-long with active disease occurring at the time of initial infection or the reactivation of tachyzoites from tissue cysts.^{8,10} Clinical signs of toxoplasmosis vary depending on host species, immune status, and age, as well as, strain and location of replicating tachyzoites within the body.^{8,10} Toxoplasmosis has been diagnosed in a wide variety of non-domestic mammals and birds^{3,4,8,10,13,19,21,22,26}, but clinically significant disease has not been reported in red pandas.^{6,17} Red pandas have been confirmed as an intermediate host for *Toxoplasma gondii* via mouse bioassay, but infection was not confirmed histologically in the panda.³⁰ Polymerase chain reaction (PCR) was also negative in this case.

Red pandas (*Ailurus fulgens*) are herbivorous carnivores with a diet primarily of bamboo, but that may also include small mammals, eggs, birds, and berries.^{1,20} Their distribution in Asia is closely associated with temperate forests having bamboo thickets in the Himalayan ecosystem.²⁰ Due to environmental changes, they have recently been added Appendix I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and many are kept in captivity for breeding.⁵ In captivity, the median life expectancy is 11.5 years and they are considered geriatric when older than 10 years of age.^{2,6} This report outlines the clinical signs and pathologic description of toxoplasmosis in four geriatric red pandas.

CASE REPORTS

Three cases of presumed toxoplasmosis were identified in captive red pandas at the Chattanooga Zoo (Chattanooga, Tennessee, USA) over a five-year period. At the time of their diagnoses, all three red pandas were housed in an indoor-outdoor exhibit, and had been at the facility for 2 - 15 years. Their diet consisted of Mazuri brand Leaf Eater Biscuits (Mazuri Exotic Animal Nutrition, St. Louis, MO 63166, USA), several varieties of bamboo, and occasional grapes or apples. Immunohistochemistry (IHC) for *T. gondii*, *Neospora caninum*, and *Sarcocystis* spp. was performed on stored pathology slides. Two cases were positive for *T. gondii* and the third for *Sarcosytis* spp.

A review of the Red Panda Species Survival Plan (SSP) pathology database (1992-2012) contained five additional cases of presumed toxoplasmosis. Medical records were not available for one case. Three presumptive cases were identified from the Smithsonian Conservation Biology Institute (SCBI) (Front Royal, Virginia, USA). Tissues were not available for immunohistochemical testing for one of these cases. Of the remaining two cases, one tested positive for *T. gondii* on IHC. The other was negative for *T. gondii* and was considered to be *Sarcosytis* spp. based on rosette formation and the morphology of immature schizonts (A. Cartoceti, pers. comm.) A fourth case was identified from the Cincinnati Zoo and Botanical Gardens (Cincinnati, Ohio, USA). This case had previously been confirmed with IHC. None of the suspected cases were positive for *Neospora caninum* on IHC.

The clinical histories of the four animals are summarized in Table 1 and the pathologic findings are summarized in Table 2.

Case 1

A 16-year-old male red panda presented with anorexia of three days' duration. Upon physical
80 examination, muffled lung sounds were noted over right hemithorax and thoracic radiographs
revealed a generalized bronchointerstitial pattern and a small amount of pleural effusion in the
right hemithorax. Fluid obtained via thoracocentesis was consistent with a modified transudate
with no cytological evidence of infectious organisms or neoplasia. Complete blood count (CBC)
values were within reference intervals.²⁸ Abnormal serum chemistry values included aspartate
85 aminotransferase (AST) of 204 U/L (reference interval²⁸ = 30-147 U/L) and chloride of 90
mEq/L (reference interval²⁸ = 92-122 mEq/L).

Treatment was initiated with dexamethasone (DexaJect SP, 4 mg/mL, Henry Schein,; 0.1
mg/kg i.m. once) and 200 mL lactated Ringer's solution (Lactated Ringer's Injection, USP,
Hospira, Lake Forest, IL 60045, USA) s.c. Antibiotic therapy was started with enrofloxacin
90 (enrofloxacin antibacterial injectable solution, 2.27%, Putney, Northwich, CW9 7UA, UK; 4.9
mg/kg) i.m. then continued p.o. (22.7 mg enrofloxacin flavored tablet, Putney; 4.9 mg/kg s.i.d)
and clindamycin (125 mg clindamycin capsules, USP, Lannett, Philadelphia, PA 19154, USA;
27.2 mg/kg p.o. b.i.d). The panda rapidly deteriorated over the next two days with worsening
dyspnea and tachypnea, before euthanasia was performed.

