Anaplasma phagocytophilum in White-tailed Deer

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We examined the reservoir potential of white-tailed deer for Anaplasma phagocytophilum. Results suggest that white-tailed deer harbor a variant strain not associated with human infection, but contrary to published reports, white-tailed deer are not a reservoir for strains that cause human disease. These results will affect surveillance studies of vector and reservoir populations.

Anaplasma phagocytophilum is an obligate intracellular bacterium and the etiologic agent of human granulocytic anaplasmosis (formerly known as human granulocytic ehrlichiosis). From 1999 to 2003, a total of 1,686 cases of human anaplasmosis were reported in the United States, and >95% of these cases occurred in northeastern or upper midwestern states. Transmission within and between reservoir populations in these regions occurs by Ixodes scapularis ticks (1,2). Infections occur in humans who have been fed upon by infected nymphal or adult ticks. No evidence shows that A. phagocytophilum is transmitted transovarially within the tick population; thus, both infected reservoirs and ticks that can transmit the infection must be available to maintain the agent in nature. Three mammalian species are reservoir competent: the white-footed mouse (Peromyscus leucopus), raccoon (Procyon lotor), and gray squirrel (Sciurus carolinensis), although serologic and molecular evidence has suggested that numerous other small, medium, and large mammals may also be reservoirs (1,3).

Every examined A. phagocytophilum sample from a patient with a confirmed case of human granulocytic anaplasmosis from the northeastern or upper midwestern United States has shown identical 16S rRNA sequences. This sequence, referred to as the A. phagocytophilum human anaplasmosis (AP-ha) signature sequence, differs by 2 bp from the sequence of the 16S RNA gene of a variant strain, AP-Variant 1. Recent studies that compared the prevalence of AP-ha to AP-Variant 1 in tick populations showed the variant to be the predominant strain at 2 of 3 sites and suggest that AP-Variant 1 is common in nature (4,5).

The white-footed mouse serves as a natural reservoir for AP-ha, and laboratory studies have shown that numerous inbred strains of mice (e.g., Balb/C, C3H, DBA/2) are also highly susceptible to infection (1,6,7). In contrast, AP-Variant 1 does not infect the white-footed mouse, DBA/2, and severely immunocompromised (SCID) mice (8). These results suggest that rodents are not a natural reservoir for AP-Variant 1 and that alternative reservoir species exist in nature. Previous reports have identified 3 white-tailed deer (Odocoileus virginianus) from Maryland and 2 white-tailed deer in Wisconsin that harbored an agent with a 16S rRNA gene sequence identical to that of AP-Variant 1 (4,9,10). Several previous studies have also suggested that white-tailed deer are a reservoir for the human agent AP-ha (10–12). These results led to the current study, which was conducted to investigate the relative potential for white-tailed deer to be a reservoir for AP-ha, AP-Variant 1, or both strains. We examined blood samples from white-tailed deer to determine the strains of A. phagocytophilum with which these deer were infected and the ticks feeding on these deer to identify strains to which they were exposed.

The Study

I. scapularis ticks were collected from white-tailed deer during controlled hunts at Ridley Creek State Park in Delaware County, Pennsylvania, in December of 2000, 2001, and 2002. Blood samples were also collected from the deer in 2001 and 2002. An unusually high percentage of ticks collected in December 2000 were positive for A. phagocytophilum (68 [49.6%] of 137); identification was based on polymerase chain reaction (PCR) amplification of a 546-bp portion of the 16S rRNA gene, as previously described (Table) (9). Further analysis showed a strong correlation between the sex of a tick and the probability of being positive for A. phagocytophilum. Most of the positive ticks were females; 62 (84.9%) of the 73 female ticks were positive, compared with 6 (9.4%) of 64 males. DNA sequencing showed that more infected female ticks were positive for AP-Variant 1, while only 9 (14.5%) of 62 were infected with AP-ha. Blood samples were not collected from white-tailed deer in 2000.

