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## TOXOPLASMOSIS IN PALLAS CATS

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**Abstract:** Toxoplasmosis, characterized by antibody titers up to 16 million, isolation of *T. gondii* from blood, and demonstration of cyst forms of *T. gondii* in the tissues of neonatal kittens, occurred in pallas cats (*Felis manul*) in a zoo in California. Infections were clinically inapparent, except in the kittens, which developed fatal encephalitis, pneumonitis, hepatitis, myocarditis and nephritis. A possible source of infection was feral pigeons, which once formed a major part of the cats' diet.

### INTRODUCTION

In a recent survey of exotic animals in zoos and in the care of private owners, we found serological evidence of infection with *Toxoplasma gondii* in 33% of the animals.<sup>8</sup> High prevalence of seropositive animals of the same species from the same location and high antibody titers suggested concentrations of toxoplasma infection among these captive animals.

Of special interest were pallas cats in a central California zoo that had unusually high serum antibody titers to *Toxoplasma*. The zoo had obtained these cats from an animal import dealer approximately 1 month prior to our tests.

Pallas cats, sometimes called steppe cats, inhabit the rocky, arid areas of the high mountains and steppes of Turkestan, western Siberia, Mongolia and Tibet.<sup>1,9</sup> They are approximately the size of a large domestic cat (2.7 to 3.6 kg) and have long, silvery-gray fur. The broad head with a low forehead and widely separated ears of pallas cats give them a distinctive appearance among the Felidae (Fig. 1). Although little is known

of the biology of this relatively rare and reclusive cat, their chief food is believed to be the pika, and other small rodents and birds. Pallas cats are prized by zoos and a special effort is being made to breed this species in captivity.

### MATERIALS AND METHODS

One male and two female adult Pallas cats were observed over a 10-month period and blood samples were collected at selected intervals for serotesting. The cats were handled with a net and heavy gloves. On one occasion they were given 2.27 mg (per kg body weight) of ketamine hydrochloride<sup>1</sup> intramuscularly. This made them tractable for approximately 30 min. The sera were tested by the microtiter indirect hemagglutination (IHA) test using commercial<sup>2</sup> antigen.<sup>5,7</sup> The test was carried out in a clear microtiter plate and after 2 hours incubation at room temperature, the results were read over a concave mirror.

Pallas cats that died were examined by necropsy and selected tissues, fixed in

<sup>1</sup> Vetalar, Parke Davis

<sup>2</sup> Industrial Biological Laboratories Inc., Rockville, Maryland 20850.

10% formalin, were sectioned and stained with hematoxylin and eosin or with periodic acid-Schiff, methenamine-silver and gram-Weigert's stains for histopathologic examination.

Tissues were tested for the presence of *T. gondii* by inoculating *Toxoplasma*-free mice intraperitoneally with 1.0 ml of a 20% tissue suspension in 0.85% saline. The mice were bled by cardiac puncture after 30 days and the sera were tested for specific antibodies.

Feral pigeons (*Columbia livia*) were captured on the zoo grounds by the use of grain baits containing 3% alpha-chloralose. Blood samples for serology were taken from either the brachial or jugular veins and brain tissues were inoculated into mice.

#### RESULTS

The serum antibody titers of the pallas cats are summarized in Table 1. The male cat had a steady increase in IHA antibody titer to a high of 1:131,072 in January, 1974. No obvious signs of illness were seen during the period of ob-

servation. In August, 1973, cat #2 was pregnant and several fetuses were palpable. By September she had reached a peak antibody titer of 1:16,777,216. In October, however, the fetuses could no longer be palpated. It appeared that the fetuses had been aborted and eaten, or possibly resorbed sometime in September.

Cat #3 was not bled in July because she was in advanced pregnancy and we chose not to disturb her. However, titers of 1:128 were detected in August and September. In July, three kittens in apparently good health were born. At 6 weeks of age, however, the kittens became ill with clinical signs referable to the central nervous system, which included left head tilt, and died within 2 to 3 days.

