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# Littermate cats rescued from a shelter succumbed to acute, primary toxoplasmosis associated with TOXO DB genotype #4, generally circulating in wildlife

E.E.V. Crouch[a], L.D. Mittel[b], T.L. Southard[a], C.K. Cerqueira-Cézar[c], F.H.A. Murata[c], O.C.H. Kwok[c], C. Su[d], J.P. Dubey[c,*]

[a] Department of Biomedical Sciences, College of Veterinary Medicine, Cornell University, 240 Farrier Road, Ithaca, NY 14853, USA
[b] Animal Health Diagnostic Center, Department of Population and Medicine, College of Veterinary Medicine, Cornell University, Ithaca, NY 14850, USA
[c] United States Department of Agriculture, Agricultural Research Service, Beltsville Agricultural Research Center, Animal Parasitic Diseases Laboratory, Beltsville, MD 20705, USA
[d] Department of Microbiology, The University of Tennessee, Knoxville, TN 37996. USA

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ABSTRACT

Cats are important in the epidemiology of *Toxoplasma gondii* infection because they are the only hosts that can excrete the environmentally resistant oocysts in the environment. Although exposure is common (approximately 30% of cats in the USA), clinical toxoplasmosis is relatively rare. Here, we report overwhelming disseminated toxoplasmosis in two litter mate 8-week-old kittens, thought to have acquired toxoplasmosis postnatally. Five domestic shorthair kittens, approximately 2–3 weeks of age, and the queen were found in upstate New York by a rescue group in spring of 2018. The kittens and queen were placed in a foster home for approximately 4–5 weeks and then transferred to a shelter. Two kittens died unexpectedly following a short illness. Postmortem examination of the two deceased kittens revealed overwhelming toxoplasmosis and the presence of entero-epithelial stages in small intestine, suggestive of recent ingestion of infected tissues. Antibodies to *T. gondii* were found in the deceased kittens and the queen but not in the three asymptomatic littermate kittens. No obvious cause of immunosuppression was demonstrated. Genetic typing of *T. gondii* from DNA extracted from liver and lungs of both kittens revealed Toxo DB #4 genotype, commonly found in wildlife. Owners and veterinarians should be aware of dangers of feeding raw meat to cats and contact with infected cat feces. Procedures to safely handle *T. gondii* infected feces in hospital setting are outlined.

## 1. Introduction

Toxoplasmosis in cats is of clinical and public health importance because felids (domestic and wild) are the only hosts that can excrete the environmentally resistant oocysts that can survive outdoors for months [1]. A cat can excrete millions of oocysts in a few days, and cats can excrete oocysts more than once in their life [2–4].

Although clinical toxoplasmosis in cats is relatively rare, cats of any age can die of toxoplasmosis [5]. Cats can acquire *T. gondii* infection by eating infected animal tissues, by ingestion of sporulated oocysts, or transplacentally [1]. Transplacental toxoplasmosis is considered uncommon in cats and epidemiological data suggest that most cats become infected postnatally when they begin to hunt for food [1]. Oocysts have low infectivity for cats compared to other hosts [6]. A review of felid clinical toxoplasmosis indicates that toxoplasmosis in newly weaned cats (8–10 weeks old) is rare [1,7]. Since 2010, clinical feline toxoplasmosis in cats has been reported infrequently worldwide [8–11].

In nature, approximately 30–50% of cats are exposed to *T. gondii* infection [1]. The reason why some cats develop clinical toxoplasmosis is not fully understood, although the age of the cat, concurrent infections, and immunosuppression are known to be factors. Toxoplasmosis is most severe in neonatal cats, with no difference in the severity of primary toxoplasmosis between aged cats and younger mature cats [5]. Of 100 cats with clinical toxoplasmosis, only 10 were within the 10- to 16-year-old category [5]. Concurrent infections were noted in 13 cats, but there were no cases of overwhelming toxoplasmosis associated with concurrent infections [5]. Although feline immunodeficiency virus (FIV) is related to human immunodeficiency virus and many AIDS

* Corresponding author at: USDA-ARS, Beltsville Agricultural Research Center, Animal Parasitic Diseases Laboratory, Building 1001, Beltsville, MD 20705-2350, USA.
*E-mail address:* jitender.dubey@ars.usda.gov (J.P. Dubey).

