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Transovarial Transmission of *Francisella*-Like Endosymbionts and *Anaplasma phagocytophilum* Variants in *Dermacentor albipictus* (Acari: Ixodidae)

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Abstract

Dermacentor albipictus (Packard) is a North American tick that feeds on cervids and livestock. It is a suspected vector of anaplasmosis in cattle, but its microbial flora and vector potential remain underevaluated. We screened *D. albipictus* ticks collected from Minnesota white-tailed deer (*Odocoileus virginianus*) for bacteria of the genera *Anaplasma, Ehrlichia, Francisella*, and *Rickettsia* using polymerase chain reaction (PCR) gene amplification and sequence analyses. We detected *Anaplasma phagocytophilum* and *Francisella*-like endosymbionts (FLEs) in nymphal and adult ticks of both sexes at 45 and 94% prevalences, respectively. The *A. phagocytophilum* and FLEs were transovarially transmitted to F_1 larvae by individual ticks at efficiencies of 10–40 and 95–100%, respectively. The FLEs were transovarially transmitted to F_2 larvae obtained as progeny of adults from F_1 larval ticks reared to maturity on a calf, but *A. phagocytophilum* were not. Based on PCR and tissue culture inoculation assays, *A. phagocytophilum* and FLEs were not transmitted to the calf. The amplified FLE 16S rRNA gene sequences were identical to that of an FLE detected in a *D. albipictus* from Texas, whereas those of the *A. phagocytophilum* were nearly identical to those of probable human-nonpathogenic *A. phagocytophilum* WI-1 and WI-2 variants detected in white-tailed deer from central Wisconsin. However, the *D. albipictus A. phagocytophilum* sequences differed from that of the nonpathogenic *A. phagocytophilum* variant-1 associated with *Ixodes scapularis* ticks and white-tailed deer as well as that of the human-pathogenic *A. phagocytophilum* ha variant associated with *I. scapularis* and the white-footed mouse, *Peromyscus leucopus*. The transovarial transmission of *A. phagocytophilum* variants in *Dermacentor* ticks suggests that maintenance of *A. phagocytophilum* in nature may not be solely dependent on horizontal transmission.

Keywords

Ixodid tick; *Anaplasma*; *Francisella*-like; transovarial transmission

Dermacentor albipictus (Packard) is a North American tick (Acari: Ixodidae) commonly known as the moose or winter tick. Although the tick infests cattle, its primary hosts are members of the deer family (Cervidae), and it is best known as a parasite of moose (*Alces alces*). *D. albipictus* has a one-host life cycle, meaning that after larvae attach to a host, all subsequent life stages through female repletion are completed on that host. During winter, heavily infested moose may carry tens of thousands of ticks, but deer, elk, and caribou are less heavily parasitized (Samuel 2004). Moose that carry a heavy burden of ticks may suffer a

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decline in condition, caused by anemia and hair loss from excessive grooming. Because of their white appearance, animals afflicted in this way have been referred to as “ghost moose” (Samuel 2004). The range of *D. albipictus* was traditionally considered to encompass the subarctic regions of Canada and Alaska and the forested mountain regions of the northern and western states of the United States. However, recent surveys of ticks infesting deer have shown that *D. albipictus* occurs throughout the approximate range of white-tailed deer (*Odocoileus virginianus*) even into the southern states (Amerasinghe et al. 1992, Luckhart et al. 1992, Kollars et al. 1997, Clark et al. 1998, Oliver et al. 1999, Williams et al. 1999, Kollars et al. 2000, Samuel 2004, Cortinas and Kitron 2006). The western range of the tick also overlaps that of mule deer and black-tailed deer (*O. hemionus hemionus* and *O. hemionus columbians*, respectively), which have been shown to serve as hosts of *Anaplasma* and *Ehrlichia* spp. (Foley et al. 1998).

