Ehrlichia chaffeensis
Infection of Sika Deer, Japan

Makoto Kawahara, Tomoko Tajima, Harumi Torii, Mitsutaka Yabutani, Joji Ishii, Makiko Harasawa, Emiko Isogai, and Yasuko Rikihisa

To determine whether Ehrlichia chaffeensis exists in Japan, we used PCR to examine blood from sika deer in Nara, Japan. Of 117 deer, 36 (31%) were infected with E. chaffeensis. The E. chaffeensis 16S rRNA base and GroEL amino acid sequences from Japan were most closely related to those of E. chaffeensis Arkansas.

Human infection with Ehrlichia chaffeensis causes human monocytic ehrlichiosis (HME), an influenza-like illness. Severity of the disease varies from mild to severe and can even cause death (1). HME cases have been reported primarily in the southeastern and south-central regions of the United States (1).

The organism, E. chaffeensis, until recently has been reported only in the United States; however, numerous reports now indicate that Ehrlichiae spp. closely related or identical to E. chaffeensis exist throughout the world (1,2). In the United States, E. chaffeensis has been most frequently identified in the lone star tick (Amblyomma americanum) (3). E. chaffeensis DNA has also been detected in A. testudinarium and Haemaphysalis yeni ticks from southern People’s Republic of China (4), in H. longicornis ticks from South Korea (5), and in A. parvum ticks in Argentina (6). Other than A. americanum, the role of these tick species as E. chaffeensis vectors has not been investigated. The whitetailed deer (Odocoileus virginianus) is the only vertebrate species currently recognized as a complete and sufficient host for maintaining the transmission cycle of E. chaffeensis (3,7). To look for molecular evidence of E. chaffeensis in sika deer (Cervus nippon) in Japan, we examined blood specimens by using PCR amplification of the 16S rRNA and groEL genes.

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The Study

In Nara, Japan, to prevent injuries to park visitors, the Foundation for the Protection of Sika Deer in Nara Park captured 97 pregnant female sika deer in April 2005 and 20 male sika deer with antlers in November 2005. Blood specimens were collected from all 117 deer, and buffy coat fractions were stored at –80°C for further analysis. Genomic DNA was extracted from the buffy coat fractions by using a QIAmp tissue kit (QIAGEN, Valencia, CA, USA). Nested PCR amplification of genomic DNA was performed by using primer pairs designed to amplify the E. chaffeensis 16S ribosomal RNA (rRNA) gene and the E. chaffeensis groEL gene (Table).

Of the 117 specimens, 36 (31%) yielded E. chaffeensis 16S rRNA amplification products and 35 (30%) yielded E. chaffeensis groEL amplification products. Of 36 16S rRNA–positive specimens, 33 were positive for groEL (92% concordance rate). Of 35 groEL-positive specimens, 33 were positive for 16S rRNA (94% concordance rate). The E. chaffeensis sequences were conserved in that all sequences obtained from sika deer in Nara were nearly identical to those of a representative strain that we named NS101 and submitted to GenBank (accession no. AB454074). The sequence of the 16S rRNA gene from E. chaffeensis NS101 was most closely related (99.6% identity; 5 bases of 1,333 bp that can be aligned for comparison differed) to that of 5 human isolates: Arkansas (GenBank accession no. M73222) (8), Sapulpa (U60476) (9), 91HE17 (U23503) (10), St. Vincent (U86665) (11), and Jax (U86664) (11); the next closest sequence was from an E. chaffeensis isolate from A. testudinarium ticks in China (GenBank accession no. AF147752) (99.2% identity; 10 bases of 1,333 bp that can be aligned for comparison differed).

When E. chaffeensis NS101 was compared with Ehrlichia spp. previously identified in Japan, the sequence of the 16S rRNA gene (a 1,328-bp segment that can be aligned for comparison) of E. chaffeensis NS101 was 98.9%, 98.9%, and 98.6% identical to that of E. muris AS145 strain, Ehrlichia sp. HF565 (the HF strain, Ixodes ovatus Ehrliehia) and Candidatus Ehrlichia shimanensis TS37, respectively. Phylogenetic analysis concurred with the observation that E. chaffeensis from Nara sika deer has the highest identity to the E. chaffeensis human isolates from the United States (Figure 1).