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patients die of toxoplasmosis, there are only a few cases of clinical toxoplasmosis reported in FIV-infected cats [11,12].

*Toxoplasma gondii* strains are genetically diverse. Recently, attention has been focused on atypical genotypes of *T. gondii* that have caused fatal toxoplasmosis in adult immunocompetent humans in French Guiana [13]. Whether *T. gondii* genetic makeup plays a part in the pathogenesis of clinical feline toxoplasmosis is not well understood. Few strains associated with clinical feline toxoplasmosis have been genotyped. In this respect, the fatal case reported from Switzerland [14] in a 10-year-old domestic immunocompetent cat had a type II *T. gondii* isolate, not different from *T. gondii* strains circulating in the European cat population [14]. Clinical toxoplasmosis was diagnosed antemortem in a 6-month-old cat from Beltsville, Maryland; the cat was excreting *T. gondii* oocysts; the viable *T. gondii* strain (TgCatUs9) was Toxo DB genotype type 4 [15].

Here, we report overwhelming disseminated toxoplasmosis in two littermate 8-week-old kittens, thought to have acquired toxoplasmosis postnatally. The genotype from these cats was identical to that typed from the Beltsville cat [15].

## 2. Materials and methods

### 2.1. Case history

Five domestic shorthair kittens, approximately 2–3 weeks of age, and the queen were found in Upstate New York by a rescue group in spring of 2018. The kittens and queen were placed in a foster home for approximately 4–5 weeks. Contrary to the expectations of the rescue group, the kittens and queen were housed in a shed with free access to the outdoors. It is unknown what food was available to the queen. Upon learning that the cats were not contained, the rescue group brought the kittens and queen to the permanent rescue shelter, where they were caged indoors and fed commercial cat food.

Upon arrival to the shelter on June 9, 2018, the queen and kittens were placed in isolation and the kittens were weaned. They were tested for gastrointestinal parasites, and screened for ringworm by Wood's Lamp. All kittens were treated for fleas upon arrival. Fecal examination performed at the Animal Health Diagnostic Center (AHDC) at Cornell University found *Toxocara cati* in the kittens, and *Capillaria* sp., tapeworms, and hookworms in the queen. The queen tested negative for feline leukemia (FeLV) and feline immunodeficiency virus (FIV). The queen was treated orally with fenbendazole for 14 days, vaccinated and spayed. The kittens were dewormed with fenbendazole. Vaccines were administered to kittens 6 days after arrival at the shelter due to concern of a possible upper respiratory tract infection at admission.

The smallest of the 5 kittens (Kitten #3, male) was less playful than litter mates. Consequently, Kitten #3 was not vaccinated. At the time of the vaccinations of the other kittens, Kitten #3 was found to have lack of rear limb coordination and a temperature of 37 °C. The kitten was administered subcutaneous fluids, Clavamox®, Nutrical® and provided a heating pad for additional warmth. Kitten #3 continued to decline, became anorexic, lethargic, and "glassy-eyed"per the shelter staff. He died 8 days after arriving at the shelter (June 17, 2018). Kitten #5 developed similar clinical signs to Kitten #3 seven days post arrival, with the body temperature fluctuating between 38.5 and 39.7 °C, and she died 9 days post arrival (June 18, 2018). The age at death was estimated to be 8–9-weeks-old.

Both kitten carcasses were stored at 4 °C prior to necropsy at the AHDC at Cornell University, College of Veterinary Medicine. The cause of death was determined to be toxoplasmosis. Upon receiving the necropsy results, the three remaining kittens and queen were started on Clindamycin as per recommended dosing by the shelter. The queen was administered Clindamycin for 21 days and was adopted out on 7 days after arrival to the shelter. The remaining kittens were treated for 28 days. Kittens received the remainder of their vaccines, were treatment for gastrointestinal parasites, and tested negative for FeLV and FIV. They were transferred to another shelter and adapted. Blood samples from the queen and the three surviving kittens were obtained on July 3, 2018 (16 days after the death of kitten #3) for *Toxoplasma gondii* testing.

### 2.2. Necropsy

Both kittens were necropsied on June 19, 2018. The female kitten (Kitten #5) was necropsied the day after death and had minimal autolysis. Samples of lung, liver, kidney, spleen, heart, stomach, urinary bladder, umbilical cord, duodenum, large intestine, brain, adrenal, pancreas, thyroid, parathyroid and adrenal glands were fixed in 10% neutral buffered formalin (NBF). Samples of brain, liver, lungs, kidney, adrenal, and spleen were collected from Kitten #3 and fixed in NBF. Additionally, pleural and abdominal effusion fluids, lungs, liver and other tissues were collected fresh and stored at −18 °C.

