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Toxoplasmosis in a Free-ranging Mink

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ABSTRACT: A free-ranging mink (*Mustela vison*), estimated to be 3 mo old, was found on the campus of Michigan State University, East Lansing, Michigan; it exhibited clinical signs of left hind limb lameness, ataxia, head tremors, and bilateral blindness. Histologically, the animal had a mild, nonsuppurative meningoencephalitis and severe chorioretinitis with intralesional bradyzoites and tachyzoites. Protozoal organisms were identified as *Toxoplasma gondii* based on histology, immunohistochemistry, and polymerase chain reaction. To the authors' knowledge, this is the first report of clinical toxoplasmosis in a free-ranging mink.

Key words: Encephalitis, mink, *Mustela vison*, retinitis, *Toxoplasma gondii*.

On 25 August 2004, a free-ranging mink (*Mustela vison*), estimated to be 3 mo old, was found on the campus of Michigan State University (MSU), East Lansing, Michigan (42°43′44.6″N, 84°28′53.7″W) near the Red Cedar River. The animal exhibited clinical signs of central nervous system disease including head tremors and ataxia, as well as left hind limb lameness. The mink was later determined to be blind in both eyes based on the absence of menace response, and running into the walls and a food dish in its temporary holding cage. The animal was euthanatized and submitted to the Diagnostic Center for Population and Animal Health (DCPAH) at MSU for complete necropsy examination. Differentials for neurologic disease in this young mink included viral diseases such as canine distemper, West Nile virus, and rabies; parasitic diseases such as toxoplasmosis, neosporosis, and *Baylisascaris* nematode migration; and cranial trauma.

Significant gross lesions were limited to the respiratory tract. The lungs were diffusely dark red, poorly collapsed, and frothy fluid leaked from the cut section. There were multifocal sharply demarcated, firm, spherical nodules measuring approximately 1.5–2.0 cm in diameter located in the caudal lung lobes. On cut section, a clear gelatinous mass exuded from the cavities. Two flukes were recovered from each cavity. The adult flukes were brown, oblong, and measured approximately 10 × 6 mm. No gross lesions were observed in other organs, including the brain and the eyes.

The most significant histologic lesions were confined to the brain and eyes. The retina of both eyes had locally extensive areas of severe inflammation and associated degeneration and disruption of all cell layers (Fig. 1); however, the retina remained attached to the choroid. The inflammatory infiltrate within the retina consisted of moderate numbers of macrophages and lymphocytes and low numbers of neutrophils. The underlying choroid had a milder infiltrate composed predominantly of mononuclear leukocytes. Within the inflamed regions, there were moderate numbers of macrophages that had phagocytosed individual protozoal tachyzoites. These protozoal organisms were basophilic, ovoid to round, and measured 2–4 μm in diameter. Scattered cells contained low numbers (4 to 12) of tachyzoites within pseudocysts in their cytoplasm (Fig. 2).

The cerebrum had a mild diffuse lymphocytic and plasmacytic meningitis and mild lymphocytic perivascular cuffing in the neuropil. In addition, there were scattered aggregates of glial cells, lymphocytes, and rare neutrophils within the neuropil of the gray matter. While in-

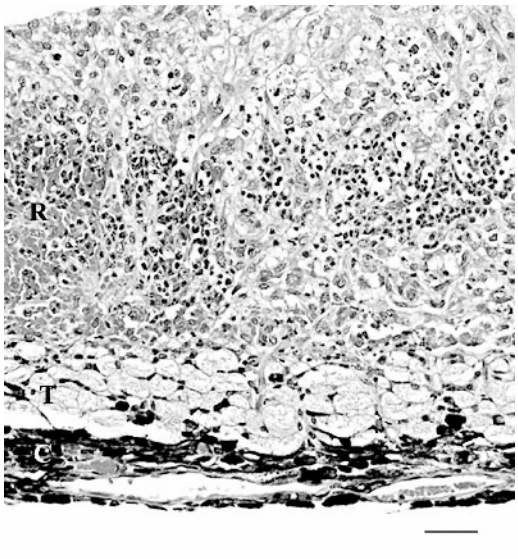

FIGURE 1. Retina (R) from a mink with severe lymphocytic infiltrates and retinal degeneration. Underlying the retina are the large foamy cells of the tapetum lucidum (T), which overlies the heavily pigmented choroid (C). Hematoxylin & eosin staining. Bar=80 μm.