In 2001, both ticks and white-tailed deer blood samples were collected. Similar to the year 2000 tick results, a higher percentage of female ticks (50 [38.8%] of 129) than male ticks (13 [20%] of 65) were positive for A. phagocytophilum. Likewise, DNA sequencing showed that more infected female ticks were positive for AP-Variant 1 (74%) than for AP-ha (26%). Of the 38 white-tailed deer blood samples collected in 2001, 11 (28.9%) were positive for
in a study in which serologic testing showed that 8% of

white-tailed deer in Wisconsin were positive for *A. phago-

cytophilum*, the only 2 samples that were PCR amplified

and sequenced were identical to AP-Variant 1 (10). Strong

antigenic cross-reactivity of the AP-ha and AP-Variant 1

strains would not be surprising, considering their 16S

rRNA genes are >99% identical.

Seroprevalence studies for infectious agents using ani-

mal reservoir and host populations and PCR amplifications

from vector species are commonly used to assess the dis-

ease risk for humans in a particular region, particularly for

viral and bacterial zoonotic agents. Our results show that

animal seroprevalence studies for *A. phagocytophilum*

must be carefully evaluated to determine whether the agent

inducing the immune response is truly infectious in humans.

Our results further show that while PCR studies of
ticks may identify *A. phagocytophilum*, DNA sequenc-

ing of the PCR products is necessary to differentiate AP-ha

and AP-Variant 1 and therefore to assess the potential for

human infections. These issues were not addressed in ear-

lier studies and likely resulted in overestimation of the

prevalence of AP-ha in nature and in the implied risk for

human anaplasmosis. Therefore, future studies of host or

vector populations must be evaluated and interpreted care-

fully, with the knowledge that non–disease-causing variant

strains may influence results.

While our results suggest that white-tailed deer are a

reservoir for AP-Variant 1, additional studies that examine

the interaction of AP-Variant 1 with white-tailed deer pop-

culations in other parts of the United States are needed to
determine if they correspond to our results from

Pennsylvania. Differences in AP-Variant 1 strain composi-
tion or local white-tailed deer or *I. scapularis* tick popula-

tions may alter the interaction of the bacterial agent,

vector, and reservoir. AP-ha strains cause a transient, rela-
tively mild febrile illness with no overt signs of disease in

immunocompetent mouse species, including the natural

reservoir, *Peromyscus leucopus* (13). Inbred laboratory

mice infected with AP-ha may remain infected for up to 55
days (6), and previous infections induce an immune

response that is only partially protective, since mice may be

reinfected (14). We have not determined whether AP-

Variant 1 produces any disease manifestation in white-
tailed deer, although the high number of positive deer in

the current study suggests that persistent infections, rein-
fections from feeding ticks, or both mechanisms may be

involved in maintenance of AP-Variant 1 in white-tailed

deer populations.

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1605

| Year | No. ticks | AP-Variant 1, n (%) | AP-ha, n (%) |
|------|-----------|---------------------|-------------|
| 2000 | 73 F      | 53 (72.6)           | 9 (12.3)    |
|      | 64 M      | 4 (6.3)             | 2 (3.1)     |
| 2001 | 129 F     | 37 (28.7)           | 13 (10.1)   |
|      | 65 M      | 10 (15.4)           | 3 (4.6)     |
| 2002 | 4 F       | 1 (25)              | 1 (25)      |
|      | 2 M       | 0 (0)               | 1 (50)      |

*All females were either partially or fully engorged.*
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dispatches

botulism

[boch’s-ə-liz-əm]

Food poisoning with neurotoxicity caused by eating food contaminated with *Clostridium botulinum*. From the Latin *botulus*, "sausage," the disease was first recognized in Germany in persons who had eaten tainted sausage and was originally called "sausage poisoning."

Sources: Dorland’s illustrated medical dictionary. 30th ed. Philadelphia: Saunders; 2003 and Botulism in Alaska [monograph on the Internet]. [cited 2005 Aug 26]. Available from http://www.epi.hss.state.ak.us/pubs/botulism/bot_03.htm

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