### 2.3. Serological testing for T. gondii antibodies

Sera and pleural cavity effusions were tested for *T. gondii* antibodies by using the modified agglutination test as described previously [1,16]. Sera were diluted 2-fold starting at 1:10 dilution. The body fluids were diluted 2-fold starting from the undiluted sample.

### 2.4. Immunohistopathological examination

Paraffin embedded sections of tissues were cut at 5 μm thickness and examined microscopically after staining with hematoxylin and eosin stain (HE). For immunohistochemistry, paraffin embedded sections were reacted with polyclonal *T. gondii* antibodies as described previously [1] and examined microscopically.

### 2.5. T. gondii molecular characterization

DNA was extracted from frozen lung and liver tissue of both kittens by using the DNA extraction kit (Quigen Inc). Multilocus PCR-Random Fragment Length Polymorphism (RFLP) typing was performed using 10 markers as described previously [17].

## 3. Results

### 3.1. Gross findings

Gross findings of both kittens included gelatinous transformation of fat, moderate to severe thoracic and abdominal effusion, severe pulmonary edema (Fig. 1A), and hepatomegaly.

### 3.2. Histopathological findings

Histologically, the liver and adrenal glands of both kittens were variably affected by multifocal, acute, random areas of coagulative necrosis. The spleen had moderate lymphoid depletion. The lesions in the brain were small and consisted of multifocal glial nodules (Fig. 2A, B). Tachyzoites were rare in lesions (Fig. 2A, B); tissue cysts were not identified. Pulmonary edema and lymphocytic interstitial pneumonia were confirmed histologically (Fig. 1B).

In the small intestines, there were multifocal areas of necrosis in the lamina propria with many tachyzoites (Fig. 3A, B). Myriad schizonts and gamonts were seen in the superficial enterocytes (Fig. 3C, D). However, much of the surface epithelium was denuded, which was attributed to both autolysis and epithelial necrosis.

### 3.3. Immunohistochemical examination

Protozoal tachyzoites were identified in every organ but were most numerous in adrenal glands. Tachyzoites in the lungs were present

