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# The *omp-1* Major Outer Membrane Multigene Family of *Ehrlichia chaffeensis* Is Differentially Expressed in Canine and Tick Hosts

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**Sixteen of 22 *omp-1* paralogs encoding 28-kDa-range immunodominant outer membrane proteins of *Ehrlichia chaffeensis* were transcribed in blood monocytes of dogs throughout a 56-day infection period. Only one paralog was transcribed by *E. chaffeensis* in three developmental stages of *Amblyomma americanum* ticks before or after *E. chaffeensis* transmission to naïve dogs.**

*Ehrlichia chaffeensis*, an obligatory intramonocytic bacterium, causes human monocytic ehrlichiosis (HME), an emerging tick-borne zoonosis (1, 5, 19). *E. chaffeensis* has been detected in field-collected *Amblyomma americanum* ticks, white-tailed deer, and dogs (2–4, 6, 9–17, 20, 21, 24). Although *A. americanum* has been shown to transmit *E. chaffeensis* among deer (7), development of an easily accessible tick transmission model using the dog would facilitate the analysis of molecular mechanisms of ehrlichial transmission.

To date, only a few *E. chaffeensis* genes have been characterized. We recently characterized the *omp-1* multigene families encoding outer membrane protein 1 (OMP-1)-immunodominant major OMPs of *E. chaffeensis* (18). A total of 22 paralogs are clustered in the 27-kb locus of *E. chaffeensis.* There has been no report of any protein gene transcription by *E. chaffeensis* in mammals or ticks. In the present study, (i) we analyzed the transcription of the entire family of 22 *omp-1* multigenes in experimentally infected dogs and *A. americanum* ticks and (ii), since *E. chaffeensis* transmission from ticks to dogs has never been demonstrated, we examined whether *E. chaffeensis* can be transmitted from *A. americanum* to dogs.

Eight pathogen-free female dogs (1 to 2 years old) were used. All dogs were free of *E. chaffeensis* infection as determined by indirect fluorescent antibody and PCR tests of their blood specimens. Dogs 133 and 146 were each intravenously inoculated with $5 \times 10^6$ DH82 cells infected with *E. chaffeensis* Arkansas (low-passage 1993 stock). *E. chaffeensis* 16S rRNA was detected in the peripheral blood mononuclear cells (PBMCs) of both dogs by reverse transcriptase PCR (RT-PCR) starting on day 7 and continuing through day 56 postinoculation (p.i.) (8). *A. americanum* ticks at three developmental stages were attached to both dogs and removed as previously described (8). The remaining six dogs were used to reattach these infected ticks. Indirect fluorescent antibody tests, isolation of PBMCs, dissection of ticks, DNA and RNA extraction, RT-PCR, and PCR based on the 16S rRNA gene were described previously (8, 23).

The 22 pairs of primers that amplify the regions shown in Table 1 were shown to be specific for each *omp-1* by PCR using 0.5 ng of purified *E. chaffeensis* DNA as template (Fig. 1A and B). By RT-PCR using these *omp-1*-specific primers, 16 *omp-1* paralogs were found to be transcribed by *E. chaffeensis* in PBMCs from dogs 133 and 146 throughout the 56 days p.i., and transcripts of the remaining six paralogs were undetectable in either dog (Fig. 1C). To normalize levels of ehrlichial RNA present in the PBMCs at every time point in each dog, constitutively expressed *E. chaffeensis* 16S rRNA was amplified by RT-PCR in the linear range (27 cycles) using primer HE1-HE3 (2). *E. chaffeensis* 16S rRNA levels were slightly increased in both dogs from day 14 to day 56 p.i., and so was the level of expression of *omp-1* paralogs (Fig. 1C). Without addition of RT, none of the RNA specimens was positive in the RT-PCR,

