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Evidence of a Limited Schizogonous Cycle for *Cytauxzoon felis* in Bobcats Following Exposure to Infected Ticks

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ABSTRACT: Schizogonous tissue stages of *Cytauxzoon felis* (Apicomplexa: Theileridae) were not observed by microscopic evaluation of impression smears of liver, spleen, lung and lymph nodes in 10 bobcats (*Lynx rufus*) from Oklahoma with naturally occurring piroplasm infections. Schizogonous stages were observed in similar tissues from experimentally-infected bobcats at 11 days postexposure to infected *Dermacentor variabilis*, but not at 30 days following tick feeding. The schizogonous cycle of this parasite appears to be short, although the bobcat appears to be a long-term carrier.

Key words: Cytauxzoon felis, bobcat *Lynx rufus*, carrier state, *Dermacentor variabilis*, ticks.

Considerable variation is documented on the occurrence and duration of the carrier status in protozoan parasites belonging to the family Theileriidae. *Theileria parva lawrencei* infections in cattle generally result in persisting piroplasm infections with an intermittent, but inconsistent, appearance of schizonts (Barnett and Brocklesby, 1966). Infections with *T. parva parva* result in a piroplasm carrier state, notably under field conditions, but with a less consistently detectable schizogonous period (Young et al., 1981). *Theileria taurotragi* in eland also produces a piroplasm and possible schizont carrier state (Grootenhuis and Young, 1981).

Recent findings demonstrate that *Cytauxzoon felis*, a uniformly fatal disease of domestic cats, is present in North America (Wagner, 1976). Studies by Kier et al. (1982) and Glenn et al. (1983) demonstrated that bobcats (*Lynx rufus*) in North America are the natural reservoir hosts of *C. felis*. Blouin et al. (1984) successfully transmitted *C. felis* from bobcats to domestic cats with the American dog tick (*Dermacentor variabilis*) confirming the association between *C. felis* and the two felid hosts. The present study was initiated to further evaluate the developmental cycle of *C. felis* in bobcats.

Between 1982 and 1984, 10 free-ranging bobcats, which harbored natural *C. felis* infections from Oklahoma, were examined. Following varying periods of confinement ranging from one to 12 mo all bobcats were killed, examined at necropsy and tissues were examined for schizogonous tissue stages of *C. felis*. Samples from liver, lungs, spleen and lymph nodes were processed by routine histologic procedures and stained with hematoxylin and eosin. Also, impression smears were made from these tissues, stained with Diff-Quick (AHS del Caribe, Inc., Aguada, Puerto Rico, 00602) stain and examined by light microscopy. Spleen homogenates from four bobcats were inoculated into domestic cats. To further evaluate the existence of schizogonous tissue stages in bobcats, a naturally infected bobcat (No., 1) with a piroplasm parasitemia of 1% was splenectomized. At splenectomy, spleen tissue was prepared for histologic evaluation and a spleen homogenate was inoculated into a domestic cat (No. 1). The splenectomized bobcat (No. 1) was placed in a wooden box with 1,000 *Dermacentor variabilis* nymphs following the procedure of Blouin et al. (1984). Engorged ticks were transferred to a humidity chamber and allowed to molt to adults.

Two additional bobcats (Nos. 2 and 3), determined to be uninfected with *C. felis* by examination of blood films and inoculation of whole blood into domestic cats, were each placed into a wooden box for 24 hr with 400 newly molted adult *D. variabilis* which had fed previously as nymphs on bobcat No. 1. Following tick attachment the bobcats were moved to a smaller cage and monitored. On day 11 post tick-attachment, a prescapular lymph node was removed from each bobcat and divided into halves. One-half of each lymph node was used for impression smears and histologic evaluation. The second half of each lymph node was homogenized in 10 ml of RPMI 1640 medium with 25 mM HEPES buffer and L-glutamine (Gibco Laboratories, Grand Island, New York, 14072, USA) and subsequently was inoculated into a domestic cat. A second prescapular lymph node was removed from bobcat No. 3 at 30 days after tick attachment and processed in the same manner, including inoculation into a domestic cat.

Schizonts of *C. felis* were not seen in the various tissues collected from the 10 naturally infected free-ranging bobcats. Inoculation of spleen homogenates from four of these bobcats into domestic cats did not cause infections with schizogonous stages as determined at necropsy 30 to 90 days following inoculation. However, all four domestic cats did develop non-clinical piroplasm parasitemias.

Schizogonous tissue stages were not observed in histologic preparations of spleen obtained from bobcat No. 1 at the time of splenectomy. A low level piroplasm parasitemia (3–5%) was produced in the domestic cat that received the spleen homogenate and persisted until the cat was killed 90 days after inoculation. Schizogonous tissue stages could not be detected histologically in liver, spleen, or lymph node of domestic cat No. 1.

Lymph node impression smears and histologic preparations from bobcats No. 2 and 3 taken at day 11 post tick-attachment contained numerous schizogonous stages in reticuloendothelial macrophages. The domestic cats (Nos. 2 and 3) that received lymph node homogenates from bobcats Nos. 2 and 3 died with clinical signs of cytauxzoonosis 11 and 14 days after inoculation. Schizonts were present in all tissues examined. Bobcat No. 2 died 19 days post tick-attachment with typical clinical sings and microscopic evidence of cytauxzoonosis.

Lymph node homogenate from bobcat No. 3, taken at 30 days post tick-attachment and inoculated into domestic cat No. 4, induced only a low level (2–3%) piroplasm parasitemia. Schizogonous tissue stages were not seen in lymph node impression smears and histologic preparations taken from this bobcat at 30 days post tick-attachment. Schizonts were not observed in lymph node impression smears or histologic sections of liver, lungs, spleen or lymph node tissues from domestic cat No. 4 when it was killed 21 days after inoculation. Bobcat No. 3 was killed 60 days after tick attachment and histologic evaluation of all tissues failed to reveal schizonts. The piroplasm parasitemia of bobcat No. 3 at 30 days post tick-attachment was 15% and dropped to 8% at 60 days.

The schizogenous tissue phase, which is initiated by sporozoite inoculation, of *C. felis* is probably of short duration. In contrast, the piroplasm carrier state initiated by piroplasm inoculation with whole blood appears to be long lived. Although the presence of extremely low level tissue schizonts cannot be dismissed, our inability to detect this pathogenic stage in 10 naturally infected free-ranging bobcats with parasitemias and in an experimentally-infected bobcat at 60 days after tick attachment supports this conclusion. However, further studies are necessary to document transmission of piroplasms between erythrocytes in the absence of the persisting pathogenic schizogonous stage.

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