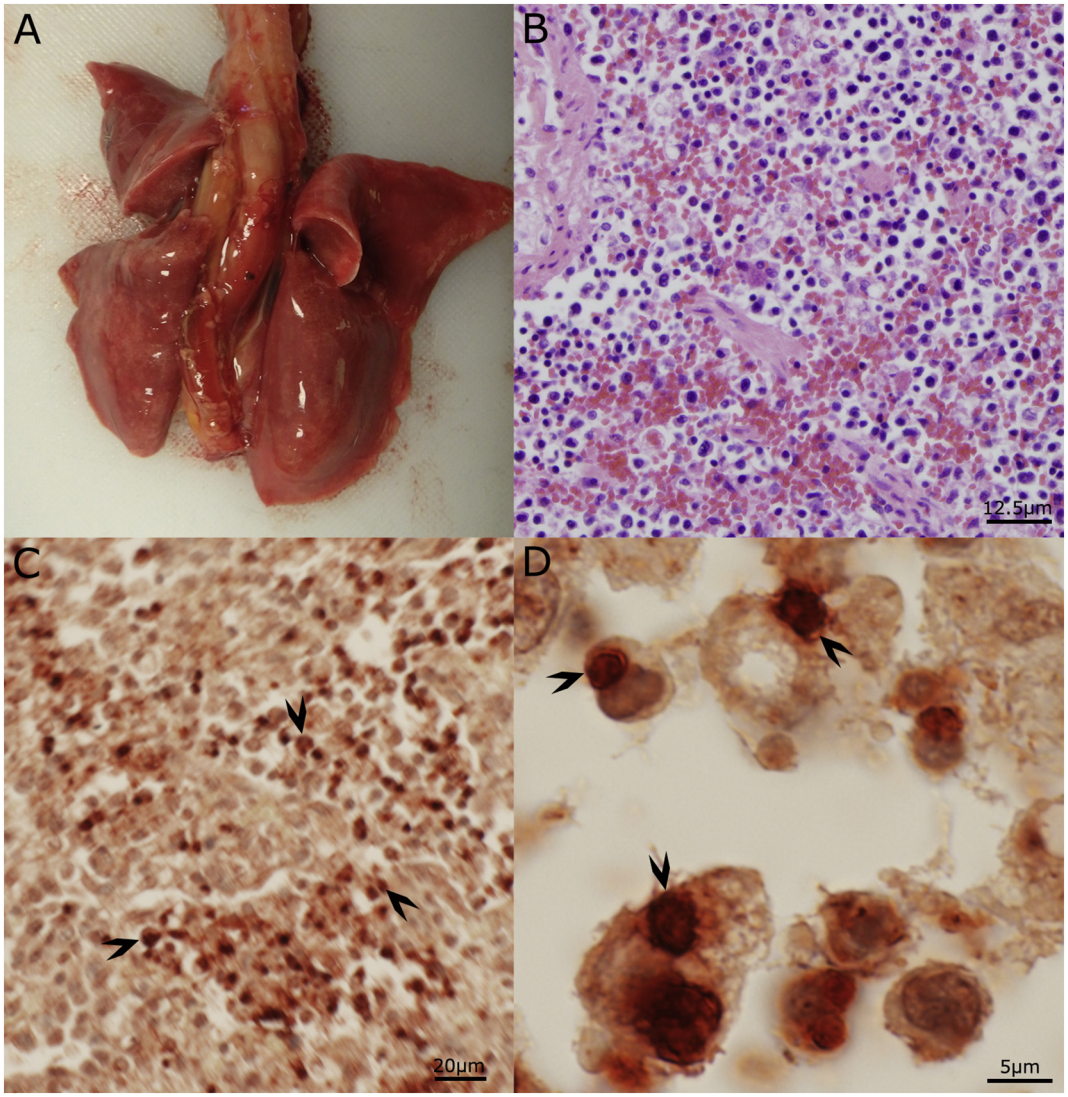


**Fig. 1.** Lungs of kittens. (A) The lung lobes are wet and heavy (pulmonary edema). (B) Mononuclear alveolar infiltrate with clear expansion of the perivascular tissue (edema). Tachyzoites are difficult to see in HE-stained section. Hematoxylin and eosin stain. (C, D) Numerous tachyzoites (arrowheads). Immunohistochemical staining with polyclonal anti-*T. gondii* rabbit antibodies.

multifocally and mostly as single organisms (Fig. 1C, D).

### 3.4. Serological findings

Antibodies to *T. gondii* were found in the queen's serum (MAT, > 1:1600) but not in 1:10 dilution of sera from the three weaned kittens that were adapted. *T. gondii* antibodies (MAT > 1:100) were detected in abdominal or pleural fluids of the two deceased kittens.

Antibodies to FIV and FeLV were not detected in any of the kittens or the queen.

### 3.5. T. gondii genotype

Both kittens had Toxo DB genotype #4 *T. gondii* (Table 1).

### 3.6. Ethics

No experiments were performed.

## 4. Discussion

Disseminated toxoplasmosis was diagnosed in two littermate kittens based on serological, immunohistochemical and molecular findings. The remaining three littermates were asymptomatic and had no evidence of *T. gondii* exposure. The queen had high levels of *T. gondii* antibodies and was asymptomatic. The diceased littermates had the same *T. gondii* genotype. Because the three remaining littermates were unaffected, these findings suggest postnatal infection. It is likely that the queen and the two diceased kittens were infected from the same source, probably an infected animal.

The lesions in both infected kittens are indicative of recently acquired infection. Both kittens had parasitic enteritis. More importantly, they had enteroepithelial stages (schizonts and gamonts) in the small intestine. Unlike tachyzoites that were present in the intestinal lamina propria and elsewhere, the enteroepithelial stages are confined to the enterocytes and are time-limited stages [18]. After the ingestion of tissue cysts in infected meat, *T. gondii* multiplies in enterocytes as schizonts and gamonts, leading to excretion of oocysts in feces; the entire cycle is terminated usually in three weeks after ingestion of infected meat. In the present cases, oocysts were not found in histological sections of intestines but may have already been excreted. Because cats are usually asymptomatic during the oocyst excretion phase, schizonts and gamonts have rarely been found histologically in cats [5].