TABLE 1. Regions selected for gene-specific PCR and RT-PCR

| Target gene | Regions for RT-PCR | |
|---|---|---|
| | Nucleotide position[a] | Amplicon size (bp) |
| *omp-1M* | 1275–1666 | 392 |
| *omp-1N* | 2932–3261 | 330 |
| *omp-1Q* | 4198–4749 | 552 |
| *omp-1P* | 4861–5285 | 425 |
| *omp-1T* | 5789–6269 | 481 |
| *omp-1U* | 6726–7007 | 282 |
| *omp-1V* | 7670–7892 | 223 |
| *omp-1W* | 9211–9659 | 449 |
| *omp-1X* | 9953–10500 | 548 |
| *omp-1Y* | 10811–11072 | 262 |
| *omp-1S* | 11685–11932 | 248 |
| *omp-1H* | 12596–12878 | 283 |
| *omp-1Z* | 13469–13807 | 339 |
| *omp-1A* | 14687–14868 | 182 |
| *omp-1B* | 15774–16138 | 365 |
| *omp-1C* | 17053–17282 | 230 |
| *omp-1D* | 18200–18445 | 246 |
| *omp-1E* | 19229–19594 | 366 |
| *omp-1F* | 20680–21015 | 331 |
| *p28* | 21826–22248 | 423 |
| *p28-1* | 23029–23373 | 345 |
| *p28-2* | 23708–24028 | 321 |

[a] Nucleotide position is based on the 27-kb *omp* locus of *E. chaffeensis* (18).

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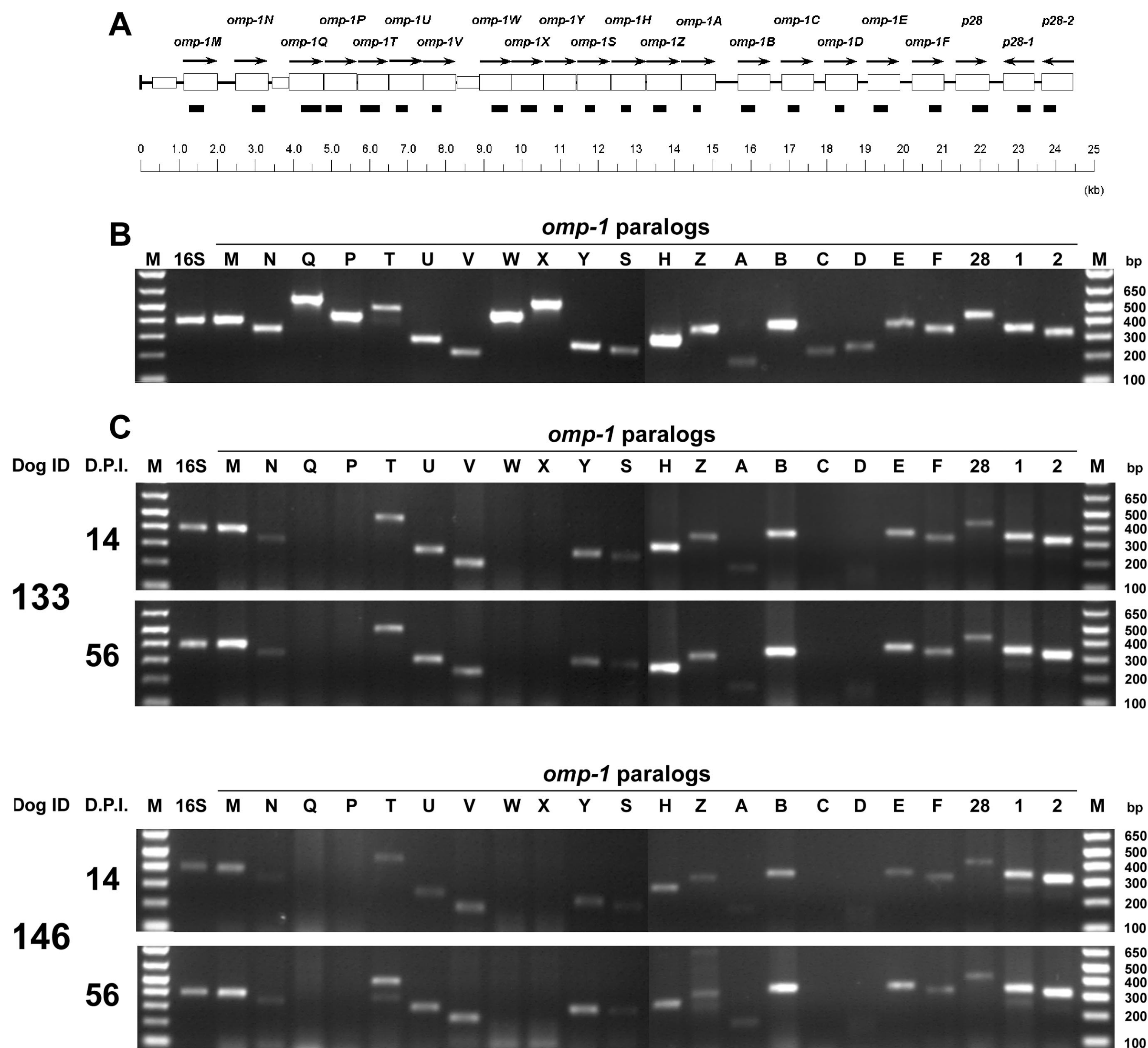
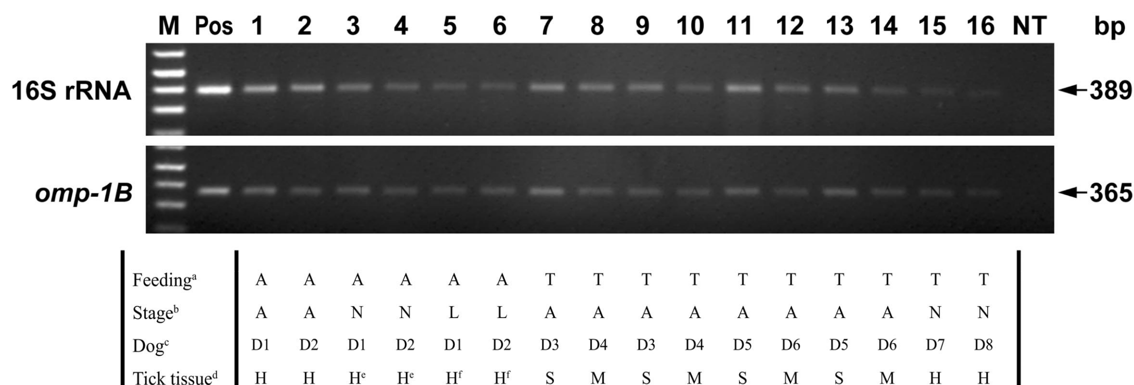