Ticks are hosts of a greater variety of microbes than any other blood-feeding arthropod group and are a major source of established and emerging zoonotic diseases (reviewed in Jongejan and Uilenberg 2004, Telford and Goethert 2004). Tick-borne bacteria include species of the genera *Anaplasma, Borrelia, Coxiella, Ehrlichia, Francisella*, and *Rickettsia* that are known pathogens of humans or livestock, whereas other members of those genera are apparently adapted to a relatively benign co-existence with ticks (Munderloh and Kurtti 1995, Munderloh et al. 2005). New or expanded pathogen transmission cycles that involve tick-borne bacteria are believed to arise because of natural and human-driven environmental forces such as climate change, land development, and livestock shipment that alter habitat, vector, and host interactions (Telford and Goethert 2004, Harrus and Baneth 2005, Parola et al. 2005, Jones et al. 2008). That paradigm is consistent with the increasing prevalence in the United States of human granulocytic anaplasmosis, Lyme disease, and tularemia in concert with expanding populations of deer and ticks (Madhav et al. 2004, Rand et al. 2004, Piesman and Gern 2004, Eisen 2007, Paddock and Yabsley 2007, O’Toole et al. 2008).

Despite the wide distribution of *D. albipictus* in association with expanding deer populations, its propensity to feed on livestock, and its recent importation into Europe on an infested horse (Lillehaug et al. 2002), few studies have focused on the tick’s microbial flora or its potential as a vector of zoonotic pathogens. The Lyme disease pathogen, *Borrelia burgdorferi*, was detected in *D. albipictus* populations in Connecticut and Oklahoma (Magnarelli et al. 1986, Kocan et al. 1992). Although the tick may feed on humans more often than commonly believed (Goddard 2002), it has not been shown to transmit *Borrelia* spirochetes. *Anaplasma marginale* was detected in *D. albipictus* that were suspected to transmit it in anaplasmosis-infected cattle herds in Idaho, Oregon, Utah, and Oklahoma (Zaugg 1990, Ewing et al. 1997). The tick has been shown to transmit *A. marginale* among cattle under experimental conditions (Stiller et al. 1981, Stiller and Johnson 1983). *Francisella*-like endosymbionts (FLEs) were detected in *D. albipictus* populations in Texas and the Canadian province of Alberta but were not present in ticks collected from five other locations (Scoles 2004).

In this report, we describe results of polymerase chain reaction (PCR) and sequence analyses designed to screen *D. albipictus*, one of the three most common tick species present in Minnesota and the upper Midwest, for presence of tick-borne bacteria from genera of medical and veterinary significance. This study was a continuation of our effort to characterize the bacterial community within the tick population parasitizing the state’s white-tailed deer herd (Noda et al. 1997, Massung et al. 2007), with a particular focus on ticks recovered from deer at Camp Ripley, a large military reservation in the approximate center of Minnesota. The annual state-sponsored archery hunt for deer at Camp Ripley offers an opportunity to regularly sample the bacteria community in *Ixodes scapularis* and *D. albipictus* ticks parasitizing the same deer population, to determine the host specificity of members of that bacterial community, and to eventually address issues regarding their possible interactions.

Materials and Methods

Ticks and DNA Preparation

Dermacentor albipictus ticks were collected from white-tailed deer at hunter check stations in October 2005 and 2006 at Camp Ripley, MN (latitude: 46.090975, longitude: −94.354986) and November 2006 and 2007 at St. Croix State Park, MN (latitude: 46.050145, longitude: −92.60979). Ticks were rinsed in 1:50 diluted bleach and maintained at room temperature in vials held at 97.5% humidity in glass humidors. Blood-fed adult female ticks were maintained in individual vials until completion of oviposition 17–34 d after collection. Larvae (F_1) hatched 6–8 wk later. To obtain F_2 larvae, populations of F_1 larvae shown by PCR to be infected with FLEs and *Anaplasma phagocytophilum* or infected with FLEs and uninfected with *A. phagocytophilum*, were shipped to the USDA–ARS, Animal Disease Research Unit at Pullman, WA. They were conditioned at 25°C for 4 wk under a long-day photoperiod (16-h light/8-h dark) followed by 2 wk at 15°C under a short-day photoperiod (8-h light/16-h dark) to simulate fall to winter transition and were returned to the long-day regimen to simulate transition to spring before infestation of a calf (USD–ARS hemoparasite barn at Moscow, ID, following procedures approved by the University of Idaho Institutional Animal Care and Use Committee). At day 14 after tick attachment, engorged F_1 nymphs were removed for analysis while nymphs remaining on the calf molted to adults. Adult females fed to repletion by days 22–29 and were held individually in vials in an incubator for production of F_2 larvae.

