Rickettsia felis in Fleas, France

To the Editor: Rickettsia felis belongs to the spotted fever group of rickettsia. The pathogenic role of this intracellular Proteobacteria in humans has been reported in patients from the United States (Texas) (1), Mexico (2), Germany (3), Brazil, and France (4). R. felis is widely distributed, is associated with blood-sucking arthropods, and has been isolated from fleas in several countries (5).

To obtain new information about the distribution of R. felis in France and potential vectors/reservoirs of this emerging pathogen, 550 fleas were collected from 82 dogs and 91 cats in 7 widely distributed locations in France (Bordeaux, Toulouse, Cosnes-Cours sur Loire, Dijon, Moulins, Limoges, and Aix-en-Provence). Specimens were collected by combing, recorded, and stored at –20°C. Samples were shipped on dry ice to the entomologic laboratory of the Institute of Comparative Tropical Medicine and Parasitology in Munich, Germany, and species identification was performed by using...
light microscopy and following the determination key of Hopkins and Rothschild (6). Because infestation levels varied (1–150 fleas/animal), we randomly analyzed 1–8 fleas (mean 3.4) from each host animal.

We homogenized fleas individually in 80 μL of phosphate-buffered saline by using 5-mm steel beads in a RETSCH Tissue Lyser Mixer Mill 300 (QIAGEN, Hilden, Germany). A total of 100 μL of ATL buffer and 20 μL of proteinase K (QIAGEN) were added, and the homogenate was incubated at 56°C in a thermomixer (Eppendorf, Hamburg, Germany) until the tissues were lysed. DNA was extracted from each flea by using a QIAamp DNA Mini Kit (QIAGEN) according to the manufacturer’s instructions (tissue protocol) and stored at −20°C until used in a PCR.

PCR amplification of rickettsial DNA was performed by using previously described oligonucleotide primer pairs Rp CS.877p/Rp CS.1258n targeting the citrate synthase (gltA) gene and, for the positive samples, Rr 190.70p/Rr 190.602n targeting the outer membrane protein A (ompA) gene (7). Amplification was conducted in 50-μL volumes that contained 5 μL of DNA, 30 μL of distilled water, 10 μL of 5× Taq buffer (Roche, Mannheim, Germany), 3 μL of 25 mmol/L MgCl₂ (Roche), 1 μL of 10 mmol/L deoxynucleotide triphosphates (Roche), 0.25 μL of each primer (100 μM), and 0.5 μL (5 U/mL) of Taq polymerase (Roche). Conditions for the gltA and ompA PCRs were as described by Bertolotti et al. (8). Negative and positive controls were included in all PCRs. All PCR products were separated by electrophoresis on 1.5% agarose gels at 100 V for 60 min and examined under UV light. For both genes, positive samples were purified by using the QIAquick PCR Purification Kit (QIAGEN) and sent for sequencing to the MWG Biotech Company (Martinried, Germany). Sequences were compared with those of previously characterized rickettsia in GenBank by using basic local alignment search tool (BLAST) (www.ncbi.nlm.nih.gov) analysis.

Five species of fleas were identified: *Ctenocephalides felis* (500, 224 from dogs and 276 from cats), *C. canis* (37 from dogs), *Pulex irritans* (11 from dogs), *Spilopsyllus cuniculi* (1 from a cat), and *Archaeopsylla erinacei* (1 from a cat). Five dogs had mixed populations of fleas; 3 of these had *P. irritans* and *C. felis*, and 2 had *C. felis* and *C. canis*. One cat had *P. irritans* and *C. felis*, and another cat had *S. cuniculi* and *C. felis*. A total of 52 (19%) of the 272 fleas from dogs and 44 (16%) of the 278 fleas from cats were positive for both the gltA and ompA genes. Positive samples were obtained from all locations. Prevalence ranged from 6% (Dijon) to 43% (Toulouse) for dogs and from 3% (Moulins) to 37% (Bordeaux) for cats (Table). Of 550 fleas, 96 were positive for both genes (gltA and ompA) and 3 of 5 species of fleas were infected: 10 with *C. canis*, 85 with *C. felis*, and 1 with *A. erinacei*. All sequences matched gltA and ompA genes from *R. felis* (similarity 99%–100%).

Our investigation provides new information about distribution of *R. felis* and widespread flea infection with *R. felis* in France. A total of 88% of infected fleas were *C. felis*, but we found infected *C. canis* in Bordeaux and Toulouse and infected *A. erinacei* in Limoges. We report the presence in France of *R. felis* in *C. canis* and *A. erinacei* in France. *R. felis* in dog fleas in Uruguay and in hedgehog fleas in Algeria has been reported (9,10). Our findings indicate that these 2 flea species may be vectors of human *R. felis* rickettsiosis in France.