FIG. 1. (A) RT-PCR region in the *omp-1* multigene cluster of *E. chaffeensis.* The open boxes and arrows show respective *omp-1* paralogs and their orientation. Closed boxes indicate amplified RT-PCR region as indicated in Table 1. (B) PCR utilizing purified *E. chaffeensis* DNA (0.5 ng) as template and gene-specific primer pairs showing the strength and specificity of each reaction. The amplified products were resolved on agarose gels containing ethidium bromide. *omp-1* genes were identified on the top in the order of their genomic localization. M, molecular size markers (1-kb plus DNA marker [Life Technologies]). Nucleotide base pair sizes of the marker are indicated on the left. (C) Expression of mRNA of *omp-1* paralogs and 16S rRNA of *E. chaffeensis* in the PBMCs of two dogs infected by intravenous inoculation of *E. chaffeensis*. Total RNA was extracted and subjected to RT-PCR. The amplified products were resolved on agarose gels containing ethidium bromide. *omp-1* genes were identified on the top in the order of their genomic localization. M, molecular size markers (1-kb plus DNA marker [Life Technologies]); D.P.I., days p.i. Nucleotide base pair sizes of the marker are indicated on the right.

indicating the absence of DNA contamination (data not shown). *Ehrlichia canis*, the agent of canine monocytic ehrlichiosis, is phylogenetically and biologically closely related to *E. chaffeensis*. This finding is similar to that with a *p30*-immunodominant major OMP multigene family of *E. canis* that has a gene structure and arrangement similar to those of the *omp-1* gene family (18). Like *omp-1* genes, the same set of *p30* genes is expressed by *E. canis* in PBMCs regardless of the individual dog or infection time period (23).

