Evaluation of C3H/HeJ mice for xenodiagnosis of infection with 
*Ehrlichia chaffeensis*

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Abstract. Because mice are experimentally susceptible to infection with *Ehrlichia* species, C3H/HeJ mice were evaluated as a potential xenodiagnostic model for detection of infection with and isolation of *E. chaffeensis*. Intraperitoneal inoculation of mice with *E. chaffeensis*-infected DH82 cell cultures produced seroconversion, with peak serum antibody titers of 1:256, at high dosages (>1.9 × 10⁴ infected cells) but not at low dosages (1.9 or 1.9 × 10⁷ infected cells). *Ehrlichia chaffeensis* was not reisolated from blood samples collected from inoculated mice on postinoculation day 21. Nested polymerase chain reaction (PCR), using primers specific for *E. chaffeensis*, was positive for only 2/70 (2.9%) tissue samples. A field evaluation in which C3H/HeJ mice were inoculated with blood and lymph node suspensions from 5 seropositive white-tailed deer, including 3 deer that were PCR positive for *E. chaffeensis*, failed to produce seroconversion in mice. The lack of seroconversion at low dosages, the failure to reisolate at any dosage, and the inability to confirm infection in PCR-positive field samples suggests C3H/HeJ mice are not a sensitive model for xenodiagnosis or detection of *E. chaffeensis*.

Since first described in 1986, more than 400 cases of human monocytic ehrlichiosis have been documented by serologic analysis at the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia. To date, however, only 3 isolates of the causative agent, *Ehrlichia chaffeensis*, have been obtained from humans using DH82 canine macrophage cell cultures. Isolation of *E. chaffeensis* and related ehrlichiae is considered difficult.

A human isolate of *E. chaffeensis* has been successfully reisolated from experimentally infected dogs and white-tailed deer (*Odocoileus virginianus*), the latter being a suspected reservoir host. Researchers reisolated *E. chaffeensis* 6 times from the blood of 2 deer. However, attempts to culture ticks and blood or other tissues from wild white-tailed deer have encountered problems such as cell culture contamination with exogenous bacteria and trypanosomases (*Trypanosoma cervi*).

Laboratory rodents have been used to study the immunopathology of ehrlichial infections of veterinary medical importance such as *E. risticii*. Laboratory C3H/HeJ strain mice, which are unable to undergo macrophage activation, were susceptible to infection with *E. risticii*. In a recent study, C3H/HeJ mice and white-footed deer mice (*Peromyscus leucopus*), but not LVG strain hamsters or red-backed voles (*Clethrionomys gapperi*), were susceptible to experimental infection with *E. chaffeensis*.

Because of complications and contaminants associated with performing culture work with wild white-tailed deer, a pilot study was conducted to evaluate C3H/HeJ mice as a xenodiagnostic tool for detection of *E. chaffeensis* infection. The specific aims of this study were to use serologic, polymerase chain reaction (PCR), and cell culture techniques to monitor the response of C3H/HeJ mice to inoculation with *E. chaffeensis* and to apply this xenodiagnostic procedure in a pilot study that utilized tissues from an *E. chaffeensis*-infected white-tailed deer population.

Materials and methods

Experimental inoculation of C3H/HeJ mice. Five 6-wk-old C3H/HeJ strain mice were obtained and housed at animal resources facilities, College of Veterinary Medicine, University of Georgia. *Ehrlichia chaffeensis* was cultured in DH82 canine macrophage cells as previously described. Uninfected DH82 cells served as the negative control inoculum.

An *E. chaffeensis* inoculum was prepared from 1 25-cm² culture flask. Cells were harvested when >80% were infected with *E. chaffeensis* as determined by direct fluorescent antibody (FA) analysis using a human anti-*E. chaffeensis* conjugate obtained from the CDC and diluted 1:50 in phosphate-buffered saline (PBS) (pH 7.2). Cells were collected using a cell scraper, concentrated by centrifugation, and resuspended in 1 ml of minimum essential medium. A portion of the suspension was used to quantify the number of cells, using a Coulter counter. Serial 10-fold dilutions were made in PBS from the original infected cell suspension for inoculation into mice.

Each mouse was given an intraperitoneal 0.2-ml inoculation of *E. chaffeensis*-infected cells. Two mice received 1.9
× 10^6 cells, 2 received 1.9 × 10^5 cells, 3 received 1.9 × 10^6 cells, 3 received 1.9 × 10^5 cells, and 3 received 1.9 cells. A single control mouse received an inoculum of 2.7 × 10^6 uninfected DH82 cells.

Blood was collected retroorbitally from all mice prior to inoculation, and all mice were marked by ear punch for identification. Mice that received the same inoculation dilution were cohoused and provided a liberal food and water supply. Mice were euthanized with CO₂ on postinoculation day (PID) 21, and blood was collected via cardiac puncture. Blood was divided into 2 fractions; 1 was placed in heparinized tubes, and 1 was placed in tubes without an anticoagulant. Serum was collected for serologic examination, and heparinized blood was prepared for culture and PCR analysis.