To prepare ticks for dissection of internal tissues, they were surface disinfected for 5 min in 0.5% bleach and 5 min in 70% ethanol followed by two rinses in sterile water. Unfed ticks and blood-fed adult females (after oviposition) were dissected with sterile scalpels in a laminar flow hood, and internal tissues were collected in sterile microcentrifuge tubes. DNA was isolated by incubation of the tissues overnight at 55°C in 200 μl Puregene DNA Purification System lysis buffer (Gentra Systems, Minneapolis, MN) supplemented with 16 μg/ml RNase A (Gentra Systems) and 1 mg/ml proteinase K (Fisher, Pittsburgh, PA). Samples from spent (postoviposition) females were treated with 200 μl Puregene RBC lysis solution, and those still containing blood-meal impurities, as evidenced by brown discoloration, were subjected to a second round of extraction before precipitation of DNA from the lysates and resuspension in 200 μl hydration buffer according to the manufacturer's protocol. Because that method was labor intensive and gave low yields of DNA, ticks collected in 2006 and 2007 were surface disinfected, transferred to a sterile 2-ml microcentrifuge tube containing one 3.2-mm stainless steel bead (Biospec, Bartlesville, OK) and 10 1-mm glass microbeads (Research Products International, Mount Prospect, IL) in 180 μl QIAamp DNA Mini Kit tissue lysis buffer (Qiagen, Valencia, CA), and beaten in a mini-bead beater (Biospec) for 3 min. The homogenates were held at −20°C for 5 min to reduce foaming and centrifuged at 10,000*g* for 1 min to pellet debris. The supernatants were adjusted to 1 mg/ml proteinase K and incubated overnight at 55°C, and DNA was recovered from spin columns in 200 μl elution buffer according to the manufacturer's protocol. However, DNA from spent females was eluted in 30 μl elution buffer and reincubated in 200 μl tissue lysis buffer as above for 2 h before final recovery from spin columns. Larvae, as pools of 10 each or as individuals, were homogenized in 50 μl STE buffer (100 mM NaCl; 1 mM EDTA; 10 mM Tris/HCl, pH 8.0) containing 0.75 mg/ml proteinase K by grinding in microcentrifuge tubes with sterile pestles rotated by hand. The homogenates were incubated for 1 h at 37°C, boiled 3 min to denature the proteinase K, and centrifuged at 18,300*g* for 1 min to pellet debris (Noda et al. 1997). The larval supernatants were used directly in subsequent PCR procedures.

PCR Detection of Bacterial DNA

Tick extracts were screened for DNA of *Anaplasma*, *Ehrlichia*, and *Francisella* spp. by PCR amplification of the bacterial 16S rRNA gene and for *Rickettsia* spp. by amplification of the

17-kDa surface antigen gene. The PCR reactions were catalyzed by *Taq* or GoTaq polymerases (Promega, Madison, WI) in 50 μl manufacturer's 1× reaction buffer that contained 2–3 μl of tick extract and 25 pmol of each primer (Integrated DNA Technologies, Coralville, IA). The reactions were run in a Robocycler thermocycler (Stratagene, La Jolla, CA), and the products were electrophoresed on 1.0% agarose gels and stained with SYBR Green (Molecular Probes, Eugene, OR) to visualize DNA. To verify that DNA in the tick extracts was PCR competent, we used the 16S+1/16S-1 primer pair specific for a 460-bp tick mitochondrial 16S rRNA gene product (Black and Piesman 1994).