*E. chaffeensis* 16S rRNA was detected in all 16 different groups of tick tissues (salivary glands, midgut, or whole body of ticks at three different developmental stages) using 16S rRNA prior to (8) and after attachment to six naïve dogs (Fig. 2), indicating that the tick infection was stable. Of 22 *omp-1* para-




| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Feeding[a] | A | A | A | A | A | A | T | T | T | T | T | T | T | T | T | T |
| Stage[b] | A | A | N | N | L | L | A | A | A | A | A | A | A | A | N | N |
| Dog[c] | D1 | D2 | D1 | D2 | D1 | D2 | D3 | D4 | D3 | D4 | D5 | D6 | D5 | D6 | D7 | D8 |
| Tick tissue[d] | H | H | H[e] | H[e] | H[f] | H[f] | S | M | S | M | S | M | S | M | H | H |

**[a] A: acquisition feeding , T; transmission feeding**
**[b] When attached. A: adult, N: nymph, L: larvae**
**[c] D1: dog 133, D2: dog 146, D3: dog 85, D4: dog 87, D5: dog 150, D6: dog 350, D7: dog AAX, D8: dog BKF**
**[d] H: half body, S: salivary gland, M: midgut**
**[e] Tissue of adults molted from nymphs**
**[f] Tissue of nymphs molted from larvae**

FIG. 2. Transcriptional profiles of the *omp-1* paralogs by RT-PCR in 16 different specimens of ticks. Total tick RNA was extracted and subjected to RT-PCR. The panels show ethidium bromide-stained RT-PCR products of *E. chaffeensis* 16S rRNA and *omp-1B*. M, molecular size markers (1-kb plus DNA marker [Life Technologies]); Pos, positive control utilizing *E. chaffeensis* DNA as template; lanes 1 to 16, different specimens of ticks; and NT, no template. Nucleotide base pair sizes of amplified product are indicated on the right.

logs examined by RT-PCR, *omp-1B* was the only *omp-1* transcript detected in all 16 groups of tick tissues (Fig. 2). Without RT, all of these tick tissues were negative by RT-PCR using 16S rRNA or *omp-1* paralog primers, indicating the absence of DNA contamination (data not shown). No ehrlichial 16S rRNA or transcripts of *omp-1* paralogs were detected in control uninfected tick tissues. It was previously demonstrated that only one *p30* paralog, *p30-10*, the ortholog of *omp-1B*, is expressed by *E. canis* in *Rhipicephalus sanguineus* ticks and that, of all 22 *p30* paralogs examined, only *p30-10* expression is up-regulated in DH82 cells at 25°C in culture compared to its expression at 37°C (23). Present results support our speculation that expression of *p30-10* and *omp-1B*, in *E. canis* and *E. chaffeensis*, respectively, is induced in ticks, since the temperature is lower in ticks than in mammals.

To estimate the detection limit of the RT-PCR, *omp-1*-specific transcripts were generated in vitro. *omp-1B* and *omp-1C* were selected as representatives, because *omp-1B* was universally expressed in both dogs and ticks (Fig. 1 and 2) and because *omp-1C* was the weakest amplicon detected by the gene-specific primers (Fig. 1B). The template for the transcription was prepared by PCR using the following primer pairs: for *omp-1B*, forward primer 5′-<u>TAATACGACTCACTATAGGG</u>AACGACAGCAGAGAAGGC-3′ and reverse primer 5′-GCGGAAACTTCTGGTGTG-3′, which was 220 bp downstream of the 3′ end (nucleotide 16138) of the RT-PCR region; for *omp-1C*, forward primer 5′-<u>TAATACGACTCACTATAGGG</u>CTTCAAGTCATGCTGATGC-3′ and reverse primer 5′-ATGATGGTGTAGCAAACGC-3′, which was 301 bp downstream from the 3′ end (nucleotide 17282) of the RT-PCR region. The T7 binding site sequences of the above primers are underlined. Specific transcripts of *omp-1B* and *omp-1C* generated in vitro as previously described (23) were 10-fold serially diluted and used in RT-PCR against a background of the total RNA from $2.5 \times 10^6$ uninfected dog PBMCs to mimic the experimental conditions (Fig. 3). Under our standard RT-PCR conditions, 365- and 230-bp cDNA fragments of *omp-1B* and *omp-1C*, respectively, were detected to levels of $10^3$ and $10^4$ transcripts, respectively. The detection limit per reaction of nested PCR based on the 16S rRNA gene was 48 fg of DNA from *E. chaffeensis*-infected DH82 cells (8). This amount of DNA corresponds to 250 *E. chaffeensis* genomes in $2.5 \times 10^6$ PBMCs. All dog and tick specimens examined in the present study were positive for 16S rRNA gene-based nested PCR. This means that at least 250 *E. chaffeensis* genomes were present in each specimen. Therefore, when the *omp-1* paralogs were not detectable in these specimens by RT-PCR, the transcript number was less than 4 to 40 per *E. chaffeensis* genome.

