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SUSCEPTIBILITY OF *AMBLYOMMA AMERICANUM* TO NATURAL AND EXPERIMENTAL INFECTIONS WITH *THEILERIA CERVI*

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ABSTRACT: One hundred fifty *Amblyomma americanum* were examined between March and September 1986 from Cookson Hills Wildlife Refuge in eastern Oklahoma (USA). Of these ticks, 11% (17 of 150) were infected with *Theileria cervi*. Field-collected nymphal ticks had an 8% (3 of 37) prevalence of infection averaging 1.0 infected acini/nymph. Female ticks had a 16% prevalence of infection averaging 1.6 infected acini/female; *T. cervi* was not observed in salivary glands of field-collected male ticks. When laboratory reared *A. americanum* nymphs were allowed to feed on experimental white-tailed deer (*Odocoileus virginianus*) with varying *T. cervi* parasitemias (<1, 2, 6 and >20%), only ticks which fed on deer with parasitemias >1% became infected. Although prevalence and intensity of infection varied in the infected ticks, there was no significant difference in prevalence of infection between males and females. However, females did acquire significantly greater intensities than males. The data from these studies confirm that *T. cervi* overwinters in *A. americanum* and suggests that the prevalence, intensity and abundance of infection of *T. cervi* in ticks is influenced by the parasitemia of the deer host. Furthermore, fawns may play a more important role in the epidemiology of *T. cervi* transmission than do adult deer because of the coordination between tick activity patterns and deer fawning.

Key words: *Theileria cervi*, *Amblyomma americanum*, white-tailed deer, *Odocoileus virginianus*, susceptibility, field study, experimental infection.

INTRODUCTION

Theileria cervi is an intraerythrocytic protozoan parasite of white-tailed deer (*Odocoileus virginianus*) found throughout the southern United States. The first report of *Theileria* sp. from white-tailed deer in North America was by Kreier et al. (1962). Subsequently, numerous reports have documented its host range (Robinson et al., 1967; Barker et al., 1973) and have implicated the lone star tick (*Amblyomma americanum*) as the vector (Kuttler et al., 1967; Barker et al., 1973; Kocan et al., 1987). Although the biology of the lone star tick in eastern Oklahoma is well documented (Clymer et al., 1970; Semtner et al., 1971; Durham et al., 1976; Patrick and Hair, 1977) the prevalence of *T. cervi* infections in field-collected ticks has not been studied nor has susceptibility of ticks to infection following feeding on deer with different levels of parasitemias been investigated.

The present study was undertaken to document the prevalence, intensity and abundance of *T. cervi* in field-collected ticks (nymphs and adults) from Cookson Hills Wildlife Refuge in eastern Oklahoma (USA) and to determine the susceptibility of ticks exposed to deer with varying parasitemias of *T. cervi*.

MATERIALS AND METHODS

One hundred fifty *Amblyomma americanum* were examined after weekly collections (March to September 1986) from Cookson Hills Wildlife Refuge, located in southeastern Cherokee County and southwestern Adair County in eastern Oklahoma (USA; 35°41′N, 94°48′W). Unfed nymphs and adults were collected using a CO_2 baited trap (Wilson et al., 1972) from an ecotone (prairie–woods interface) area. Ticks were transported to the Medical Entomology Tick Laboratory (Oklahoma State University, Stillwater, Oklahoma 74078, USA) and placed in stockinette cells (ABCO Dealers, Inc., Milwaukee, Wisconsin, 53217, USA) attached to shorn domestic sheep. Ticks were allowed to feed for 6 days after which they were removed. Ticks that did not attach after the initial 24 hr were discarded. After removal, the dorsal and ventral halves of the exoskeleton of fed ticks were separated with a razor blade and the salivary glands removed with fine forceps. Salivary glands were teased onto microscope slides, air dried, stained with methyl green-pyronin (Walker et al., 1979)

TABLE 1. Prevalence, intensity and abundance of *Theileria cervi* infections in field-collected *Amblyomma americanum* from Cookson Hills Wildlife Refuge, Oklahoma.