dividual tachyzoites were rare compared to the number found in the retinal tissue, there were moderate numbers of larger, round, 20–60 μm diameter cysts, which

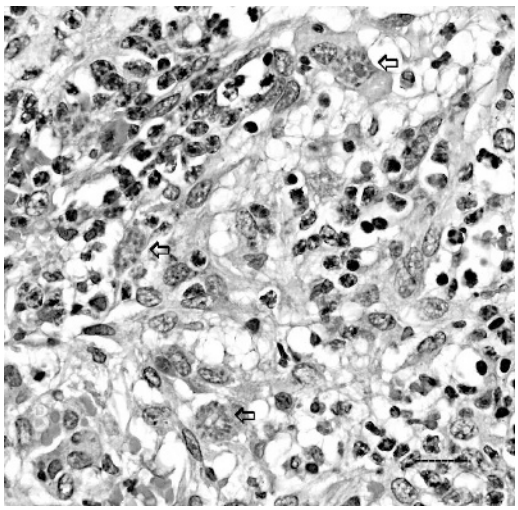

FIGURE 2. Higher magnification of retina from Figure 1. Several macrophages containing *Toxoplasma gondii* tachyzoites (arrows) are present. Hematoxylin & eosin staining. Bar=25 μm.

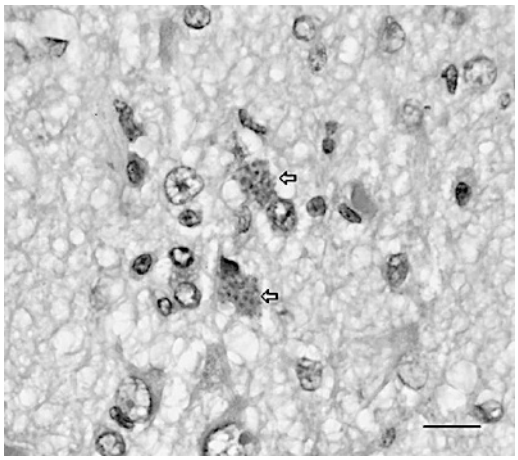

FIGURE 3. Cerebrum from a mink containing *Toxoplasma gondii* encysted bradyzoites within glial cells (arrows). Hematoxylin & eosin staining. Bar=20 μm.

were scattered throughout the gray matter associated with areas of inflammation (Fig. 3). These organisms were consistent with Apicomplexa protozoal organisms, such as the bradyzoite stage of *Toxoplasma gondii* or *Neospora caninum*.

There were numerous basophilic, oval parasitic cysts present within the sarcoplasm of myocytes in the periorbital musculature. These cysts were approximately 60–90 μm in diameter, up to several hundred μm in length, and contained numerous, intracytoplasmic, ovoid, basophilic organisms. These protozoal cysts were morphologically consistent with the sarcocyst stage of *Sarcocystis* spp. and were uniformly negative on immunohistochemical staining for both *Toxoplasma* and *Neospora*. These organisms were not associated with any inflammatory infiltrates, nor was there any evident pathology in the adjacent muscle tissue.

An additional microscopic finding unrelated to this mink's neurologic problems was a moderate to severe, multifocal, chronic pneumonia. The pneumonic areas were associated with the presence of adult and larval forms of nematodes within bronchioles and alveolar spaces, and the presence of adult trematodes within

bronchioles, and trematode eggs in alveolar spaces. The adult nematodes had a smooth cuticle, lateral cords, accessory hypodermal cords, a large intestine, and two genital tracts that contained multiple larvae; nematodes were morphologically consistent with *Protostrongylus* spp. (Bowman, 1999b). Adult trematodes were characterized by tegumental spines, vitellaria found within the parenchyma, and brown pigment in their intestinal tract. The trematode eggs measured approximately 100 × 50 μm in dimension, with unilateral opercula and round refractile yellow capsules; trematodes were morphologically consistent with *Paragonimus kellicotti* (Bowman, 1999b). There were no significant histologic lesions in the stomach, bladder, small intestine, large intestine, spleen, liver, adrenal glands, heart, or kidneys.