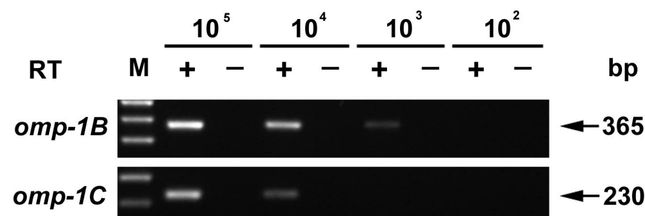



FIG. 3. Estimation of RT-PCR sensitivity in detecting the transcripts of *omp-1* paralogs. +, RT-PCR analysis was performed using decreasing amounts of in vitro generated transcripts as template; −, shown is the result of an identical reaction without the addition of RT as control for DNA contamination. The transcript numbers are shown at the top. *omp-1* genes are identified on the left. M, molecular size markers (1-kb plus DNA marker [Life Technologies]). Nucleotide base pair sizes of amplified product are indicated on the right.

To test *E. chaffeensis* transmission from ticks to dogs, approximately 150 nymphs infected as larvae were placed on dogs BKF and AAX, respectively; 50 and 60 adult ticks infected as nymphs were placed on dogs 150 and 350, respectively; and 50

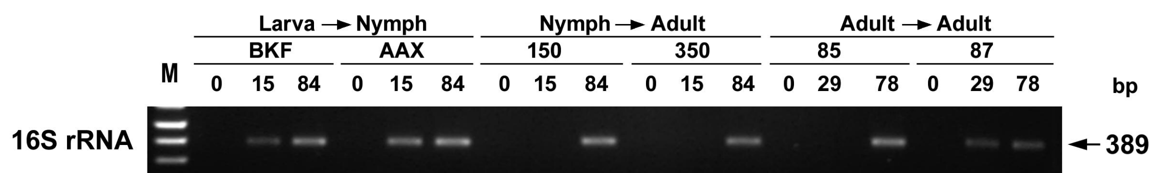



FIG. 4. Expression of 16S rRNA of *E. chaffeensis* in the PBMCs of six dogs infected by tick attachment. Total RNA was extracted and subjected to RT-PCR. The amplified products were resolved on agarose gels containing ethidium bromide. Stages of ticks fed on naïve dogs, dog identifications, and days postattachment are indicated in this sequential order at the top. Larva→Nymph, nymphs infected as larvae; Nymph→Adult, adults infected as nymphs; Adult→Adult, adult males infected as adults; and M, molecular size markers (1-kb plus DNA marker [Life Technologies]). Nucleotide base pair size of amplified product is indicated on the right.

and 70 male ticks infected as adults were placed on dogs 85 and 87, respectively. Ticks were allowed to feed for 7 days and were then removed. *E. chaffeensis* 16S rRNA was detected by RT-PCR in the PBMCs, starting from days 15 to 36 through 153 (dogs 85 and 87) or through day 159 (dogs 150, 350, BKF, and AAX) postattachment (only representative time points are shown in Fig. 4). In addition, *groEL* transcripts of *E. chaffeensis* were detected using primers described previously (22) on day 35 or 36 in the 16S rRNA-positive samples (data not shown), indicating that *E. chaffeensis* was transmitted among dogs by *A. americanum* ticks.

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