The lesions in the lungs, liver, intestines, and brain were of especial

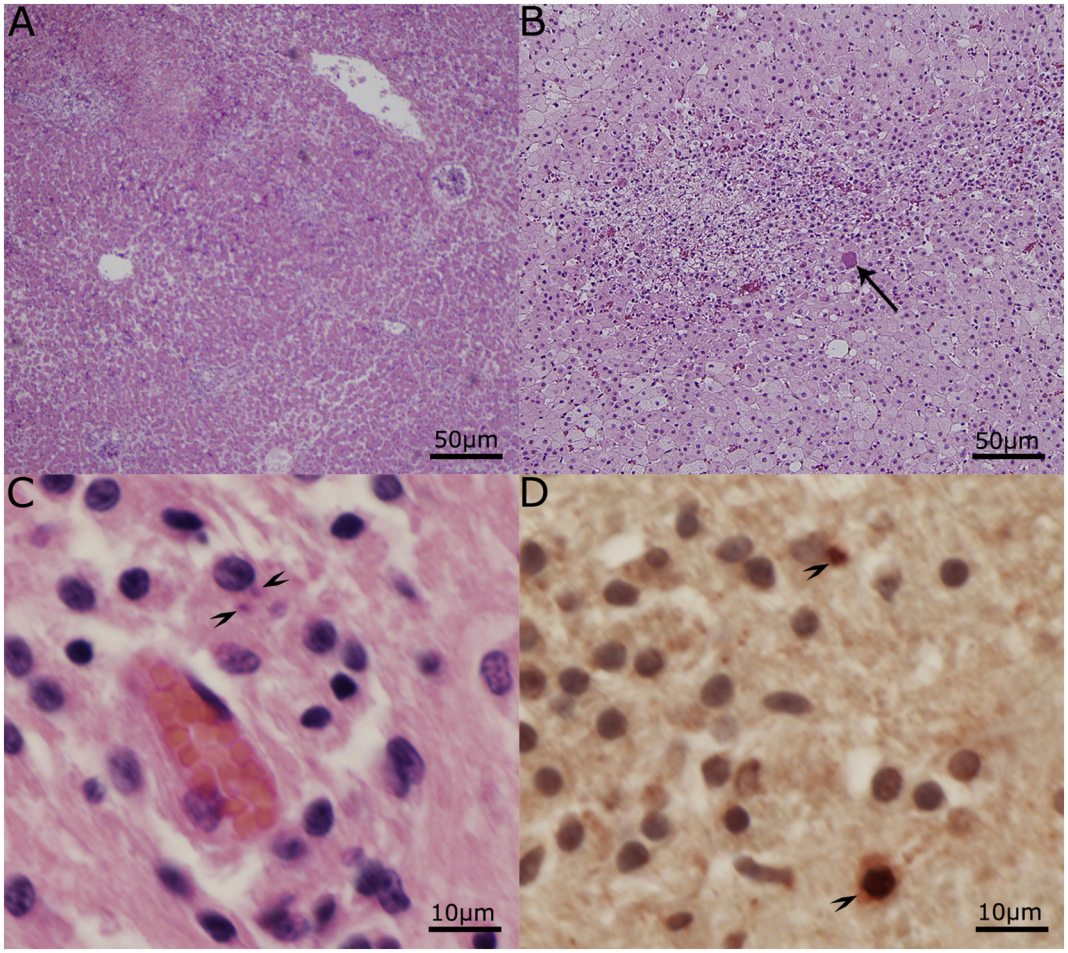


**Fig. 2.** Toxoplasmosis in liver and adrenal gland of cats. A, B, C stained with hematoxylin and eosin (HE) stain. D- Immunohistochemistry with polyclonal anti-*T. gondii* rabbit antibodies. (A) Liver with multifocal necrosis (arrow). (B) Adrenal gland with necrosis and infiltration of monouclear cells. Arrow points to a group of tachyzoites. (C, D) Mononuclear cell infiltration and tachyzoites (arrowheads).

interest. Death was attributable to pulmonary edema and necrotizing hepatitis associated with intra-lesional tachyzoites. The lesions in brain were small, but were present in all sections of brain. Only a few tachyzoites were detected in brain and tissue cysts were not found. These findings indicate recently acquired infection [18].