Sex/stage	Sample size	Prevalence (%)	Intensity	Abundance
Female	87	16	10.0 (3.9)*	1.6 (6.5)*
Male	26	0	0.0 (0.0)	0.0 (0.0)
Nymph	37	8	1.0 (0.0)	0.1 (0.3)
Total	150	11	8.3 (2.0)	1.0 (3.1)

* Standard deviation.

and examined with a light microscope at 100× magnification for the presence of acini infected with *T. cervi*. To confirm the presence of *T. cervi* in the field-collected ticks, a sporozoite stabilate was prepared from salivary glands and injected into a susceptible white-tailed deer. All deer were <6 mo old. Procedures for the preparation of the sporozoite stabilate and maintenance of the deer were described previously (Kocan et al., 1987).

Two naturally infected, spleen-intact and two experimentally infected, splenectomized white-tailed deer were used to determine susceptibility of laboratory-reared *A. americanum* to infection following feeding on deer with varying levels of parasitiemia. Because spleen-intact deer >6 mo old seldom have *T. cervi* parasitemias greater than 2% (Barker et al., 1973), experimentally infected deer were splenectomized in order to produce the higher parasitemias. Spleen-intact deer with parasitemias of <1% and 2%, and splenectomized deer with 6% and >20% parasitemias were housed at Oklahoma State University (Stillwater, Oklahoma 74078, USA; Kocan et al., 1987). Each deer was placed into a wooden box (1.8 × 1.2 × 1.0 m) with screen ventilation holes along with approximately 1,000 *A. americanum* nymphs. After 12 hr, infested deer were removed to rubber-coated expanded metal cages (2.0 × 1.7 × 1.1 m) placed inside metal pans. The margins of the pans were taped with double sticky tape to prevent the escape of ticks. Every effort was made to attend to the comfort and well being of the deer; animals were removed from cages during twice daily cage cleanings and food and water were provided *ad libitum*. Replete nymphs were collected from the pans daily, placed in paper cartons and stored in a humidity chamber (90 to 98% humidity, 25 C with a 14-hr light–dark photophase; Patrick and Hair, 1977). The levels of parasitemia that were used in the different infestation groups were determined for each deer by examination of thin blood smears prior to and at the completion of tick feeding.

TABLE 2. *Theileria cervi* in field-collected *Amblyomma americanum* from Cookson Hills Wildlife Refuge, Oklahoma.

Month	Total number ticks recovered	% Infected (all ticks)	% Females infected	% Males infected	% Nymphs infected
March	33	9	17	0	0
April	53	11	13	0	16
May	27	19	27	0	0
June	17	6	0	0	0
July	6	0	0	0	0
August	14	14	0	0	14
September	0	0	0	0	0

The molted adult ticks were placed in stockinettes attached to sheep and allowed to feed for 6 days. Salivary glands were removed as before from ticks of each infestation group (<1, 2, 6 and >20% parasitemia). The prevalence, intensity and abundance of infection were determined for each group (Margolis et al., 1982). Statistical analysis using analysis of variance (ANOVA; Bancroft, 1968; PC version SAS, 6.2, 1988, SAS Institute, Cary, North Carolina 27511, USA) was used to compare intensity data following analysis using Bartlett's Test of heterogenicity (Steel and Torrie, 1980) and rank transformation (Conover and Iman, 1981). Values of $P < 0.05$ were considered significant.

RESULTS

The captive deer inoculated with the sporozoite stabilate made from salivary glands of field-collected *A. americanum* developed a <1% *T. cervi* parasitemia 11 days after exposure.