Anaplasma/Ehrlichia DNA was detected using the PER1/PER2 primers specific for a 451-bp product (Goodman et al. 1996). Positive control reactions contained an extract of tick ISE6 cells infected with *A. marginale* isolate Am291 (Munderloh et al. 1996). *Francisella* DNA was detected by PCR using the F5/F11 primers specific for a 1,142-bp product (Forsman et al. 1994). Positive control reactions contained an extract of a *D. andersoni* tick (Baldridge et al. 2004), infected with the "DAS" FLE (Niebylski et al. 1997). Extracts were screened for DNA of *Rickettsia* spp. using the Rr17kDa1/Rr17kDa2 primers specific for a 432-bp product (Williams et al. 1992). Positive control reactions contained an extract of an *I. scapularis* tick infected with a rickettsial endosymbiont (Noda et al. 1997). All reaction cycle parameters were similar or identical to those in the references for the above primers. Amplification products of the predicted size were excised from agarose gels, purified using the QIAquick gel extraction (Qiagen), and sequenced as a necessary precaution to confirm genus/species identity (Massung and Slater 2003, Kugeler et al. 2005, Sikutova et al. 2007). Gel-purified *Taq* or GoTaq-amplified products were sequenced on an ABI 377 automated sequencer at the University of Minnesota Advanced Genetic Analysis Center. High-fidelity *PfuTurbo* Hotstart DNA Polymerase (Stratagene) amplified products from pooled larvae extracts were cloned with the Topo TA Cloning Kit for Sequencing (Invitrogen, Carlsbad CA). Sequences were aligned using the Clustal W program.

Results

Detection of Bacterial DNA in Extracts of *D. albipictus* Ticks Collected in 2005

As described below, DNA of FLEs and *A. phagocytophilum* variants was detected in tick extracts that were first confirmed as PCR competent using tick mitochondrial 16S rRNA gene primers (Black and Piesman 1994), but DNA of *Ehrlichia* and *Rickettsia* spp. was not detected. All but one, or 95%, of 20 extracts from spent adult female ticks collected from five deer at Camp Ripley, MN, in 2005 supported amplification of a product that co-migrated on agarose gels with the predicted 1.14-kbp product produced in FLE-positive control reactions. Six adult male and five nymphal ticks were also PCR positive for FLEs (Table 1). Thus, 97% of all the ticks were FLE positive. The single negative female (F5–5) was one of five ticks collected from deer 5. To test for transovarial transmission (TOT) of the FLEs, we screened extracts of two pools of 10 F_1 larvae from each of 16 ticks that oviposited in separate containment vials. The larvae had therefore never fed on a deer. Consistent with TOT, all larval extracts, including two from female F5–5 progeny, were PCR positive for FLEs (Table 1), suggesting that F5–5 itself and therefore 100% of the ticks collected from deer were FLE positive. We confirmed FLE detection by sequencing the *Taq*PCR products from four adult *D. albipictus* extracts. The sequences were identical and, when used as a query to BLASTn search the GenBank database, produced the highest alignment scores with 16S rRNA gene sequences amplified from FLEs previously detected in *Dermacentor* ticks. We sequenced 12 cloned PCR products amplified from larval pool extracts with high-fidelity *PfuTurbo* Hotstart DNA Polymerase and found that they were polymorphic at three nucleotide positons. The most common FLE sequence from the Minnesota *D. albipictus* was identical to that of an FLE detected in a *D. albipictus* tick collected from a horse in Texas (accession no. AY375395). That sequence occupied the most

distal position in an FLE phylogenetic tree rooted with *Francisella tularensis* as an outgroup (Scoles 2004).

Ten of 22 extracts from spent adult female ticks collected from the five Camp Ripley deer supported amplification of a product that co-migrated on agarose gels with the predicted 0.45-kbp product produced in *Anaplasma/Ehrlichia* spp.-positive control PCR reactions, whereas 5 adult male extracts were PCR negative and 1 of 5 nymphal extracts was positive (Table 1). Thus, 34% of the 32 tick extracts were PCR positive. Of five ticks collected from deer 5, none were PCR positive, whereas 25– 80% of ticks from the other deer were PCR positive. Consistent with TOT, 42% of extracts from two pools of 10 F_1 larvae from each of 18 ticks were PCR positive (Table 1). Sequences of the 16S rRNA gene PCR products from eight larval pool extracts produced the highest BLASTn alignment scores with *A. phagocytophilum* variants WI-1 and WI-2 (accession nos. DQ426991 and DQ426992) previously detected by PCR in white-tailed deer in Wisconsin (Michalski et al. 2006). Sequencing of 36 clones of *PfuTurbo* Hotstart Polymerase-amplified products from larval pool extracts of progeny of three females showed that 24 clones shared an identical 374 nucleotide sequence (*A. phagocytophilum* variant MN-1), nine shared a sequence (*A. phagocytophilum* variant MN-2) that differed from the first by one nucleotide, and three differed at up to three more nucleotides. An alignment (Fig. 1) of a 224-bp region of the 16S rRNA gene containing polymorphisms that discriminate between *A. phagocytophilum* variants (Michalski et al. 2006) showed that the *A. phagocytophilum* variants MN-1 and MN-2 from *D. albipictus* were identical, respectively, to *A. phagocytophilum* variant WI-1 and WI-2 from white-tailed deer and were divergent from the two *I. scapularis*--associated variants: the presumed nonpathogenic *A. phagocytophilum* variant 1, also associated with white-tailed deer, and the human pathogenic *A. phagocytophilum* ha associated with the white-footed mouse, *Peromyscus leucopus* (Massung et al. 2003,2007).