In the present study, both kittens had overwhelming disseminated toxoplasmosis. Such severe toxoplasmosis is rare in cats [5]. Why these cats suffered from severe illness is unknown; there was no evidence of immunosuppressive etiology. The genotype of *T. gondii* may be a factor. There is a limited number of *T. gondii* that have been genotyped in North America, especially from cats. One of the reasons for lack of genotyping data is that only a few *T. gondii* are present in tissues of asymptomatic animals and not enough parasite DNA can be extracted for genetic typing. Fortunately, good quality DNA could be extracted from lungs and livers of both kittens in the present study for genotyping. A recent review of multilocus PCR-RFLP genotyping of 13 samples from cats revealed that genotype #4 was found in six cats [19]. Genotype #4 is closely related to clonal type II (collectively #1 and #3), and is considered non-virulent to mice. However, the results from this study indicate that genotype #4 strains could cause severe toxoplasmosis in kittens. The genotype in cats in the present study was identical to the pet cat in Maryland that had acute toxoplasmosis and was excreting *T. gondii* oocysts [15].

In the present study, the surviving asymptomatic cats were treated with clindamycin. Clindamycin hydrochloride (25–50 mg/kg, orally, or intramuscularly, every 8–12 h) has been used to treat experimentally [20] or naturally infected cats [21]. Clindamycin administration can depress or stop oocyst shedding in cats, if given prophylactically [20]. However, it is ineffective against tissue cysts. Thus, it cannot eliminate infection. It is unlikely that the clindamycin treatment affected the antibody responses of the remaining cats; they were seronegative and were probably never exposed to *T. gondii*.

A history of kittens having access to infected meat increases the potential of acute fatal toxoplasmosis. Although this fatal condition is not common, it should be on the differential diagnosis for veterinarians seeing acutely ill kittens about 8 weeks of age. This condition is of high concern in feral kittens that eat infected meat caught hunting or being fed by the queen. Stress may have played a part in the immune compromise of these two kittens. The stress associated with movement to a foster home, transfer to a shelter and weaning may have been a cause of a stress- associated immune compromise. A definitive diagnosis of fatal, primary postnatally acquired toxoplasmosis can only be made by histological examination of appropriately collected tissues.

Cats can excrete very large numbers of *T. gondii* oocysts in a matter of few days [1,18], without any clinical signs. At any given time, only 1% of cats have been found excreting oocysts and, as indicated earlier, there are no previous documented cases of diarrhea in cats during the time of oocyst shedding. Perhaps the greatest concern is the management of cats that may be found excreting oocysts in veterinary clinics. Practical advice to contain *T. gondii* oocysts provided earlier by Dubey and Prowell (2013) [15] is repeated here. In hospitalized situations, cats are usually housed in cages with newspaper as bedding. *T. gondii* oocysts are excreted unsporulated (not infective) in feces and it takes about a day for sporulation and infectivity to be attained. Therefore,