95 On gross necropsy examination the animal was overweight (body condition score 4/5) with
mild muscle wasting. Sternal, retropharyngeal, and tracheobronchial lymph nodes were enlarged.
Multifocal to coalescing white-tan nodules were found throughout all lung lobes. The lungs were
firm and congested. The liver was diffusely yellow to tan with rounded edges.

Histologically, intracellular and extracellular protozoal organisms were within the brain,
100 heart, lung, liver, lymph nodes, spleen, and adrenal glands. Myocardial degeneration, necrosis,

and loss with aggregates of lymphocytes and plasma cells were associated with cardiomyocytes containing numerous 2 μm oval to elongate tachyzoites (Figure 1A). Within the lung, macrophages and bronchial epithelial cells multifocally contained previously described protozoal tachyzoites. Multifocal areas of necrosis were throughout the cerebrum and cerebellum, spleen, lymph nodes, adrenal glands, and liver. Macrophages in these organs often contained previously described protozoal organisms.

A section of lung tissue was digested with proteinase K (20 mg/mL) overnight at 55 °C in 200 mL lysing buffer and the DNA extracted using a commercial kit (Qiagen, Valencia, CA 91354, USA). Extracted DNA was used in a *Toxoplasma gondii*-specific PCR. A diagnostic repetitive 529-bp non-coding region was amplified with 5 μL DNA in a 25- μL reaction with the use of primers TOX4 and TOX5.¹¹ *Toxoplasma gondii* PCR of lung was positive and amplified the 529-bp non-coding region.⁹

Serological testing was performed on lung fluid using the modified agglutination test (MAT).⁷ The lung fluid was positive for IgG (512) (reference interval (H. Wyrosdick, pers. comm.) - ≥ 32 considered positive for *T. gondii*) and negative for IgM (<512) (reference interval (H. Wyrosdick, pers. comm.) - ≥ 512 is suspect for *T. gondii*) antibodies.

Case 2

A 16 year old male red panda with a chronic history of intervertebral disc disease between the 2nd and 3rd lumbar vertebrae, presented for sudden and severe weakness, anorexia, and mild dyspnea. Radiographs were considered normal, except for collapse of the intervertebral disc space between the 2nd and 3rd lumbar vertebrae. Abnormal CBC values included a basophil count of 0.48×10^3 (reference interval²⁸ = $0.000-0.427 \times 10^3$) and an eosinophil count of 0.58

x10³ (reference interval²⁸ = 0.010-0.496 x10³). Abnormal serum chemistry values included alanine aminotransferase of 263 U/L (reference interval²⁸ = 17-169 U/L). Humane euthanasia
125 was performed the same day. MAT for *T. gondii* was performed on a stored frozen serum from a year previous and a fresh serum sample. Results were ≥ 8192 for IgG and > 512 for IgM for both samples.

On gross necropsy the animal was overweight (body condition score 4/5). The lungs were diffusely firm and contained multifocal to coalescing 0.1 to 2.0 cm in diameter yellow-tan
130 nodules that extended into the parenchyma of all lung lobes. Similar nodules were within all lobes of the liver. The intervertebral disk space between the 2nd and 3rd lumbar vertebrae was markedly narrowed. Bridging spondylosis was present on the ventral vertebral bodies of the 2nd and 3rd lumbar vertebrae.

Histologically, intracellular protozoal organisms were within the lung and liver. Alveolar
135 macrophages were occasionally distended by numerous 2 μ m oval to elongate protozoal tachyzoites (Figure 1B). Multifocal to coalescing, random areas of coagulative necrosis and increased numbers of lymphocytes, plasma cells, and neutrophils were throughout the liver. Hepatocytes adjacent to areas of necrosis occasionally contained numerous previously described intracytoplasmic tachyzoites (Figure 1C). Wallerian degeneration characterized by matter
140 vacuolation with occasional swollen axons (spheroids) and multifocal clusters of lymphocytes and eosinophils were within the spinal cord.