Four months after giving birth to the kittens, the dam died. Death was apparently the result of wounds incurred in fighting. Serum from heart blood taken during necropsy had a titer of 1:16,384 for *Toxoplasma*. Although toxoplasmosis was not the apparent cause of death, *T. gondii* was isolated by mouse inoculation of blood from the cat's heart.



FIGURE 1. Adult pallas cat.

TABLE 1. Indirect hemagglutination antibody titers to *Toxoplasma gondii* in pallas cats.

Cat #	Sex	Month and titer <sup>1</sup>						
		July, 1973	Aug	Sept	Oct	Nov	Jan, 1974	May
1	male	≥4096	8,192	32,768	32,768	ND	131,072	131,072
2	female	≥4096	32,768	16,777,216	524,288	ND	8,388,508	32,768
3	female	ND	128	218	ND	16,384 <sup>2</sup>	—	—

<sup>1</sup> Reciprocal serum titer  
<sup>2</sup> Post mortem blood sample  
ND = not done

### Pathology

Necropsy and histopathology of two of the kittens revealed multiple foci of hepatic necrosis and nonsuppurative hepatitis, severe interstitial pneumonia with focal necrosis, focal interstitial nephritis, marked reticuloendothelial hyperplasia in spleen and lymph nodes, focal myocardial necrosis and nonsuppurative myocarditis, focal subacute nonsuppurative necrotizing encephalomyelitis and nonsuppurative meningitis. In one kitten, the cortices of long bones were extremely thin. *Toxoplasma* organisms were evident in sections of the liver, lung, kidney, brain and spinal cord. The lesions were typical of acute to subacute toxoplasmosis (Fig. 2, 3 and 4).

The pathological findings on the dam (female cat #3) were extensive suppurative and necrotizing cellulitis and myositis involving the subcutis and superficial muscles of the left side of the head and neck, over the left scapula and

humerus and extending down the left foreleg. Many bacteria, believed to be *Clostridium* spp. were seen in histology. These lesions probably resulted from bite wounds, since there were multiple widespread skin wounds. Careful observation of all other tissues, including numerous brain sections, did not reveal *Toxoplasma* organisms even though there was mild encephalitis characterized by nonsuppurative perivascular cuffing. Mice inoculated with heart blood collected at necropsy responded with IHA titers exceeding 1:256. Attempts to isolate the organism from the cat's brain tissues were negative.

The pallas cats had a preference for eating fresh-killed pigeons to the point that other food was rejected. However, over a period of 2 to 3 months, the cats were fed increasing portions of commercial diet until they became accustomed to it. Their present food consists entirely of commercial feline diet.<sup>[3]</sup>

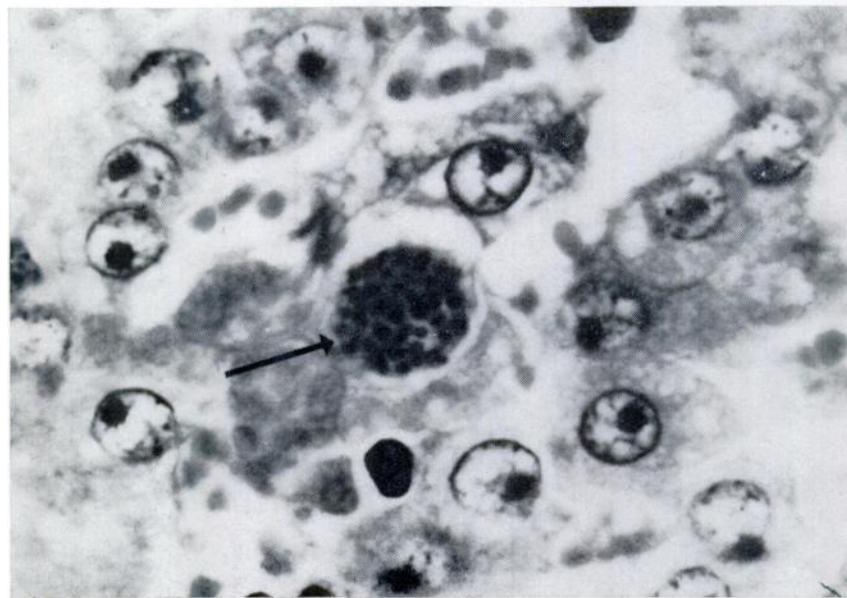


FIGURE 2. *Toxoplasma gondii* (Arrow) in liver of pallas kitten, H&E X 1000.