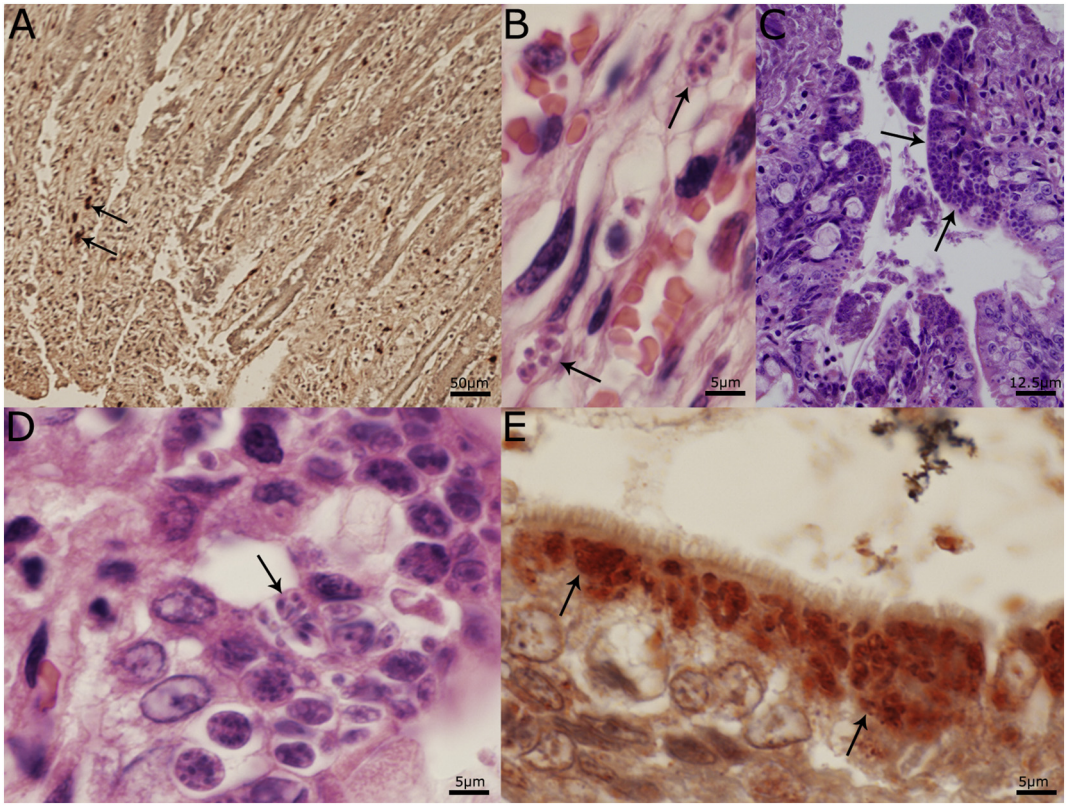


**Fig. 3.** Toxoplasmosis in intestines of cats. A, E. Immunohistochemistry with polyclonal anti *T. gondii* rabbit antibodies. B, C, D Hematoxylin and eosin stain (HE). (A) Small intestine of Kitten #5: The epithelium is denuded. Many tachyzoites are seen within the lamina propria (arrows). (B) Two groups of tachyzoites (arrows) in the lamina propria. (C, D) Intact surface epithelium of Kitten #5 heavily infected with schizonts and gamonts (arrows). (E) Schizonts and gamonts (arrows) in surface epithelium below the brush border.

**Table 1**
Genotyping of *T. gondii* isolates from 2 naturally infected cats.

| Type *T. gondii* strain/Sample ID | Genetic markers | | | | | | | | | | | ToxoDB genotype |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | SAG1 | 5′-3′ SAG2 | alt-SAG2 | SAG3 | BTUB | GRA6 | C22-8 | C29-2 | L358 | PK1 | Apico | |
| GT1 | I | I | I | I | I | I | I | I | I | I | I | #10 |
| PTG | II or III | II | II | II | II | II | II | II | II | II | II | #1 |
| CTG | II or III | III | III | III | III | III | III | III | III | III | III | #2 |
| TgCaCg1 | I | II | II | III | II | II | II | u-1 | I | u-2 | I | #66 |
| MAS | u-1 | I | II | III | III | III | u-1 | I | I | III | I | #17 |
| TgCatBr5 | I | III | III | III | III | III | I | I | I | u-1 | I | #19 |
| TgCatBr64 | I | I | u-1 | III | III | III | u-1 | I | III | III | I | #111 |
| TgRsCr1 | u-1 | I | II | III | I | III | u-2 | I | I | III | I | #52 |
| Present study | | | | | | | | | | | | |
| Kitten #3-lung | II or III | II | II | II | II | II | II | II | I | II | I | #4 |
| Kitten #3–liver | II or III | II | II | II | II | II | II | II | I | II | I | #4 |
| Kitten #5-lung | II or III | II | II | II | II | II | II | II | I | II | I | #4 |
| Kitten #5-liver | II or III | II | II | II | II | II | II | II | I | II | I | #4 |

feces, including bedding, should be removed daily to prevent sporulation. Cleaning and disinfection of infected cat cages is also problematic because none of the commonly employed disinfectants kill *T. gondii* oocysts. However, *T. gondii* oocysts are killed by exposure to temperatures higher than 70 °C. Although steaming the infected cage is an option, there is also the danger of aerosolization of fecal matter and oocysts and spreading infection on the floor. Use of hot air (eg. hair dryer, heat lamp) is one of the options to kill oocysts in the infected cage. Pouring boiling water in the infected cage is another option. During these cleaning operations, the operator should wear gloves, goggles, and protective clothing. The persons attending infected sick cats and the owners of the cat should be informed of the possible consequences of *T. gondii* infection so that they can seek medical advice.

## Declaration of Competing Interest

None.

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