Indirect FA tests were performed as previously described.³ Serum was screened at a dilution of 1:32 in PBS on spot slides of E. chaffeensis-infected DH82 cells. Samples exhibiting fluorescence were evaluated at serial 2-fold dilutions. Results were recorded as the reciprocal of the highest dilution at which specific staining of the ehrlichial organisms was made using fluorescein isothiocyanate (FITC)-labeled goat anti-mouse IgG diluted 1:50 in PBS (pH 7.2).

Isolation of E. chaffeensis from C3H/HeJ mice was attempted as described previously.² Whole blood collected in heparin was mixed 1:3 with lysing buffer (150 mM NH₄Cl, 0.7 mM KH₂PO₄, and 3 mM ethylenediaminetetraacetic acid (EDTA)-Na₂) and incubated at room temperature for 5 min with frequent inversion. The suspension was centrifuged at 1,500 × g for 5 min to concentrate leukocytes. This procedure was repeated 2 more times with lysing buffer as a wash. Uninfected DH82 canine macrophage cells from a 25-cm² flask were suspended in 5 ml of fresh medium and added to the pellet. The entire suspension was then put back into the flask. Twice weekly, the supernatant from the culture flask was decanted, and 5 ml of fresh medium was added.

Starting at day 5, cultures were examined twice weekly for evidence of infection using direct FA staining as previously described.⁴ Culture supernatant was collected and centrifuged at 1,500 × g for 5 min, and cells were resuspended in 1 ml of medium. Approximately 0.1 ml of the suspension was cytocentrifuged onto glass slides. Slides were allowed to air dry for 15 min, fixed in acetone for 15 min, and dried at room temperature for 15 min. Conjugate for the direct FA consisted of FITC-labeled rabbit anti-deer IgG diluted 1:100.

Cells were harvested from the deer blood and prescapular lymph nodes within 12 hr and processed for culture as described above. Isolated cells from the blood, prescapular lymph nodes, and spleen were evaluated as above by PCR for the presence of E. chaffeensis.

A fraction of cells isolated from blood and prescapular lymph node from each deer was resuspended in 0.25 ml PBS and inoculated intraperitoneally into 1 C3H/HeJ mouse (1 mouse per tissue, 3 mice/deer). Blood was collected retroorbitally on PID 15, and on PID 30, mice were euthanized by CO₂ and blood was collected by cardiac puncture. Serum was separated for indirect FA analysis. Any tissue-inoculated mice that seroconverted were euthanized, and cardiac blood, mesenteric lymph nodes, and spleen samples were collected for confirmatory in vitro isolation attempts and for PCR analysis as previously described.

Results

Experimental inoculation of C3H mice with E. chaffeensis. Mice that received the 3 highest dilutions of E. chaffeensis-infected cells seroconverted by PID 21 (Table 1). The geometric mean titers for mice that received 1.9 × 10⁶, 1.9 × 10⁵, and 1.9 × 10⁴ cells were 181 (titer = 256, 128), 128 (titer = 128), and 81 (titer = 128, 64, 64), respectively. Mice in the 2 lowest dilution and negative control groups were all seronegative at 1:32 on PID 21. One of the 2 mice that received 1.9 × 10⁵ E. chaffeensis-infected cells was
Table 1. Results of inoculation of C3H/HeJ mice with *Ehrlichia chaffeensis*-infected DH82 canine macrophages.

<table>
<thead>
<tr>
<th>No. mice</th>
<th>Inoculum*</th>
<th>Serology†</th>
<th>PCR‡</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>B</td>
</tr>
<tr>
<td>2</td>
<td>$1.9 \times 10^6$</td>
<td>128, 256</td>
<td>0/1</td>
</tr>
<tr>
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<td>$1.9 \times 10^5$</td>
<td>128</td>
<td>0/1</td>
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<td>3</td>
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<td>$1.9 \times 10^5$</td>
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<td>3</td>
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</tr>
<tr>
<td>1</td>
<td>$2.7 \times 10^6$</td>
<td>&lt;32</td>
<td>ND</td>
</tr>
</tbody>
</table>

* *E. chaffeensis*-infected DH82 canine macrophages determined to be >80% infected by results of direct FA analysis and counted with a Coulter counter. Negative control was inoculated with uninfected cells.
† Reciprocal of dilution of serum that produced specific fluorescence of ehrlichial organisms using an indirect FA test.
‡ Nested PCR analysis using ECB/ECC and HE1/HE3 primer pairs and analyzed with gel electrophoresis. No. positive/no. tested. B = blood; LN = lymph node; SP = spleen; LU = lung; LI = liver; KI = kidney; ND = not done.