Detection of Bacteria in Extracts of *D. albipictus* Ticks Collected in 2006 and 2007

Extracts of adult female and male ticks collected from 11 deer in 2006 at Camp Ripley were 93 and 66% PCR positive for FLEs, respectively (Table 1), indicating an FLE prevalence in adult ticks of 91 versus 97% in 2005, a statistically insignificant difference (*t*-test, $P > 0.05$). All 11 deer produced PCR-positive ticks, whereas 100% of larval pool extracts were PCR positive, again consistent with TOT of FLEs. Six adult females collected in 2006 and 2007 from four deer at St. Croix State Park, and pools of their F_1 larvae, were PCR positive for FLEs (Table 1), indicating the presence of FLEs and TOT in a *D. albipictus* population from a location ≈150 miles southeast of Camp Ripley.

Fifty-nine percent of Camp Ripley female ticks collected in 2006 were PCR positive using the *Anaplasma/Ehrlichia* primers, whereas three males were PCR negative (Table 1), indicating a prevalence of 53 versus 37% in 2005 ($P > 0.05$). Ticks collected from 2 of the 11 deer were not PCR positive. Thirty-eight percent of larval pool extracts were PCR positive versus 42% of larval pools in 2005 ($P < 0.05$), consistent with TOT at a similar efficiency. Two of the six adult female ticks collected at St. Croix State Park were PCR positive (Table 1), as were three of their six F_1 larvae pools, whereas larvae from the other four ticks were PCR negative. Sequence analyses yielded the *A. phagocytophilum* variant MN-1 and MN-2 sequences detected in 2005.

TOT by Individual Ticks to F_1 Larvae

To further assess efficiency of TOT, we screened individual extracts of 20 larvae from each of four females that were PCR positive for both *A. phagocytophilum* and FLE. All but 1 of the 80 larvae were PCR positive for FLEs, indicating TOT efficiencies of 95–100% by all four

females (Table 1). In contrast, 26 larvae were PCR positive for *A. phagocytophilum* and individual female TOT efficiencies ranged from 10 to 40%.

Trans-Stadial and TOT to F_2 Larvae

To assess transstadial (TST) of FLEs and *A. phagocytophilum* in F_1 ticks and TOT to F_2 larvae, we infested a calf with two groups of separately confined F_1 larvae and recovered F_1 nymphs and adult females that produced F_2 larvae. One group of infesting F_1 larvae was PCR positive for FLEs and PCR negative for *A. phagocytophilum*, whereas the second group was PCR positive for both. Two weeks after infesting the calf, we recovered F_1 nymphs that molted from the FLE-positive/*A. phagocytophilum*--positive larvae group and all tested FLE positive, but only 7% were *A. phagocytophilum* positive (Table 1), consistent with efficient TST of FLEs, whereas that of *A. phagocytophilum* was not. All F_1 nymphs recovered from the FLE-positive/*A. phagocytophilum*–negative larvae group tested PCR positive for FLE and PCR negative for *A. phagocytophilum*, consistent with TST of FLE, as well as absence of *A. phagocytophilum* infection by horizontal transmission through the calf from the separately confined *A. phagocytophilum*--positive larvae group. We obtained F_2 larvae from 15 of 21 engorged adult female ticks matured from the calf-fed FLE-positive/*A. phagocytophilum*--positive larvae group. All extracts of F_2 larvae pools from those ticks were PCR positive for FLE and PCR negative for *A. phagocytophilum* (Table 1). The results were consistent with TST of FLE to F_1 adults and TOT to F_2 larvae but the *A. phagocytophilum* did not undergo TOT, and the ticks again did not become infected by horizontal transmission through the calf from the *A. phagocytophilum*--positive group of infesting larvae. To further assess infection status of the calf, we collected blood from it at days 18 and 39 after attachment of ticks. Tick ISE6 cell cultures were inoculated with the 18-d blood, but bacteria did not appear over a period of 2 mo, whereas cultures inoculated with *A. marginale* isolate Am291 or the “DAS” FLE from *D. andersoni* (Niebylski et al. 1997) supported vigorous growth of those bacteria. Blood collected at both time points was screened by PCR with the F5/F11 and PER1/PER2 primer pairs but did not support amplification of the predicted products, whereas control blood samples spiked with “DAS” or isolate Am291 did. The results of both assays were consistent with absence of FLEs and *A. phagocytophilum* in the calf blood.