Of the 150 ticks that were examined from Cookson Hills Wildlife Refuge, the prevalence of infection with *T. cervi* for all ticks examined was 11% (17 of 150). The overall intensity of infection was 8.3 infected acini/infected tick and the abundance of infection was 0.95 infected acini/tick examined (Table 1). Female ticks had the highest prevalences, intensities and abundances. Of the 87 females collected, 16% (14 of 87) were infected with *T. cervi* with a mean of 10 infected acini/infected female, and an abundance of infection of 1.6 infected acini/female examined. The greatest intensity of *T. cervi* infection was in a field-collected female with 48 infected

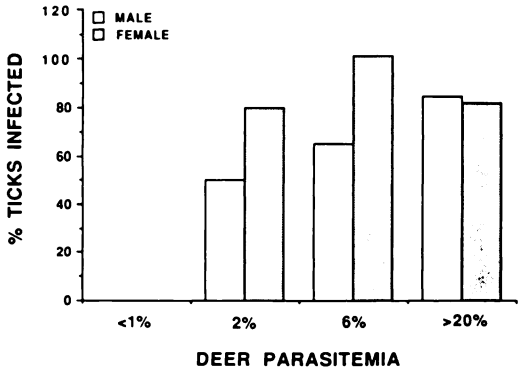

FIGURE 1. Prevalence of infection in adult *Amblyomma americanum* after exposure to white-tailed deer with varying levels of *Theileria cervi* parasitemia.

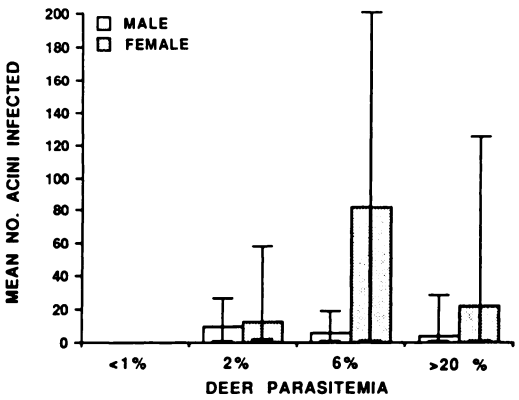

FIGURE 2. Mean intensity of *Theileria cervi* infection in adult *Amblyomma americanum* after exposure to deer with varying levels of parasitemia (vertical lines represent the range).

acini. *Theileria cervi*-infected acini were not found in the 26 males examined. Infected females were first collected during the second week of March and continuously thereafter until July. Adults were not collected in August (Table 2).

Nymphal ticks had the next highest prevalence of infection of the field-collected ticks with 8% (3 of 37) infected and with a mean intensity of infection of 1.0 infected acini/infected nymph and an abundance of 0.08 infected acini/nymph examined. Infected nymphs were collected in April and August.

When laboratory-reared ticks were allowed to feed on deer with varying levels of parasitemia, only ticks feeding on deer with parasitemias >1% became infected. In the groups in which ticks fed on deer with parasitemias >1%, both male and female ticks became infected. The greatest prevalence of infection (100%) was seen in the ticks that fed on the deer with a 6% parasitemia (Fig. 1).

Among experimentally infected ticks, both males and females became infected but the degree of infection was variable (Fig. 2). However, females had significantly higher intensities than males (Table 3). Females feeding on the deer with a 6% parasitemia had infections with a maximum of 212 infected acini per tick. Ticks that fed on deer with >20% parasitemia acquired a high intensity of infection, but the intensity of infection was not significantly different than that seen in the ticks that fed on the deer with a 2% parasitemia. The greatest intensity of infection was seen in females that fed on the deer with a 6% parasitemia. In this group, the intensity was significantly greater than any other group (Table 4). The mean intensity observed for male and female ticks from this group was 12.3 and 82.7 infected acini, respectively. This group also had the greatest variation in intensities in female ticks, ranging from 1 to 212 infected acini/tick and was the only group in which all experimentally exposed ticks became infected.

DISCUSSION

Data from field-collected and experimentally infected *A. americanum* suggest that the epidemiology of *T. cervi* is correlated with the seasonal activity patterns of the tick, environmental conditions and the *T. cervi* parasitemias in the deer. The presence of *T. cervi* in the wild tick population closely followed the adult tick spring–summer activity pattern for southeastern Oklahoma (Hair and Bowman, 1986). Collection of ticks began in early March and ended in late August; the first infected tick was observed in mid-March. Based on lone star tick feeding behavior and activity patterns (Patrick and Hair,

TABLE 3. Results of ANOVA on intensity of *Theileria cervi* infections in experimentally infected male and female *Amblyomma americanum* using rank transformed data.