Immunohistochemistry for *Toxoplasma gondii* (Polyclonal *T. gondii* antibodies; 1:2,000 dilution; University of California-Davis, Davis, CA 95616, USA) showed scattered moderate to

strong intracellular and extracellular positive labelling within areas of necrosis within the liver
145 (Figure 1D).

Case 3

A 17 year old male red panda presented with gradual exophthalmos of the left eye over the
course of two months. Physical exam found decreased body condition and left ocular corneal
pigmentation, but no other significant findings. An aspirate of the left globe yielded
150 serosanguinous fluid, which was submitted for cytology and culture. Amikacin (Amikacin
sulfate, 250 mg/ml, Henry Schein; 15 mg/kg) was infused into the globe, and penicillin G
procaine (Combi-Pen-48, 300,000 units/mL, Bimeda, Inc., Oakbrook Terrace, IL 60181, USA;
22,000 units/kg s.c.) was administered. Cytology revealed neoplastic cells consistent with
melanoma. Euthanasia was performed due to advanced age and poor prognosis.

155 On gross necropsy the red panda was in good body condition. The liver was noted with
occasional pale, round foci up to 0.9 cm in diameter, and a single cyst 5 cm in diameter
containing clear fluid was noted in the right lateral hepatic lobe. The kidneys had several cortical
cysts measuring up to 0.7 cm in diameter that contained clear fluid. The left eye contained a soft
tan-brown mass that filled the anterior and posterior chambers as well as most of the vitreous
160 chamber, and displaced the lens against the retina. The right eye was grossly normal.

Histopathology confirmed the left ocular mass as a malignant melanoma. The right eye was
noted with aggregates of mononuclear inflammatory cells, predominantly plasma cells in the
choroid and retina, with rare protozoal cyst-like structures. The brain was noted with multifocal
neural lipofuscinosis. Immunohistochemistry of sections of the brain and right retina were
165 positive for *T. gondii*. Pancreatitis and interstitial nephritis were also noted and were considered

significant enough to cause clinical signs if the panda had not been euthanized. Retinitis and dermatitis were both considered secondary to immune suppression.

No serum was available for *Toxoplasma* serology.

Case 4

170 An 11 year old female red panda presented for weight loss of two months duration and recent lethargy. Physical examination was largely normal, except for a significant flea burden, pale mucus membranes, and an enlarged spleen. CBC abnormalities included a severe microcytic anemia with a hematocrit of 10% (reference interval²⁸ = 26-49%). Abnormal serum chemistry values included hypoalbuminemia of 2.0 g/dL (reference interval²⁸ = 2.3-3.9 g/dL) and 175 hypocholesterolemia of 84 mg/dL (reference interval²⁸ = 117-277 mg/dL). Whole-body radiographs confirmed splenomegaly and revealed multiple areas of discospondylosis. Treatment consisted of topical fipronil (Frontline 0-22#, Merial, Duluth, Georgia 30096, USA), 250 mL 0.9% NaCL (0.9% Sodium Chloride Injection, USP, Hospira) s.c., and iron dextran (iron hydrogenated dextran injection, 100 mg/mL, Henry Schein; 100 mg i.m. once).

180 Nine days later, the panda was anesthetized again due to worsening condition, anorexia, and suspected ascites. Fluid obtained via abdominocentesis was consistent with a modified transudate with no cytological evidence of infectious organisms or neoplasia. The panda did not recover from anesthesia.

On gross necropsy examination, the mucus membranes were pale, and an excessive 185 amount of flea feces was throughout the hair coat. The abdomen was distended and the subcutaneous tissues were edematous. Submandibular, axillary, sternal, and mesenteric lymph nodes were enlarged. A 1 cm long brown area was on the ventral brainstem. The liver had

rounded edges and was diffusely mottled orange-red. The spleen was enlarged and firm. An 11x2.5 cm, dark red, cystic mass compressing the right lung lobes was in the mediastinum.