For immunohistochemical examination, paraffin-embedded sections were incubated with polyclonal goat antibodies against *Neospora caninum* and *T. gondii* (both diluted 1:100) (VMRD, Inc., Pullman, Washington, USA). A biotin-streptavidin immunoperoxidase labeling procedure (Dako Corp., Carpenteria, California, USA) was used to demonstrate immune reactions. Positive immunohistochemical controls included tissues that contained different protozoal parasites to which the appropriate antisera were added. For negative controls, the primary antibodies were replaced with homologous nonimmune antisera. While in our experience, these two polyclonal antibodies usually provide highly specific staining results, in this case, the bradyzoites and tachyzoites identified in the brain and retina reacted strongly for both anti–*N. caninum* and anti–*T. gondii* polyclonal antibodies. This cross-reactivity problem was only encountered with this lot of antibodies, and it necessitated additional and more specific diagnostic tests to differentiate the two protozoal agents.

Three thin slices of formalin-fixed brain tissue (4 × 4 × 0.5 mm each) from separate areas of the brain were tested. Formalin was removed by repeated washes in phosphate buffered saline, and DNA was extracted using a commercial kit (DNeasy Tissue Kit, QIAGEN Inc., Valencia, California, USA). The DNA was tested for an 115 base pair (bp) target sequence of the 35-fold repetitive B1 gene of *T. gondii* using the primers TOXOB22 and TOXOB23 (Bretagne et al., 1993). In addition, DNA was tested for a 337 bp target sequence of the Nc5 region of *N. caninum* using the primers Np21plus and Np6plus (Muller et al., 1996). The DNA from the mink brain tissue and *T. gondii* positive control DNA produced a 115 bp band after polymerase chain reaction with *T. gondii* specific primers; the DNA from brain tissue produced no bands, and *N. caninum* positive control DNA produced a 337 bp band after polymerase chain reaction with *N. caninum*–specific primers (Fig. 4).

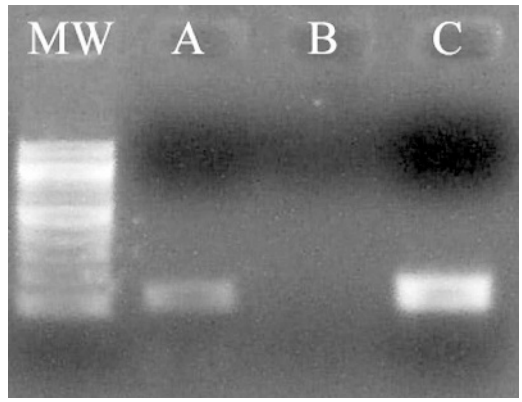

FIGURE 4. Agarose gel showing polymerase chain reaction amplicons from the brain of a mink. Lane MW contains a 100 base pair molecular weight marker; lane A brain sample is from the mink; lane B is negative control; lane C is positive DNA control for *Toxoplasma gondii*.

Toxoplasma gondii is an enteric coccidian of the domestic cat (*Felis catus*) and other members of the family Felidae. The sexual cycle of *T. gondii* occurs in cats; thus, they are the only known definitive host, and therefore only infected cats shed oocysts of this parasite in their feces. Fully

sporulated oocysts, which can be present in the cat feces, the soil, and/or contaminated water and food, are infective upon ingestion to essentially all warm-blooded mammals, including cats (Turner, 1978; Jones et al., 1997; Bowman, 1999a; Webb et al., 2005). Infection is established not only by ingestion of oocyst, but also by ingestion of tissue cysts from an intermediate host or by transplacental transmission of trophozoites from dam to fetus (Franti et al., 1976; Dietz et al., 1993). Most mammals and birds, as well as cats, can serve as intermediate hosts. *Toxoplasma* has also been identified in the milk and semen of mammals, which can then serve as a potential source of infection (Jones et al., 1997).