Treatment	Mean square	*F* value	Probability > *F*
Sex (male versus female)	976.5	9.72	0.0030*
Intensity	3,935.0	39.15	0.0001*
Intensity–sex interaction	1,196.8	11.91	0.0011*

* Statistically significant at $P < 0.05$.

TABLE 4. Mean number of infected acini in experimentally infected male and female *Amblyomma americanum* after feeding on *Theileria cervi*-infected deer with different levels of parasitemia.

Parasitemia levels	Males ($\bar{x}$ infected acini)	Females ($\bar{x}$ infected acini)
<1%	0	0
2%	6.2 (7.7)*	12.3 (9.7)
6%	6.7 (4.0)	82.7 (26.0)
>20%	11.4 (7.7)	28.5 (9.7)*

* Standard deviation.

* Statistically significant at $P < 0.005$ using ANOVA following rank transformation.

1977) this tick either (1) was infected as a nymph while feeding the previous year and which then molted to an adult and remained in diapause until the spring of the following year or (2) was a "second year" active adult that became infected two summers previously. This finding supports and extends those of Durham et al. (1976) and Barker et al. (1973) that *T. cervi* can overwinter in female lone star ticks. Since female ticks were the most heavily infected life history stage recovered from the field, this indicates that overwintering females, which were infected as nymphs, may be important in transmitting *T. cervi* to the deer host. Although infected field-collected nymphs were observed, it presently is unknown whether or not nymphs are able to transmit the parasite to the deer host especially considering their different feeding behavior and short engorgement time.

Although no field-collected male *A. americanum* were found infected, laboratory studies showed that males are capable of acquiring heavy infections. Additionally, Kocan et al. (1988) documented that males can acquire infections and some males require less feeding time than females to produce mature sporozoites. The absence of infected males among field-collected ticks may be related to the small sample of males examined or to different feeding and behavioral patterns between male and female ticks.

Our results suggest that a minimum piroplasm parasitemia in deer is necessary for infection of ticks. Conversely, Buscher and Tangus (1986) working with the transmission of *Theileria parva* in *Rhipicephalus appendiculatus* found no correlation between the level of parasitemia of the host and the degree of infection in adult ticks which fed on these hosts. Purnell et al. (1974) working with the same host–parasite system, observed a difference in the degree of infection in adult ticks, but only in those that fed on animals with >40% parasitemia.

The present study indicates that the level of parasitemia can be an important factor in infection of ticks. In ticks allowed to feed on deer with varying levels of parasitemia, only those that fed on a deer with a parasitemia >1% became infected, suggesting that a >1% level of parasitemia of *T. cervi* is necessary for infection.

Previous studies indicate that under natural conditions, adult deer harbor parasitemias of <1% (Barker et al., 1973). However, the results of our study show that laboratory-reared lone star ticks feeding on deer with <1% parasitemia do not become infected. Therefore, it appears that the role of the adult deer as a source of infection for ticks may be minimal. However, these findings do not preclude the possibility that unknown fluctuations in parasitemia levels may occur in adult deer during the tick feeding season. Present data indicates that 1- to 2-wk-old fawns can be experimentally infected with *T. cervi*. These fawns had parasitemias as high as 27% (Barker et al., 1973). Because fawns

seldom move during the first few weeks after birth (Jacobson, 1979) and often hide in ecotone areas that provide the most suitable habitat for ticks (Hair and Bowman, 1986), fawn behavior may increase the chances of their becoming infested and as a result, infected with *T. cervi*. These fawns, with potentially higher parasitemias, much like the experimentally infected, splenectomized adult deer in the present study, could serve as ideal hosts for nymphs and the perpetuation of the *T. cervi* life cycle.

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