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## Theileriosis in a White-tailed Deer (*Odocoileus virginianus*) Fawn

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**ABSTRACT:** A white-tailed deer (*Odocoileus virginianus*) fawn was collected in Missouri (USA) and submitted for diagnostic evaluation. Necropsy and histologic examination revealed severe *Amblyomma americanum* infestation, pronounced icterus, and marked hemosiderin deposition in the liver and spleen. Whole blood evaluation revealed a normocytic normochromic anemia and a piroplasm parasitemia of approximately 70%. The piroplasm was identified as *Theileria cervi* by polymerase chain reaction and sequencing of the V4 variable region of the 18S rRNA gene from a paraffin-embedded section of lung. Although *T. cervi* parasitemias have been commonly reported in healthy white-tailed deer, the severe parasitemia in this fawn may have contributed to overt clinical disease, perhaps as part of a combined malnutrition and parasitemia syndrome.

**Key words:** *Amblyomma americanum*, lone star tick, *Odocoileus virginianus*, Theileriosis, *Theileria cervi*, white-tailed deer.

*Theileria cervi*, an intraerythrocytic hemoprotozoan parasite of white-tailed deer (*Odocoileus virginianus*), was first reported in North America by Kreier et al. (1962). Subsequent work has implicated *Amblyomma americanum*, the lone star tick, as the principle vector for *T. cervi* (Kuttler et al., 1967; Samuel and Trainer, 1970; Barker et al., 1973; Davidson et al., 1983; Kocan et al., 1987; Laird et al., 1988; Waldrup et al., 1992). This parasite has been reported in white-tailed deer collected in Missouri (Kreier et al., 1962), Oklahoma (Barker et al., 1973; Laird et al., 1988), Texas (Robinson et al., 1967; Waldrup et al., 1992), and Alabama (Kingston, 1981; Davidson et al., 1983), as well as Arkansas, Florida, Georgia, South Carolina, Maryland, and Virginia (Davidson et al., 1983), but its role as a primary pathogen remains unclear.

Although mortality in fawns experimentally infested with heavy burdens of *Thei-*

*leria*-infected lone star ticks has been reported (Barker et al., 1973), other investigators have reported equal mortality in fawn study groups highly infested with *A. americanum* regardless of *T. cervi* infection (Hair et al., 1992). *Amblyomma americanum* infestation alone as a primary cause of neonatal fawn mortality caused by blood loss (Emerson, 1969) or tissue destruction and secondary infection (Bolte et al., 1970) has been proposed. However, several researchers have suggested that *T. cervi* may be pathogenic to deer when high population density and poor nutrition coincide with additional hardships such as secondary infection, other principle disease, or heavy parasitism (Robinson et al., 1967; Barker et al., 1973; Davidson et al., 1983). Here we report a case of a white-tailed deer fawn with an extreme *T. cervi* parasitemia, which may have contributed to overt clinical disease, perhaps as part of a combined malnutrition and parasitemia syndrome.

In June 1996, a lone white-tailed deer fawn was noted beside a refuge service road in the Peck Ranch Wildlife Management Area in Carter County, Missouri (USA; 37°02'N, 91°09'W). Three other fawns had been found dead or dying in the same area during the previous week. The fawn was observed in situ for 24 hr and was collected and held for an additional 48 hr. It was then transferred to a local veterinary clinic where it was humanely euthanized because of extreme lethargy and labored breathing. Blood and tick samples were collected and submitted with the carcass to the Southeastern Cooperative Wildlife Disease Study (SCWDS, The University of Georgia, Athens, Georgia, USA) for necropsy.

The fawn was a 6.1 kg male estimated to be  $\leq 2$  mo of age based on tooth eruption patterns, size, and weight. The carcass was thin and infested with numerous nymphal and adult *A. americanum* ticks, primarily about the head and ears. Gross examination revealed pronounced icterus, an enlarged friable liver, and edematous lungs.

