Failure to Detect Infection in Fallow Deer (Cervus dama) Exposed to Theileria cervi from White-tailed Deer

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Failure to Detect Infection in Fallow Deer (*Cervus dama*) Exposed to *Theileria cervi* from White-tailed Deer

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ABSTRACT: A frozen stabilate was produced from *Theileria cervi* sporozoites in salivary glands of adult *Amblyomma americanum*. The stabilate was inoculated into three fallow deer (*Cervus dama*) and two white-tailed deer (*Odocoileus virginianus*). Following inoculation, the white-tailed deer developed parasitemias as determined by blood smear examination at 11 and 13 days postexposure. Repeat examination of blood from the three fallow deer for 30 days postexposure failed to reveal observable piroplasms. These findings indicate that fallow deer are not as susceptible to the *Theileria cervi* found in white-tailed deer from North America. Thus, there are some questions regarding the taxonomic position of this organism.

Key words: *Theileria cervi*, fallow deer, *Cervus dama*, white-tailed deer, *Odocoileus virginianus*, experimental infection, *Amblyomma americanum*.

The first report of a *Theileria* sp. from white-tailed deer (*Odocoileus virginianus*) in North America was by Kreier et al. (1962). Schaeffler (1962) classified this parasite as *T. cervi* based on a comparison of the piroplasms from white-tailed deer and those described originally by Bettencourt et al. (1907) from fallow deer (*Cervus dama*). Kuttler et al. (1967a) and Barker et al. (1973) demonstrated that *Amblyomma americanum* transmit *T. cervi* to white-tailed deer with a prepatent period ranging from 14 to 21 days.

Although several serologic studies have compared antigenic responses to *T. cervi* in white-tailed deer with those induced by other *Theileria* spp. (Schaeffler, 1963; Kuttler et al., 1967b) there have been no attempts to determine if *Theileria* sp. found in North America is the same as the organism described by Bettencourt et al. (1907) from Europe. This study was undertaken in an effort to better understand the relationship between *Theileria* sp. in white-tailed deer and Old World cervids.

The deer used in this study consisted of three fallow deer and two white-tailed deer; all ≤1 yr old. All deer were born and maintained in captivity at Oklahoma State University, Deer Research Facility, Payne County, Oklahoma, USA. All deer were maintained in either individual 3 × 6 m pens or together in an adjoining 14 × 13 m outdoor pen. Uninfected, unchallenged deer of both species were housed also in the same or adjoining pens. Deer were determined to be free of *Theileria* sp. infections because they were born and raised in captivity and we were never able to demonstrate piroplasms in erythrocytes after repeated examinations of blood smears. Additionally, the experimental facility where the study was conducted is at the far western edge of the known range of *A. americanum* (Hair and Bowman, 1986) and natural transmission of *T. cervi* has not been observed in captive deer during the 10-yr existence of the facility.

In order to produce a readily available and uniform method of infection, a frozen stabilate was produced from *T. cervi* sporozoites in salivary glands of adult *A. americanum*. The stabilate was made by allowing 2,000 laboratory reared *A. americanum* nymphs to feed on a naturally infected, splenectomized white-tailed deer. At the time of tick feeding the parasitemia was increasing, beginning at 12% and increasing to 25% by tick repletion. After repletion, ticks were placed in an acaradarium and allowed to molt. At 50 days

after repletion, ticks were placed in stockinettes attached to a shorn sheep and allowed to feed for 6 days. Subsequently, ticks were removed and the dorsal surface of their exoskeleton was removed with a razor blade. Salivary glands were collected with fine forceps and placed in RPMI 1640 media (Gibco Laboratories, Grand Island, New York 14072, USA). Salivary glands were ground in a hand held tissue grinder and centrifuged at 100 *g* for 5 min. Following centrifugation, the supernate was passed through a 5-μm filter and resuspended in RPMI media to establish a volume where 1 ml was equal to a 10-tick equivalent. The samples were placed in 2-ml vials containing 10% glycerine and frozen at −70 C. Electron microscopic examination of the stabilate revealed numerous sporozoites along with host cell mitochondria and cell fragments (Fig. 1). To initiate infections, enough vials were thawed and mixed together to produce a combined volume of 1 ml which each deer received by subcutaneous injection.

Following inoculation with the stabilate, the two white-tailed deer developed piroplasm parasitemias, as determined by blood smear examination at 11 and 13 days postexposure, and remained infected for at least 6 mo. Repeated biweekly examination of blood films from the three fallow deer for 30 days postexposure failed to reveal any observable piroplasms.

Our results indicate that the organism described by Bettencourt et al. (1907) from fallow deer may not be the same organism that is found in naturally infected white-tailed deer from North America. This conclusion is supported by the fact that many *Theileria* sp. from bovids vary in their ability to infect other members of the same family and that the same host species may be infected with different *Theileria* sp. in different geographic regions (Uilenberg, 1981). Until a more complete understanding of the development of this parasite in both its tick vector and definitive host is available, the taxonomic status of *T. cervi* in white-tailed deer from North America remains unresolved.

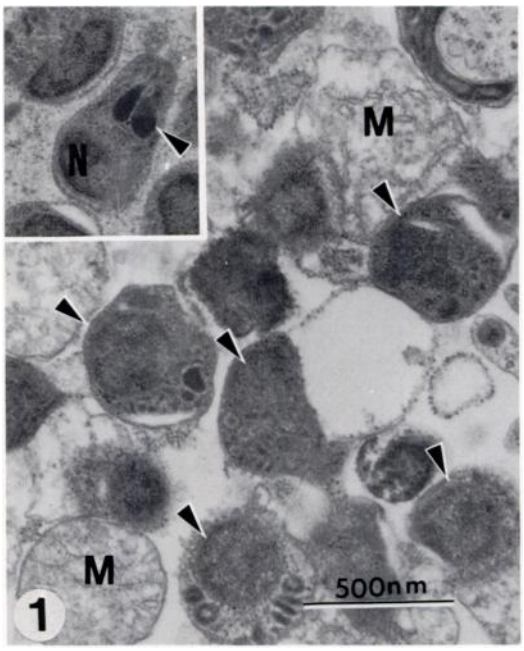

FIGURE 1. Transmission electron micrograph of tick-derived *Theileria cervi* sporozoite stabilate. Note numerous sporozoites (arrows) and host cell mitochondria (M). Insert. Individual sporozoite identifiable by typical organelles including nucleus (N) and rhoptries (arrows).

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