190 Histologically, areas of necrosis and neutrophilic and histiocytic inflammation were within the cerebrum, brainstem, heart, liver, spleen, and skeletal muscle. Infectious organisms were not apparent with routine histopathologic examination. A focal area of necrosis with degenerate neutrophils and mononuclear cells was within the brainstem. Individual neurons within the cerebral gray matter were necrotic. Within the heart, multifocal areas of myocardium were
195 infiltrated by many neutrophils and macrophages. There was degeneration and necrosis of cardiomyocytes in these areas, The mediastinal mass was composed of lymphocytes and sheets of thymic epithelial cells, consistent with a lymphocytic thymoma.

Immunohistochemistry for *Toxoplasma gondii* showed scattered intracellular and extracellular stippled positive labeling in areas of inflammation within the heart.

200 No serum was available for serologic study.

DISCUSSION

This case series describes toxoplasmosis in four geriatric red pandas. Clinical signs in cases 1 and 2 are consistent with disseminated toxoplasmosis similar to that found in domestic dogs and
205 cats.^{8,10} Multiple organ systems were affected in both cases and signs were vague. Clinical signs in case 3 were primarily attributed to metastatic melanoma, but the more-restricted dissemination most resemble the distribution of toxoplasmosis exhibited in mink with localized infection.²³ The initial clinical signs on case 4 could be attributed to thymoma and iron-deficiency anemia;

however, the ascites and rapid deterioration despite a stable hematocrit are more consistent with
210 disseminated toxoplasmosis than thymoma. There were no consistent changes on CBC and
serum chemistry values to aid in the diagnosis of toxoplasmosis in these cases, which is similar
to findings in domestic cats.¹⁶

All four red pandas had significant pathologic changes associated with toxoplasmosis. Cases
1 and 2 had extensive necrosis and inflammation in multiple organs associated with intracellular
215 protozoal tachyzoites. Case 4 also had extensive, disseminated necrosis and inflammation.
Although protozoal organisms were not apparent on routine histopathologic examination of case
4, positive labeling for *Toxoplasma gondii* was within the heart, and inflammation in other
organs most likely represents disseminated toxoplasmosis. Cases 1 and 3 had encephalitis with
intralesional *Toxoplasma gondii* tachyzoites. Inflammation was within the spinal cord of case 2;
220 however, no protozoal organisms were present on routine histopathologic examination. Rapid
replication of tachyzoites is thought to be the cause of widespread coagulative necrosis in
toxoplasmosis,²⁹ which is supported by the high number of intracellular tachyzoites present
surrounding areas of necrosis in cases 1 and 2. Cases 1 and 2 had ventral bridging spondylosis,
and cases 2 had severe intervertebral disk disease. No mention of the spinal column was
225 mentioned in the necropsy reports of cases 3 and 4, though spondylosis was noted on radiographs
of both before euthanasia. Degenerative changes of the vertebrae are likely related to geriatric
age in these pandas.

The sources of infection for these cases are unknown. Red pandas will catch and eat small
mammals and birds, but these individuals were not known predators. Oocysts could be present
230 within the soil of the outdoor exhibit. While the current exhibit design at the Chattanooga Zoo
would prevent domestic cats from easily entering the exhibit, oocysts can survive for up to a year

in soil and organic material.¹⁰ Straw is used as bedding within the den areas which could have been contaminated with cat feces. Mulch and bedding have been suspected as the source of toxoplasmosis in captive birds.¹³ Rodents fed as part of the diet were purpose-raised and should
235 have minimal exposure to oocysts. Finally, the produce fed as part of the diet were washed before feeding, which should have minimized exposure through this route. Case 3 was located in a rural area with few feral cats, making contaminated bedding or bamboo the most likely route of exposure. Rodents are not fed, and all produce offered is washed.

