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Attempted Transmission of *Ehrlichia chaffeensis* among White-tailed Deer by *Amblyomma maculatum*

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ABSTRACT: A deer was needle-exposed intravenously to *Ehrlichia chaffeensis* (Rickettsiales: Ehrlichieae) in canine macrophage (DH82) cells and 7 days later was infested with laboratory-reared *Amblyomma maculatum* (Koch) (Acari:Ixodidae) nymphs for acquisition feeding. After molting, the adult ticks were allowed to feed on a naive deer. The organism was reisolated from the needle-exposed deer by cell culture and *E. chaffeensis* DNA was detected in the deer's blood by PCR. Similar isolation/recovery techniques were used for the tick-exposed deer and no evidence of infection was found. Although these findings must be considered as preliminary owing to inadequate controls, the data suggest that *A. maculatum* is probably not a suitable vector for *E. chaffeensis.*

Key words: Amblyomma maculatum, experimental infection, Gulf Coast tick, *Odocoileus virginianus,* white-tailed deer.

Ehrlichia chaffeensis, the causative agent of human (predominantly monocytic) ehrlichiosis, is a tick-borne rickettsial agent. The disease is endemic in the Ozark Plateau and adjacent regions, including portions of Oklahoma (Lockhart et al., 1996; Murphy et al., 1998). The major wildlife reservoir host for *E. chaffeensis* appears to be white-tailed deer (*Odocoileus virginianus*) (Anderson et al., 1993; Lockhart et al., 1997; Ewing et al., 1995) and the usual vector, the lone star tick, *Amblyomma americanum* (Anderson et al., 1993; Ewing et al., 1995; Lockhart et al., 1996). Domestic dogs are also known to be susceptible to infection (Dawson and Ewing, 1992; Dawson et al., 1996; Murphy et al., 1998) and several species of mice have been experimentally infected with the organism (Telford and Dawson, 1996).

As part of an ongoing study of the life history and economic importance of *A. maculatum,* 15 adult white-tailed deer from Payne County, Oklahoma, USA (36°06′N 97°03′W) were shot and examined for the presence of ticks. Ten of these deer were also tested for *E. chaffeensis* using a nested PCR method described previously (Murphy et al., 1998). No effort was made to collect the total tick population on the deer, but those recovered were speciated and counted. All 15 deer were infested with at least one life stage of *A. americanum* and 13 were found to be infested with at least one life stage of *A. maculatum*. Two of 10 deer tested by nested PCR analysis were positive for *E. chaffeensis* DNA. Because of the broad host range and comparable geographic distribution of both *A. americanum* (Semtner et al., 1971) and *A. maculatum* (Semtner and Hair, 1973) we attempted to investigate, under experimental conditions, the possibility that *A. maculatum* can also transmit *E. chaffeensis* to white-tailed deer, as has been demonstrated for *A. americanum* (Ewing et al., 1995).

Three captive-born, 5-mo-old, laboratory-reared white tailed deer were housed in facilities for wildlife species until exposed to *E. chaffeensis* or to ticks. All three fawns were found negative for *E. chaffeensis* by nested PCR analysis prior to exposure. Needle exposure and exposure to potentially infected ticks were done in tick-free isolation facilities maintained by the Laboratory Animal Resources Unit (Oklahoma State University, Stillwater, Oklahoma, USA).

Ehrlichia chaffeensis (isolate 15B-WTD-GA) was cultured in a continuous canine macrophage cell line (DH82) as previously described (Dawson and Ewing,

1992). Cultures were maintained at the University of Georgia and shipped overnight to Oklahoma. Deer A was tranquilized by intramuscular injection with xylazine (2 mg/kg body weight) and inoculated intravenously with 2 ml of infected DH82 cells containing 1.5×10^5 cells/ml. The cells were determined to be greater than 80% infected. Blood samples were collected from deer A one week prior to and immediately before exposure and on days 7, 12, 14, 18, 21, 25 after exposure. Two blood samples (EDTA; 4 ml each) were collected at each sampling period, one for culture attempts and the other for nested polymerase chain reaction (PCR) assay. Procedure for isolation attempts followed those of Ewing et al. (1995) and PCR determinations followed those of Murphy et al. (1998).

On day 7 after needle exposure, deer A was infested with 1,500 nymphal laboratory-reared *A. maculatum*. Ticks were obtained from the Tick Rearing Facility of the Oklahoma Agricultural Experiment Station, Oklahoma State University. Procedures for infestation followed those previously described (Ewing et al., 1995). Following acquisition feeding on deer A, ticks were maintained at 90% RH, 27 C, and a 14L : 10D photoperiod until transmission feeding was attempted.

Deer A became infected and was culture positive on day 7 following needle exposure and remained culture positive through day 25 when it was euthanatized. Deer A was PCR positive on day 7 and day 25 post-exposure.

During the same time period, deer B was infested with 1,000 "clean" *A. maculatum* nymphs obtained from the same rearing facility. This deer was to serve as a tick-feeding control and, if found to be *E. chaffeensis* negative, also as one of two principals (along with deer C) in the tick-transmission portion of the trial. However, deer B died during the infestation procedure, leaving us without sufficient numbers of deer for the controls we had planned upon, necessitating a modification in protocol.

Approximately 800 of the 1,500 nymphal *A. maculatum* placed on deer A fed to repletion and molted. Of these adults, 150 were placed on deer C; procedures similar to those described by Ewing et al. (1995) were followed. Blood samples for culture attempts and PCR assays were obtained prior to exposure and on days 3, 7, 10, 14, 17, 21, 28, 31, and 35 after infestation with ticks. Procedures for culture attempts and PCR assays were the same as those described for deer A. Twenty additional unfed adult ticks (previously fed on deer A) were sent to the University of Georgia where they were individually dissected and total nucleic acid extracted by standard techniques (Sambrook et al., 1989). Ethanol-precipitated nucleic acid pellets were dissolved in 50 μl of molecular biology grade water and a nested polymerase chain reaction (PCR) was performed as previously described (Murphy et al., 1998) with 1 and 10 μl of DNA template from each tick.

All attempts to culture *E. chaffeensis* from deer C were unsuccessful and no evidence of the organism's DNA was found at any of the sampling times by PCR analysis. Attempts to detect *E. chaffeensis* DNA in the 20 adult ticks that were dissected were also unsuccessful.

Although the number of deer used in this study was small, results show that the needle-exposed deer (deer A) became infected as demonstrated by both culture and PCR methods. Adult *A. maculatum* that had fed on this deer did not transmit the infection to a naive deer. Previous studies (Ewing et al., 1995) in which the same procedures were used to study *A. americanum,* demonstrated that the lone star tick can transmit *E. chaffeensis.* In contrast, at least under the experimental conditions employed in this study, *A. maculatum* was found not to transmit *E. chaffeensis.* We are unable to explain why PCR analysis was positive on days 7 and 25 post-exposure only while culture attempts were

successful at each of the six post-exposure sampling times. Likewise, it is unknown whether passage of *E. chaffeensis* in DH82 cells affected the ability of ticks to transmit the agent as has been proposed previously in similar circumstances (Ewing et al., 1995). Unfortunately there were inadequate numbers of deer available to permit the simultaneous feeding of *A. americanum* in order to control for this eventuality. These results, although preliminary in nature, indicate that *A. maculatum* that feed on white-tailed deer in endemic areas may be unable to transmit *E. chaffeensis* even if the parasite is ingested in a blood meal.

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