Discussion

Based on results of PCR assays and sequence analyses, we showed that *A. phagocytophilum* variants and an FLE co-infect and undergo TST and TOT in *D. albipictus* collected from white-tailed deer at two locations in Minnesota. The presence of *A. phagocytophilum* variants and FLEs of uncertain pathogenic potential in ticks has unknown epidemiological implications. *D. andersoni* was the first tick implicated as a vector of the agent of tularemia in humans and livestock, *Francisella tularensis*, after the historic Rocky Mountain spotted fever (RMSF) epidemics in the Bitterroot Valley of Montana (Parker et al. 1924). Although later studies further implicated *D. andersoni* and other ticks as vectors of *F. tularensis*, the complex epidemiology of tularemia in North America has changed over the past century and involves rabbits, terrestrial rodents, aquatic mammals, lice, blood-feeding flies, and fomites in addition to ticks (Hayes 2005). At present, transmission by *Amblyomma americanum* and *Dermacentor variabilis* ticks is suspected as the main cause of human tularemia in the United States and by *D. andersoni* in recent livestock outbreaks (Eisen 2007, O’Toole et al. 2008, Petersen et al. 2009). The first described FLE, “DAS,” was also found in *D. andersoni* and was pathogenic when injected into guinea pigs or hamsters (Burgdorfer et al. 1973) but was not present in tick salivary glands and was not tick transmissible to guinea pigs in the laboratory (Niebylski et al. 1997). More FLEs were found in *D. variabilis* and *Ornithodoros* ticks (Noda et al. 1997, Sun et al. 2000, Goethert and Telford 2005), and a phylogenetic analysis showed that closely related FLEs were present in six *Dermacentor* spp., including *D. albipictus* from Texas and Alberta

(Scoles 2004). The "DVS" FLE of *D. variabilis* was present in all ticks collected at three locations in Massachusetts but was confined to ovarian tissues and Malpighian tubules, whereas the "DVF" FLE co-infected 55% of the same ticks in disseminated infections that possibly included the salivary glands (Goethert and Telford 2005). Both FLEs underwent TOT to larvae, apparently without the interference observed among *Rickettsia* spp. in ticks that may influence the epidemiology of RMSF caused by *R. rickettsii* (Burgdorfer et al. 1981, Macaluso et al. 2002).

Anaplasma phagocytophilum was first studied in the mid-20th century as a usually nonlethal pathogen that caused "tick-borne fever" and "pasture fever" in European sheep and cattle herds, respectively (Woldehiwet and Scott 1993). It is now known to infect a wide array of domestic and wild animals (Stuen 2007) and is the agent of the emergent zoonotic disease, human granulocytic anaplasmosis (Dumler et al. 2005). *A. phagocytophilum* is closely associated with ticks of the *Ixodes ricinus/persulcatus* complex, which includes *I. scapularis*, but it has been detected in *Dermacentor*, *Hemaphysalis*, and *Rhipicephalus* ticks (MacLeod 1962, Holden et al. 2003, Alberti et al. 2005, Cao et al. 2006, Barandika et al. 2008). MacLeod's pioneering studies of the tick-borne fever agent showed that it underwent TST, but not TOT, in *I. ricinus* (MacLeod and Gordon 1933, MacLeod 1936). Other early 20th century investigators obtained experimental evidence for TOT of the related anaplasmosis agent, *A. marginale*, in *Dermacentor* and *Boophilus* (*Rhipicephalus*) ticks, but other contemporaries could not do so (reviewed in Dikmans 1950,Ristic 1968). Nor could later investigators, possibly because of use of colonized ticks and *Anaplasma* strains maintained in cattle (Anthony 1968), but they did not rule out that TOT might still occur in wild ticks (Anthony and Roby 1962,Leatch 1973,Stich etal. 1989). Ourevidence for TOT of *A. phagocytophilum* in *D. albipictus* implies that its persistence in nature may not be as dependent on mammalian reservoirs as believed (Liz et al. 2002).