Routine testing for *Toxoplasma* in red pandas has been performed at the Chattanooga Zoo on
240 stored and fresh serum. The panda which tested positive on IHC for *Sarcocystis* spp. was also serologically positive for *Toxoplasma*. Six additional pandas have all tested negative for infection. This provides an overall serologic prevalence of 33%. This is similar to the prevalence found in captive red pandas in China, but higher than reported in wild pandas in China.^{18,24} Unexpectedly, while toxoplasmosis is not generally considered a problem in red
245 pandas, 2 of 3 seropositive panda at the Chattanooga Zoo were euthanized due to clinical signs caused at least in part by toxoplasmosis.¹⁷ Different strains of *T. gondii* can vary in virulence and tropism.¹⁰ *Toxoplasma gondii* DNA was not sequenced from these cases so strain type is not known.

Immunohistochemistry was critical to diagnosing *T. gondii* infection in every case. Two of
250 the 6 cases (33%) of suspected toxoplasmosis in the Red Panda SSP pathology database for which there were medical records were negative on IHC. One IHC negative panda was positive on MAT with IgG = 2048 and IgM= 512. The IgG titer was much higher than the cut-off of 32, but this animal succumbed to *Sarcocystis* infection. This comparison of IHC and serologic results is similar to that found in sheep, where only 46.2% of MAT-positive sheep were positive for *T.*

255 *gondii* on IHC.²⁷ In sheep, the MAT titration has no bearing on the likelihood of being positive
on IHC.²⁷

Sarcocystis appears to be the primary differential diagnosis for *T. gondii* in red pandas. Both
T. gondii IHC-negative red pandas are known or considered to be infected with *Sarcocystis*.
Sarcocystis has been reported in neonatal and adult red pandas, so there may be a difference in
260 age predisposition between the protozoal species since all four of these cases were geriatric.^{6,31}
Infection with both protozoa is possible. In the previously mentioned study in sheep, 46.2% of
Toxoplasma MAT-positive sheep were positive on *Toxoplasma* IHC, but 88.5% were positive on
Sarcocystis IHC.²⁷ There was no cross-reaction in individual samples for the different
protozoa.²⁷ Dual-infections were not noted in any of the red pandas tested and no infections with
265 *N. caninum* were detected.

In domestic cats, seropositivity increases with age due to increased exposure, but clinical
disease does not become more prevalent with age.^{8,10} Active infections can be diagnosed with a
positive IgM titer, but titer may be negative in up to 20% of acute cases.⁸ In the tested cases, the
IgM titer was negative at all time points. Active infections can also be diagnosed by a four-fold
270 increase or decrease in the IgG titers, but this increase may not occur in all cases and did not
occur in case 2.¹⁶ The magnitude of a titer is unimportant in diagnosing active toxoplasmosis.¹⁵

Reactivation of tissue cysts is presumed to be the cause of active disease in these cases.
There were no apparent reasons found for the reactivation of the tissue cysts in cases 1 and 2.
Immunosuppression, either pathologic or pharmacologic, is suspected to be the cause of
275 tachyzoite formation from cysts. Neoplasia and autoimmune diseases were not present in these
cases. Case 2 was diagnosed with intervertebral disc disease. Spinal cord injury can induce

immune suppression. This syndrome is poorly understood and has not been described in humans and animal models.²⁵ Neoplastic immune suppression is suspected due to metastatic melanoma in case 3 and thymoma in case 4. Age-related changes in the immune system may have also
280 played a role in the occurrence of active toxoplasmosis, since all four cases were over 10 years of age and considered geriatric

Treatment of toxoplasmosis was attempted in case one. Treatment in domestic and zoo animals can be unrewarding.^{8,14,15,22} Clindamycin and trimethoprim-sulfonamide are considered the treatments of choice in domestic animals.^{8,21} Palatability of clindamycin can be a problem in
285 domestic animals and seemed to be a problem in this case. Treatment including clindamycin was unsuccessful. Various other treatments have been attempted with some success reported when treating macropodids with atovaquone.^{8,12} Earlier diagnosis and treatment of active disease may have been more successful.