Although longer 16S rRNA gene sequences are desirable for strain comparison, the sequence of the 16S rRNA gene of E. chaffeensis from H. longicornis ticks in Korea (GenBank accession no. AY350424, 390 bp) and from A. parvum ticks in Argentina (GenBank accession no. EU826516, 470 bp) were identical to a corresponding, but incomplete, segment (358 bp and 402 bp, respectively) of the 16S rRNA gene of E. chaffeensis NS101 and Arkansas strains. An Ehrlichia sp. was detected in blood samples
from marsh deer (Blastocerus dichotomus) captured near the Parana River in southeast Brazil in 1998 (12). However, the 16S rRNA sequence from these marsh deer (GenBank accession no. DQ345720, 383 bp) shares 98.7% identity to a corresponding, but incomplete, segment (381 bp) of the E. chaffeensis NS101 and Arkansas strains. Because the sequences of the corresponding segments of Ehrlichia sp. HF565 and ‘Candidatus Ehrlichia shimanensis’ TS37 both had 99.0% identity to that of E. chaffeensis Arkansas strain, higher than the 98.7%, the Ehrlichia sp. in marsh deer from Brazil might not be E. chaffeensis.

Among the E. chaffeensis groEL sequences available in current databases, only that from E. chaffeensis Arkansas has >1,000 bp for reliable comparison. The groEL DNA sequence (1,208 bp that can be aligned) of the NS101 strain (GenBank accession no. AB454074, 1,208 bp) was closest closely related to that of E. chaffeensis Arkansas (GenBank accession no. L10917), followed by the Ehrlichia sp. HF565 strain (GenBank accession no. AB032712), and the E. muris AS145 strain (GenBank accession no. AF210459) (Figure 2). The deduced E. chaffeensis NS101 GroEL amino acid sequence (402 residues) was most closely related (99.5% identity) to that of E. chaffeensis Arkansas, followed by the Ehrlichia sp. HF565 strain (99.2% identity) and E. muris AS145 strain (99.0%).

**Table. Detection of 16S rRNA gene and groEL gene of Ehrlichia chaffeensis in sika deer, Japan**

<table>
<thead>
<tr>
<th>Target gene</th>
<th>1st PCR or nested PCR</th>
<th>Primer ID</th>
<th>Product size, bp</th>
<th>Sequence of primers (5’ → 3’)</th>
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**Conclusions**

On the basis of the long 16S rRNA and the groEL DNA sequences, our study demonstrates the presence of E. chaffeensis in sika deer from Nara, Japan. The genetic similarity of E. chaffeensis in the sika deer in Japan to strains isolated from HME patients in the United States raises the possibility that HME may exist in Japan. Of 1,803 serum samples collected from persons in metropolitan Tokyo from 1991 through 1995, when E. muris was used as antigen, 20 were seropositive (13). Because E. chaffeensis and E. muris antigens are highly cross-reactive (14), some of these persons might have been infected with E. chaffeensis.

Of the 10 tick species found on sika deer (15), the primary tick species found on sika deer in Nara is *H. longicornis*, which is known to bite humans in Japan and to be infected with *E. chaffeensis* in South Korea (5). Because sika deer are abundant and increasing throughout Japan (16), this finding highlights need to survey sika deer and humans in Japan for *E. chaffeensis* infection.

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Figure 1. Phylogenetic relationship between *Ehrlichia chaffeensis* NS101 (in boldface) and other *Ehrlichia* spp. 16S rRNA gene sequences. GenBank accession numbers are shown in parentheses. Numbers above internal nodes indicate the number of bootstrap replicates of 1,000 that supported the branch. Scale bar indicates percent sequence divergence.

Figure 2. Phylogenetic relationship between the *Ehrlichia chaffeensis* NS101 groEL sequence (1,208 bp) (in boldface) and other *Ehrlichia* spp. groEL sequences. GenBank accession numbers are shown in parentheses. Numbers above internal nodes indicate the number of bootstrap replicates of 1,000 that supported the branch. Scale bar indicates percent sequence divergence.
Ehrlichia chaffeensis Infection, Sika Deer, Japan

Acknowledgments
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Dr. Kawahara is a researcher at the Nagoya City Public Health Research Institute. His research focuses on the *Ehrlichia* and *Anaplasma* species in Japan. He discovered *Ehrlichia muris* and *Candidatus Neoehrlichia mikurensis* and characterized the HF strain and *Mycoplasma haemomuris*.

References

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