Anaplasma phagocytophilum apparently exists as a complex of variant strains or genospecies, analogous to the *B. burgdorferi* s.l. complex, that are associated with particular hosts and vary in relative pathogenicity (Massung et al. 2007). The *A. phagocytophilum* variants in the Minnesota *D. albipictus* populations were very similar to the presumed nonpathogenic *A. phagocytophilum* variant WI-1 and WI-2 strains (Fig. 1) detected in 20% of white-tailed deer from central Wisconsin but not in *I. scapularis* collected from those deer (Michalski et al. 2006). Another nonpathogenic strain, *A. phagocytophilum* variant 1, was present in 80% of the deer from the same study and in 17% of *I. scapularis*, but the remaining 83% of ticks were infected with the human pathogenic *A. phagocytophilum* ha strain. The *A. phagocytophilum* variant 1 was present in 64% of *I. scapularis* collected from deer at Camp Ripley, MN, in 2003 but the WI-1 and WI-2 variants were not (Massung et al. 2007). That study and the current study taken together imply that *A. phagocytophilum* variants circulating in *I. scapularis* and *D. albipictus* feeding on the same deer populations may be tick species specific. The FLE and *A. phagocytophilum* infection status of the deer hosting the *D. albipictus* in this study is unknown, but we note that TST and TOT of such bacteria would theoretically increase the tick's potential vector capacity. The results of this study and the presence of *A. phagocytophilum* variants WI-1 and WI-2 in Wisconsin deer but not in *I. scapularis* collected from those deer (Michalski et al. 2006) suggest that *D. albipictus* should be considered as a possible vector of *A. phagocytophilum* variants in white-tailed deer.