T. gondii is an obligate intracellular parasite. On ingestion, sporulated oocysts rupture in the intestine and release sporozoites. These enter and multiply in the cells of the intestine and associated lymph nodes to produce tachyzoites, which spread to all other tissues of the body; there they invade cells and continue to multiply (Turner, 1978; Bowman, 1999a). Tissue cysts containing bradyzoites are formed in the brain, striated muscle, liver, and other tissues; they remain viable for the life of the host and can serve as a source of recrudescence to active infection (Jones et al., 1997; Bowman, 1999a). Tachyzoite replication results in the destruction of the infected cell, which can lead to clinical disease, representative of the affected organ (Jones et al., 1997). Animal and humans with a competent immune system generally exhibit little clinical signs of illness; however immunocompromised, young, and aged individuals may develop severe illness that can result in death.

Few cases of clinical toxoplasmosis have been described in mink, and to the authors' knowledge, all previous reports have been in captive or farmed mink (Pridham, 1961; Dietz et al., 1993; Henriksen et al., 1994; Frank, 2001; Smielewska-Los and Turniak, 2004). The seroprevalence of *Toxoplasma* antibodies in both captive and free-ranging mink is well documented (Franti et al., 1976; Dietz et al., 1993; Henriksen et al., 1994; Smith and Frenkel, 1995; Smielewska-Los and Turniak, 2004) and indicates significant levels of exposure of free-ranging animals to these parasites. For example, one report describes a 66% seroprevalence to toxoplasmosis in free-ranging mink in the midwestern USA (Smith and Frenkel, 1995).

In the mink case reported here, the clinical signs and pathological findings, consisting of meningoencephalitis and granulomatous chorioretinitis with intralesional bradyzoites and tachyzoites, are consistent with toxoplasmosis. *T. gondii* infection was suspected based upon histology and immunohistochemistry, and it was confirmed with polymerase chain reaction. The significance of the pneumonia as it relates to this animal's clinical signs is undetermined. The sarcocysts in the periorbital muscles were determined to be an incidental finding. *Toxoplasma* chorioretinitis has not previously been reported in mink, but is a relatively common, local manifestation of systemic toxoplasmosis in cats, dogs, and humans (Jones et al., 1997; Lynch et al., 2004; Garweg, 2005; Webb et al., 2005), and it was likely responsible for this animal's clinical blindness. The lesions in the aforementioned species generally consist of granulomatous retinitis or chorioretinitis, as was seen with this case. In dogs, systemic toxoplasmosis is often secondary to infection with canine distemper virus (Hoskins, 2000).

While clinical toxoplasmosis, frequently accompanied by abortion in female mink, has been previously reported in captive mink, this appears to be the first report of confirmed clinical toxoplasmosis in a free-ranging mink. Since previous seroprevalence shows that mink are frequently exposed, this disease may be more common in free-ranging mink, and it simply goes unnoticed because affected animals

likely do not survive long in the wild. Alternatively, this mink may have been more likely to develop clinical signs due to its young age and heavy parasitism by other organisms, while infected adults may remain subclinical. Based upon the season of the year when this mink was found, and the animal's young age, we believe this mink had only recently separated from its dam. The advanced retinal lesions with clinical blindness suggest the possibility that toxoplasmosis was acquired in utero or during the early neonatal period. While blindness may not pose a major problem for a kit being raised by its mother, it could result in rapid starvation or death by predation once that mink is forced to survive on its own. Surprisingly, this mink was in adequate body condition, suggesting that it had only recently developed complete blindness, or perhaps had depended on scavenging food sources that required an accurate sense of smell rather than the capture of live prey, which would have required highly accurate vision.

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