This case series highlights the potential for clinical disease caused by *T. gondii* in red pandas,
290 even if infected years previously. The MAT can be used to monitor serum levels of IgG and IgM, but there is no evidence in this species that these results can be used to predict a clinical outbreak. Similar to other species, clinical signs are vague, and there are no consistent changes on CBC or chemistry panel analysis. Immunohistochemistry must be used to confirm a diagnosis, which makes an *ante-mortem* diagnosis difficult. Treatment, similarly to other species,
295 appears to be unrewarding. Clinicians should be aware of the potential for toxoplasmosis in geriatric red pandas, and make efforts to reduce risk of infection or conditions leading to immunosuppression in MAT positive individuals

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Figure 1. *Toxoplasma gondii* infection in two red pandas (*Ailurus fulgens*). **A.** Numerous round protozoal tachyzoites (arrow) are within the cytoplasm of a cardiomyocyte (case 1). H&E, bar = 20 μ m. **B.** Alveolar septa are replaced by fibrin and necrotic debris (*). Alveolar macrophages contain numerous round to elongate *Toxoplasma gondii* tachyzoites (arrow) (case 2). H&E, bar = 20 μ m. **C.** A hepatocyte contains elongate protozoal tachyzoites (arrow) (case 2). H&E, bar = 20 μ m. **D.** There is moderate to strong intracellular and extracellular positive labelling for *Toxoplasma gondii* within hepatocytes (case 2). Immunohistochemistry, bar = 20 μ m.

Table 1. Summary of clinical disease in geriatric red pandas (*Ailurus fulgens*) with toxoplasmosis.^a

Case No.	Age	Presenting signs	Radiographic abnormalities	Blood work abnormalities
1	16 years	Anorexia, dyspnea	Bronchointerstitial lung pattern, pleural effusion, discospondylosis	Increased AST, hyperchloremia
2	16 years	Weakness, dyspnea	Collapsed intervertebral disc space	Basophilia, eosinophilia, increased ALT
3	17 years	Exophthalmia	Discospondylosis	NP
4	11 years	Lethargy, flea infestation	Splenomegaly, discospondylosis, ascites	Anemia, hypoalbuminemia, hypocholesterolemia

^a AST = aspartate aminotransferase, ALT = alanine aminotransferase, NP = not performed

Table 2. Summary of pathologic findings in geriatric red pandas (*Ailurus fulgens*) with toxoplasmosis.^a

Case No.	Gross findings	Microscopic findings	Ancillary tests
1	Overweight, enlarged lymph nodes, yellow-tan pulmonary nodules, yellow-tan enlarged liver	Protozoal tachyzoites in brain, heart, liver, lymph nodes, spleen, and adrenal glands associated with necrosis and histiocytic inflammation	<i>Toxoplasma</i> PCR-positive <i>Toxoplasma</i> MAT- positive
2	Overweight, yellow-tan nodules in the lung and liver, narrowed intervertebral disc spaces and bridging spondylosis	Protozoal tachyzoites within macrophages in the lung and liver, associated with necrosis and histiocytic inflammation; white matter degeneration and eosinophilic inflammation in the spinal cord	IHC of liver- <i>Toxoplasma</i> -positive; <i>Sarcocystis</i> and <i>Neospora</i> - negative <i>Toxoplasma</i> MAT-positive
3	Pale foci in the liver, focal hepatic cyst, cortical cysts in the kidney, tan-brown mass in the left eye, right eye apparently normal	Left eye malignant melanoma, right eye retinitis and choroiditis with protozoal cysts, pancreatitis and interstitial nephritis and bacterial dermatitis	IHC of brain and right retina- <i>Toxoplasma</i> -positive; <i>Sarcocystis</i> and <i>Neospora</i> - negative
4	Flea dirt, distended abdomen, enlarged lymph nodes, enlarged liver and spleen, focal brown area on brainstem, cystic mediastinal mass	Necrosis and neutrophilic and histiocytic inflammation in the cerebrum, brainstem, heart, liver, spleen, and skeletal muscle, mediastinal thymoma	IHC of heart- <i>Toxoplasma</i> -positive; <i>Sarcocystis</i> and <i>Neospora</i> - negative

^a PCR = polymerase chain reaction, IHC = immunohistochemistry. MAT = modified agglutination test