Ehrlichia chaffeensis was not reisolated from the blood from any of the mice at PID 21. Cultures from 1 mouse inoculated with $1.9 \times 10^6$ cells, 1 with $1.9 \times 10^5$ cells, and 1 with $1.9 \times 10^4$ cells were discontinued at Pills 5, 6, and 9, respectively, because of bacterial contamination. All other cultures were maintained until day 63.

A total of 70 tissue specimens from 12 inoculated mice were examined by PCR for the presence of *E. chaffeensis* DNA (Table 1). Two tissues, spleen from 1 mouse that received $1.9 \times 10^6$ cells and lung from 1 mouse that received $1.9 \times 10^7$ cells, were positive by PCR using *E. chaffeensis*-specific primers HE1 and HE3 (Fig. 1).

Inoculation of deer tissue into C3H/HeJ mice. Deer collected from Piedmont NWR had *E. chaffeensis*-reactive antibody titers of 128–512 (Table 2). *Ehrlichia chaffeensis* was not isolated from blood or lymph node of any of the deer. The culture from the lymph node of deer 3 was discontinued at PID 12 because of bacterial contamination. All remaining cultures were examined weekly by direct FA and maintained until PID 68. Blood from 2 deer and lymph node from a third deer were positive by PCR for *E. chaffeensis* (Fig. 1). All other blood, lymph node, and spleen samples were negative by PCR for *E. chaffeensis*.

None of the C3H/HeJ mice inoculated with blood, lymph node, and spleen samples from white-tailed deer developed *E. chaffeensis*-reactive antibodies, including 3 mice inoculated with tissues that were positive for *E. chaffeensis* by PCR. The negative control mouse remained seronegative. Because none of the

Table 2. Serology and PCR results on white-tailed deer collected September 21, 1994, from Piedmont National Wildlife Refuge, Jasper and Jones counties, Georgia.

<table>
<thead>
<tr>
<th>Deer No.</th>
<th>Serology*</th>
<th>PCR†</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>B</td>
</tr>
<tr>
<td>1</td>
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<td>3</td>
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<td>512</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>256</td>
<td>–</td>
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</tbody>
</table>

* Reciprocal of dilution of serum that produced specific fluorescence of ehrlichial organisms using an indirect FA test.
† Nested PCR analysis using ECB/ECC and HE1/HE3 primer pairs and analyzed with gel electrophoresis. B = blood; LN = lymph node; SP = spleen.
mice seroconverted, no further testing was done on their tissues.

Discussion

Inoculation of C3H/HeJ mice produced equivocal results with regard to susceptibility to E. chaffeensis. Although mice that received higher doses seroconverted, the inability to reisolate E. chaffeensis and detection of E. chaffeensis DNA in only 2 of 12 inoculated mice suggests that replication of E. chaffeensis was minimal and presumably transient. Antibody titers paralleled the number of infected cells injected, suggesting that the serologic responses may have been simply a function of the number of organisms in the inocula. Conversely, the detection of E. chaffeensis DNA in the lung of 1 mouse dosed with $1.9 \times 10^2$ infected cells could be interpreted as evidence that replication occurred in that animal.

The present results are similar to those obtained by other researchers who also inoculated C3H/HeJ mice with E. chaffeensis. In that study, mice developed E. chaffeensis-reactive antibodies within 21 PIDs, were infrequently positive when tested by PCR within 30 PIDs, and were difficult to confirm as infected via reisolation (a single reisolation at PID 11). Other research indicated that C3H/HeJ become persistently infected and it was proposed that C3H/HeJ mice may be a useful animal model for the study of protective immunity against E. chaffeensis. Although the present data do not provide support for the concept of persistent infection, they do suggest that C3H/HeJ mice may be suitable for immunity studies.

Regardless of the differing results and interpretations between this and the previous study, both studies show that C3H/HeJ mice are markedly less susceptible to this infection than are white-tailed deer and dogs, the only nonrodent mammals that have been experimentally inoculated with E. chaffeensis. In those studies, E. chaffeensis was consistently reisolated from the blood of needle-exposed dogs from between PIDs 7 and 26 and from deer between PIDs 13 and 24.

The interest in utilizing mice as a xenodiagnostic aid arose because isolation of ehrlichiae in cell culture generally has proved difficult and, more specifically, because prior attempts to isolate E. chaffeensis from deer blood often had been confounded by contamination with T. cervi. The utility of C3H/HeJ mice for this purpose is best evaluated based on the results from field samples. Mice inoculated with deer tissue that was PCR positive for E. chaffeensis uniformly failed to seroconvert. Use of C3H/HeJ mice in an identical manner in another field survey also yielded negative results for 8 PCR-positive tissue suspensions and, more importantly, failed to detect infection in 5 deer blood samples from which E. chaffeensis was isolated in cell culture. Thus, C3H/HeJ mice apparently are not a sensitive xenodiagnostic tool for the detection of E. chaffeensis from field samples. Nevertheless, as proposed in previous studies, they may be useful as an experimental model for the study of immune response to E. chaffeensis.

Acknowledgements

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Sources and manufacturers