[3] Zupreem Feline diet, Hill Packing Co., Topeka, Kansas.



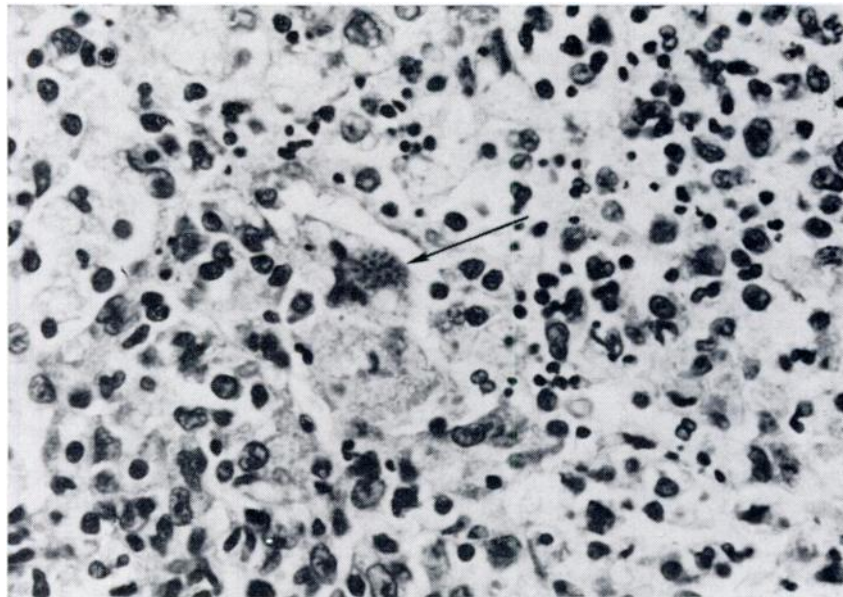


FIGURE 3. *Toxoplasma gondii* (Arrow) in lung of pallas kitten with severe interstitial pneumonia, H&E X 320.

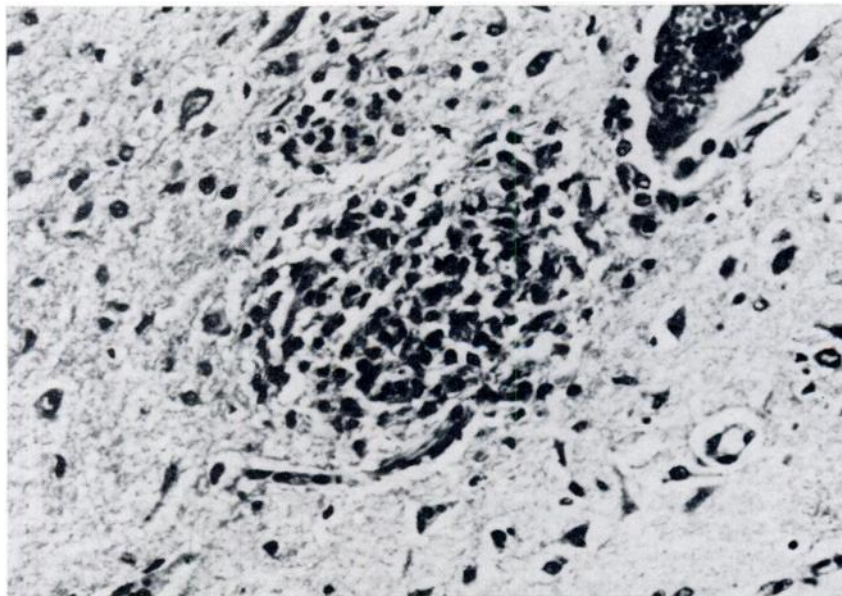


FIGURE 4. Focal encephalitis, pallas kitten, H&E X 250.