Sections of liver, spleen, lymph node, heart, tongue, intestine, rumen, pancreas, cerebellum, cerebrum, lung, and ear pinna were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned to a 5  $\mu\text{m}$  thickness, and stained with hematoxylin and eosin for histologic evaluation. Aseptically collected swabs of liver, kidney, spleen, lymph node, lung, brain, heart, urine, and conjunctiva were submitted to the Athens Diagnostic Laboratory (Athens, Georgia, USA) for aerobic bacterial culture according to standard bacteriologic techniques (Ikram and Hill, 1991). Sections of kidney and liver for *Leptospira* spp. fluorescent antibody (FA) testing and sections of lung for adenovirus FA testing were submitted to the same laboratory. For adenovirus testing, frozen sections of lung were sectioned to 8  $\mu\text{m}$  and stained with commercial FA conjugates (American BioResearch, Seymour, Tennessee, USA) that represented group 1 and group 2 bovine adenovirus. Immunofluorescence for *Leptospira* spp. was performed as described (Donahue et al., 1991). Whole blood smears were stained with Diff-Quik® (Dade Diagnostics of P.R. Inc., Aguada, Puerto Rico, USA) and examined for parasites. Impression smears of the lymph nodes, liver, and spleen were stained with Giemsa and examined for schizonts.

A complete blood count performed on EDTA-anticoagulated blood submitted with the carcass revealed a normocytic normochromic anemia and a leukocytosis characterized by a mature neutrophilia. A chemistry profile performed on serum submitted with the carcass and an analysis of urine collected postmortem revealed a

prerenal azotemia and alterations in electrolyte balance. Histologic evaluation revealed marked hemosiderin deposition in the liver and spleen. The lymph nodes showed lymphoid depletion. Multiple focally extensive areas of subacute to chronic inflammation surrounding tick bites were present in the pinna. No lesions were noted in the heart, tongue, intestine, rumen, pancreas, cerebellum, cerebrum, or lung. Fluorescent antibody testing for adenovirus and *Leptospira* spp. was negative. Bacterial cultures did not reveal any significant organisms. Whole blood smears revealed *T. cervi* in 70% of erythrocytes in accordance with previous descriptions (Kreier et al., 1962; Kingston, 1981; Telford and Forrester, 1991). Schizonts were not detected in impression smears of the lymph node, liver, and spleen.

For polymerase chain reaction (PCR), DNA was extracted from 20  $\mu\text{m}$  sections of paraffin-embedded tissues as described (Roy et al., 2004) except that DNA was extracted by using the GFX Genomic Blood DNA Purification Kit (Amersham Pharmacia Biotech, Piscataway, New Jersey, USA). Primary outside amplification for the V4 variable region of the 18S rRNA gene of piroplasms was done using 5  $\mu\text{l}$  of DNA in a 25  $\mu\text{l}$  reaction containing primers RLBH-F (GAGGTAGTGACAAGAAATAACAATA) and RLBH-R (TCTTCGATCCCCTAACTTTC) as described (Gubels et al., 1999). For the nested PCR, 1  $\mu\text{l}$  of primary product was used as template in a 25  $\mu\text{l}$  reaction containing the same PCR components and primers. An amplicon of the appropriate size (approximately 500 bp) was detected in sections of lung. Negative results from other tissues were likely due to extended fixation in formalin before being embedded. Sequencing of the amplicon from lung tissue resulted in a 489 bp product that was 100% identical with sequence from *T. cervi* previously reported from white-tailed deer in Missouri (GenBank accession number AF162433).

Marked hemosiderin deposition in the

liver and spleen, together with anemia and intense icterus, indicate a severe hemolytic anemia in this fawn. *Theileria cervi* parasitemias ranging from 1% to 60% have been reported in intact and splenectomized deer of variable age (Kreier et al., 1962; Kuttler et al., 1967; Robinson et al., 1967; Barker et al., 1973; Hair et al., 1992). Reports of clinical signs associated with *T. cervi* parasitemia are not numerous; however, the 70% parasitemia observed here is similar to the 80% parasitemia reported in a translocated captive white-tailed deer fawn exhibiting clinical signs in Texas (Chae et al., 1998). Although a common and often incidental finding in healthy white-tailed deer, the severe parasitemia in this fawn may have contributed to overt clinical disease, perhaps as part of a combined malnutrition and parasitemia syndrome.

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