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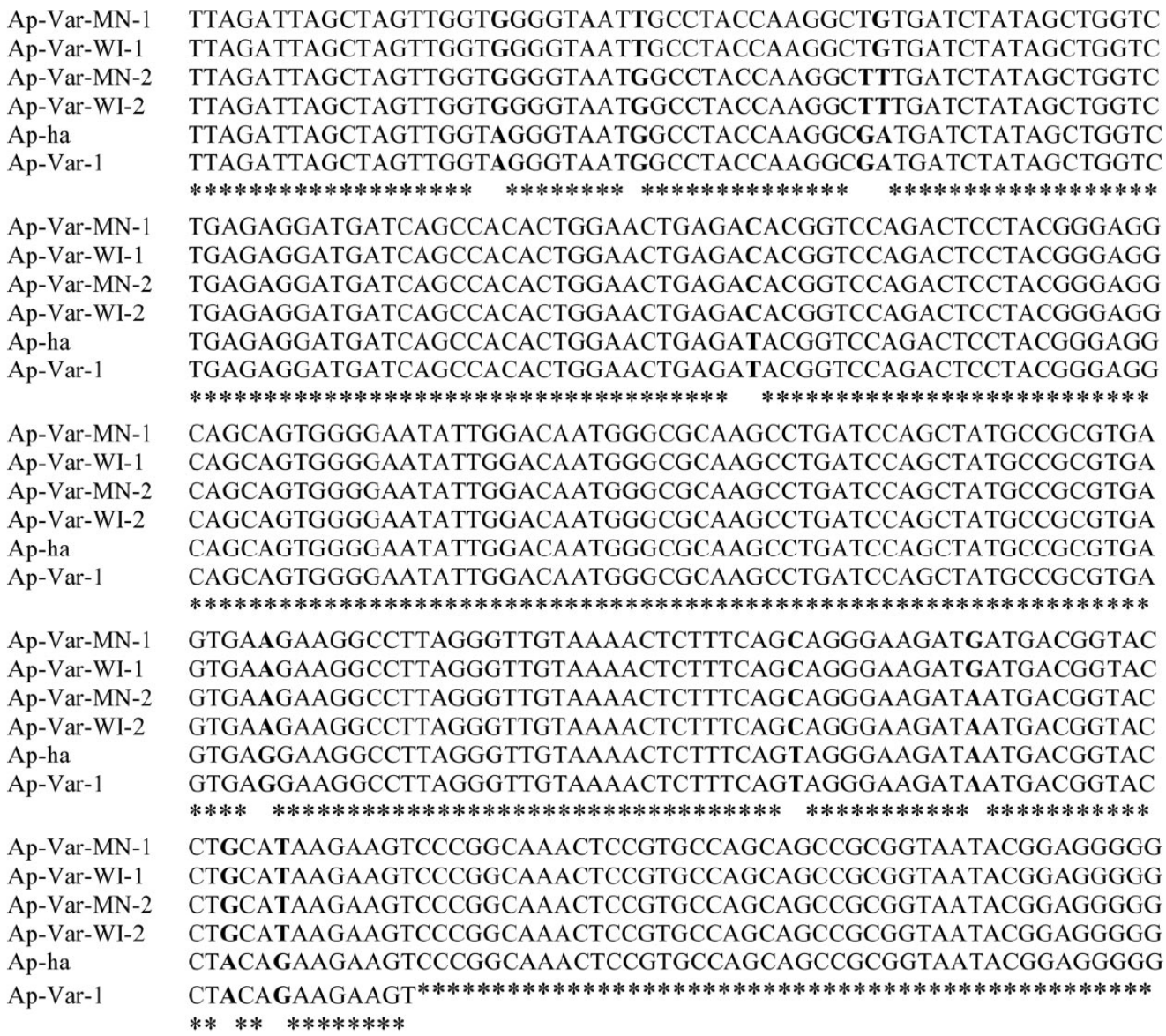

Fig. 1.

Nucleotide polymorphisms in a 224 nucleotide portion of the 16S rRNA gene sequences of *A. phagocytophilum* variants. Sequences of the *A. phagocytophilum* variant-MN-1 and MN-2 variants from *D. albipictus* were aligned to those of the *A. phagocytophilum* Variant-WI-1 and WI-2 variants (accession nos. DQ426991 and DQ426992) from Wisconsin white-tailed deer and the nonpathogenic *A. phagocytophilum* variant-1 (AY193887) and pathogenic *A. phagocytophilum* ha (U02521) variants associated with *I. scapularis*. Nucleotide polymorphisms are indicated in bold.

Table 1

Prevalence (%) of FLE and *A. phagocytophilum* in *D. albipictus* collected from Minnesota deer

Site (year)	No. deer	*Dermacentor albipictus*													
		Adult females		Adult males		Nymphs		F_1 larval pools		Individual F_1 larvae		F_1 nymphs		F_2 larval pools	
		FLE	*A. phagocytophilum*	FLE	*A. phagocytophilum*	FLE	*A. phagocytophilum*	FLE	*A. phagocytophilum*	FLE	*A. phagocytophilum*	FLE	*A. phagocytophilum*	FLE	*A. phagocytophilum*
Camp Ripley (2005)	5	(95) 19/20[a]	(45) 10/22	(100) 6/6	(0) 0/5	(100) 5/5	(20) 1/5	(100) 32/32	(42) 15/36	ND	ND	ND	ND	ND	ND
Camp Ripley (2006)	11	(93) 27/29	(59) 17/29	(66) 2/3	(0) 0/3	ND ND	ND ND	(100) 51/51	(38) 31/81	(99) 79/80	(26) 21/80	(100) 75/75	(7) 3/45	(100) 21/21	(0) 0/21
St. Croix	4	(100) 6/6	(33) 2/6	ND	ND	ND	ND	(100) 18/18	(17) 3/18	ND	ND	ND	ND	ND	ND

[a] No. PCR-positive ticks/no. ticks tested.

ND, not determined