Reservoir Competency of Goats for the Ap-Variant 1 Strain of Anaplasma phagocytophilum

Robert F. Massung

Thomas N. Mather
University of Rhode Island, tmather@uri.edu

Michael L. Levin

Follow this and additional works at: https://digitalcommons.uri.edu/cels_past_depts_facpubs

Terms of Use
All rights reserved under copyright.

Citation/Publisher Attribution
Available at: http://dx.doi.org/10.1128/IAI.74.2.1373-1375.2006

This Article is brought to you for free and open access by the College of the Environment and Life Sciences at DigitalCommons@URI. It has been accepted for inclusion in Past Departments Faculty Publications (CELS) by an authorized administrator of DigitalCommons@URI. For more information, please contact digitalcommons-group@uri.edu.
Reservoir Competency of Goats for the Ap-Variant 1 Strain of *Anaplasma phagocytophilum*

Robert F. Massung, Thomas N. Mather, and Michael L. Levin

Viral and Rickettsial Zoonoses Branch, Division of Viral and Rickettsial Diseases, CDC, Atlanta, Georgia; and Center for Vector-Borne Disease, University of Rhode Island, Kingston, Rhode Island

Received 2 September 2005/Returned for modification 8 October 2005/Accepted 8 November 2005

Field-collected ticks were used to infect goats with either Ap-ha, a strain associated with human disease, or a variant strain, Ap-Variant 1, of *Anaplasma phagocytophilum*. Goats were shown to be competent as a reservoir for Ap-Variant 1, and challenge and immunosuppression studies were used to further examine infection in the goat model.

Ap-Variant 1 represents a distinct strain of *Anaplasma phagocytophilum* that has not been associated with human disease. White-tailed deer (*Odocoileus virginianus*) appear to serve as a natural reservoir for Ap-Variant 1, and to date they are the only vertebrate species known to harbor this strain (1, 6, 7). However, deer are difficult to use in a laboratory setting and require specialized large animal facilities. Therefore, the potential for the domestic goat to serve as a smaller ruminant animal model was examined. The competency of goats as a natural reservoir for Ap-Variant 1 was also evaluated by tick-feeding and transmission studies.

Questing adult and nymphal ticks were collected by flagging from South Kingstown, Rhode Island, and allowed to feed on three pathogen-free goats. Two of the goats (A and B) became infected with *A. phagocytophilum* strains whose 16S rRNA sequences matched that of the human pathogenic strain Ap-ha. The third goat (goat C) became infected with the Ap-Variant 1 strain, as demonstrated by PCR amplification from blood and DNA sequencing, as previously described (8). EDTA-treated blood (3.5 ml) collected from goat C at 3 weeks postinfection was used to infect goat D with Ap-Variant 1. This showed that the DNA detected by PCR in the blood of goat C represented viable organisms. Larval and nymphal ticks were allowed to feed on goat C, allowed to molt, and fed on goat E, which became infected with Ap-Variant 1, thereby demonstrating the reservoir competency of goats.

Blood smears were prepared from goat D on day 14 postinjection, stained for visualization of cellular morphology, and fluorescein labeled using anti-*A. phagocytophilum* antibodies (Fig. 1). The fluorescein-labeled image (Fig. 1B) demonstrates that Ap-Variant 1 was detected only in segmented neutrophils. Blood samples from goats C, D, and E were collected sequentially and assessed for infection and serological titers. End-point titers in the sera were determined by an immunofluorescence assay using a fluorescein isothiocyanate-labeled rabbit anti-goat conjugate (Rockland Immunochemicals, Gilbertsville, PA). Table 1 summarizes the source of infection and PCR, DNA sequencing, and immunofluorescence assay results for each of the five goats. Only goat A showed signs of clinical illness, including fever, lethargy, lameness, loss of appetite, and nasal discharge. Goat E was further tested every 2 to 3 days for 3 months by use of complete blood counts with differential and serum...
chemistry profiles. Goat E had a slightly elevated temperature (103.5°F) on day 7 postinfestation only and showed mild and transient neutropenia and lymphopenia on days 10 and 7 postinfestation, respectively. Otherwise, goat E showed normal serum profiles and blood cell counts during the infection and through 3 months postinfestation.

Although each of the three goats infected with Ap-Variant 1 were PCR positive for various lengths of time, the infections appeared to be cleared because each goat was PCR negative past day 24. To assess the clearance of Ap-Variant 1 in goats, goat D was treated on day 110 postinfection and for five consecutive days with an intramuscular dose (4 mg/kg body weight) of dexamethasone as a strong immunosuppression regimen (3, 4). Goat D remained PCR negative through day 32 posttreatment, and the serum titer remained stable, at ≤128. These results suggest that goat D had cleared the Ap-Variant 1 infection.

Goat C was treated with a regimen of the antibiotic oxytetracycline (10) at months postinfection and, 25 days after treatment, infected with 50 nymphal ticks that were infected with Ap-ha. This goat became PCR positive on day 21 postinfestation the titer had risen to 1/256. Goat C remained PCR positive through day 25, and the infectious agent was confirmed to be Ap-ha by DNA sequencing. Whereas the titer of goat C was 1/32 prior to the challenge, it may be that the agent causes morbidity and mortality in a domestic or wildlife species that has not yet been examined. A. phagocytophilum in BALB/c mice and cross-protection between two sympatric strains. Infect. Immun. 72:4723–4730.

**REFERENCES**


---

**TABLE 1. Infection sources and test results for goats infected with either Ap-ha or Ap-Variant 1**

<table>
<thead>
<tr>
<th>Goat</th>
<th>Infection method</th>
<th>Infecting strain</th>
<th>Day of initial PCR-positive result</th>
<th>Days with PCR-positive result</th>
<th>Initial seroconversion (day)*</th>
<th>Maximum titer*</th>
<th>Maximum fever (°F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Field-collected ticks&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Ap-ha</td>
<td>11</td>
<td>8</td>
<td>NT</td>
<td>NT</td>
<td>103.9</td>
</tr>
<tr>
<td>B</td>
<td>Field-collected ticks&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Ap-ha</td>
<td>11</td>
<td>1</td>
<td>NT</td>
<td>NT</td>
<td>104.3</td>
</tr>
<tr>
<td>C</td>
<td>Field-collected ticks&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Ap-Variant 1</td>
<td>10</td>
<td>14</td>
<td>1/1,024</td>
<td>100.6</td>
<td>103.3</td>
</tr>
<tr>
<td>D</td>
<td>Blood from goat C</td>
<td>Ap-Variant 1</td>
<td>7</td>
<td>12</td>
<td>9</td>
<td>1/256</td>
<td>103.3</td>
</tr>
<tr>
<td>E</td>
<td>Ticks fed on goat C&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Ap-Variant 1</td>
<td>5</td>
<td>19</td>
<td>7</td>
<td>1/512</td>
<td>103.5</td>
</tr>
</tbody>
</table>

* Determined by PCR and DNA sequencing of the 16S rRNA gene.

<sup>b</sup> Adult *Ixodes scapularis* ticks collected from South Kingstown, Rhode Island.

<sup>c</sup> Fed as larvae and nymphs on goat C, allowed to molt, and fed as nymphs and adults on goat E.

<sup>d</sup> NT, not tested.

Editor: J. T. Barbieri