Inasmuch as *T. gondii* infection is common in pigeons, we examined the zoo population of pigeons for toxoplasmosis. Over a 3-month period, 26 pigeons were collected and tested for specific antibodies. Four (26%) of the pigeons were seropositive (three with a titer of 1:64 and one with a titer of 1:8192). Mice were inoculated with brain tissues of 14 pigeons but *T. gondii* was not isolated.

The final blood samples from the adult cats were collected during May, 1974. Both cats appeared to be in good health. The female (#2) cat was pregnant again and fetuses approximately 15 cm long were palpable. She gave birth to three kittens the next day. One of the kittens died after 16 days from what was believed to be neglect by the mother. However it had a titer of 1:32,768 at the time of death and *T. gondii* was isolated from the brain and the heart muscle by mouse inoculation. A second kitten died at 58 days of age and *Toxoplasma* cysts were observed in the heart muscle. The third kitten remained in good health.

The antibody titer of the male cat appeared to have stabilized at 1:131,072, whereas the titer of the female had decreased from 1:16,777,216 to 1:32,768 (9 2-fold decreases) since her interrupted pregnancy in September, 1973. A serum sample from one of her kittens taken 14 days after birth had a titer of 1:262,144.

#### DISCUSSION

Toxoplasmosis in pallas cats is probably similar to the disease in domestic cats. The disease is usually asymptomatic in adults, but can be fatal for kittens.<sup>2</sup> Acute fatal disease in immature animals is probably due to a delay in the development of cellular immunity.<sup>4</sup> Slower immunologic response could allow greater parasitization of the central nervous system and vital organs during the initial infection of *T. gondii* in young hosts. The pathology of the dead pallas kittens was similar to that found in domestic kittens experimentally infected with *T. gondii*. Domestic kittens developed hepatitis, myocarditis, pneumonitis, and encephalitis and died within 9 days.<sup>2</sup>

Evidence suggests that the two female cats contracted primary infections during pregnancy. This probably led to intra-uterine infections which later resulted in the death of the kittens from cat #3 and the interrupted pregnancy of cat #2. The continual high titers of the remaining two cats suggest a chronic latent infection. Pallas cat #3 apparently had a chronic infection which may have been reactivated by stress of her wounds at the time of death.

Primary toxoplasmosis during pregnancy may result in fetal infection and sometimes abortion. Congenital infection usually does not occur in subsequent pregnancies because of the immune status of the mother. Although toxoplasma cysts were present in two kittens from cat #2, signs typical of toxoplasmosis were not observed. Whether *Toxoplasma* played a role in causing the death of the kittens from the second pregnancy could not be determined.

The adult pallas cats in this study may have been infected with *Toxoplasma* before they were acquired by the zoo. A more likely explanation, however, would be that they became infected from eating pigeons harboring the cyst stage.

Pigeons have free access to the zoo and surrounding neighborhood and due to their gleaning food habits, could be bringing the infection into the zoo from domestic cats. Many reports of toxoplasmosis in pigeons have established that they are easily infected by the oocyst stage of *T. gondii* from cat feces.<sup>8,10</sup> Infection often leads to chronic infection with the cyst stage in the tissues of both birds and mammals. However, cysts have also been found in certain species of birds in which specific antibodies were not detected.<sup>8</sup> Cats appear to be more susceptible to the cyst stage of *T. gondii* than to oocysts, as indicated by lower dose of cysts required to produce a patent infection.<sup>10</sup> It is evident that zoos provide the necessary feline hosts, carnivores, and a concentration of susceptible animal species to perpetuate *Toxoplasma*. Further studies are needed to determine the modes of dispersal for this parasite in zoos.

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