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**Table. Prevalence of *Rickettsia felis* in fleas from dogs and cats, France**

<table>
<thead>
<tr>
<th>Locality</th>
<th>Animal</th>
<th>No. animals</th>
<th>No. fleas</th>
<th>Flea species</th>
<th>No. (%) gltA+ ompA+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aix-en-Provence</td>
<td>Dog</td>
<td>6</td>
<td>20</td>
<td><em>Ctenocephalides felis</em>, † <em>C. canis</em>, † <em>Pulex irritans</em></td>
<td>6 (30)</td>
</tr>
<tr>
<td></td>
<td>Cat</td>
<td>11</td>
<td>38</td>
<td><em>C. felis</em>, † <em>C. canis</em>, † <em>P. irritans</em></td>
<td>14 (37)</td>
</tr>
<tr>
<td>Bordeaux</td>
<td>Dog</td>
<td>14</td>
<td>67</td>
<td><em>C. felis</em>, † <em>C. canis</em>, † <em>P. irritans</em></td>
<td>8 (12)</td>
</tr>
<tr>
<td></td>
<td>Cat</td>
<td>11</td>
<td>38</td>
<td><em>C. felis</em>†</td>
<td>14 (37)</td>
</tr>
<tr>
<td>Cosnes-Cours sur Loire</td>
<td>Dog</td>
<td>15</td>
<td>44</td>
<td><em>C. felis</em>, † <em>C. canis</em></td>
<td>7 (16)</td>
</tr>
<tr>
<td></td>
<td>Cat</td>
<td>17</td>
<td>50</td>
<td><em>C. felis</em>†</td>
<td>3 (6)</td>
</tr>
<tr>
<td>Dijon</td>
<td>Dog</td>
<td>6</td>
<td>18</td>
<td><em>C. felis</em>, † <em>C. canis</em></td>
<td>1 (17)</td>
</tr>
<tr>
<td></td>
<td>Cat</td>
<td>1</td>
<td>3</td>
<td><em>C. felis</em>†</td>
<td>1 (33)</td>
</tr>
<tr>
<td>Limoges</td>
<td>Dog</td>
<td>15</td>
<td>45</td>
<td><em>C. felis</em>†</td>
<td>7 (16)</td>
</tr>
<tr>
<td></td>
<td>Cat</td>
<td>21</td>
<td>61</td>
<td><em>C. felis</em>, † <em>Archaeopsylla erinacei</em>†</td>
<td>11 (18)</td>
</tr>
<tr>
<td>Moulins</td>
<td>Dog</td>
<td>12</td>
<td>36</td>
<td><em>C. felis</em>, † <em>C. canis</em></td>
<td>5 (14)</td>
</tr>
<tr>
<td></td>
<td>Cat</td>
<td>22</td>
<td>65</td>
<td><em>C. felis</em>, † <em>Spilopsyllus cuniculi</em></td>
<td>2 (3)</td>
</tr>
<tr>
<td>Toulouse</td>
<td>Dog</td>
<td>14</td>
<td>42</td>
<td><em>C. felis</em>, † <em>C. canis</em>†</td>
<td>18 (43)</td>
</tr>
<tr>
<td></td>
<td>Cat</td>
<td>19</td>
<td>61</td>
<td><em>C. felis</em>†</td>
<td>13 (21)</td>
</tr>
</tbody>
</table>

*gltA, citrate synthase A; ompA, outer membrane protein A.
†Species positive for gltA and ompA.
Novel Nonstructural Protein 4 Genetic Group in Rotavirus of Porcine Origin

To the Editor: Infection with group A rotavirus is the main cause of acute gastroenteritis in infants and young children worldwide and in young animals of many species, including piglets. In recent years, several epidemiologic studies designed to monitor the appearance of novel or atypical rotavirus antigenic types have provided evidence for the increasing antigenic diversity of group A rotaviruses (1–3). In addition to the 2 rotavirus classification systems, VP7 (G) and VP4 (P) genes, the virus can also be classified on the basis of the nonstructural glycoprotein 4 (NSP4)–encoding gene. Sequence analyses of the NSP4 gene indicated the presence of at least 5 distinct genetic groups among human and animal rotaviruses, termed A to E (1,4,5). Among human rotaviruses, the diversity of NSP4 genes has been restricted mainly to genetic groups A and B; only a few human strains possess genetic group C. Conversely, all 5 NSP4 genetic groups (A–E) have been identified in rotaviruses of animal origins. To our knowledge, porcine rotaviruses (PoRV) have been reported to belong only to NSP4 genetic group B (1).

During an epidemiologic survey of PoRV from June 2000 through July 2001, a total of 175 fecal specimens were collected from diarrheic piglets from 6 different farms in Chiang Mai Province, Thailand. Of these, 39 (22.3%) specimens were positive for group A rotavirus (6). A novel and unusual PoRV CMP034 strain was isolated from a 7-week-old piglet during this survey. Molecular genetic characterization showed that the CMP034 strain carried a novel P[27] genotype with a new lineage of G2-like rotavirus genotype (7). We performed a molecular analysis of the NSP4 gene of this strain in comparison with those of other NSP4 gene sequences available in the GenBank database.

The full-length of NSP4 gene was amplified by NSP4–1a and NSP4–2b primer pairs (8). The PCR amplicon was sequenced in both directions by using the BigDye Terminator Cycle Sequencing kit (PerkinElmer- Applied Biosystems, Inc., Foster City, CA, USA) on an automated sequencer (ABI 3100; PerkinElmer-Applied Biosystems, Inc.). The sequence of CMP034 was compared with those of reference strains available in the National Center for Biotechnology Information GenBank database by using BLAST (www.ncbi.nlm.nih.gov/blast). The NSP4 nucleotide sequence of the CMP034 strain was deposited in GenBank under accession no. DQ534017.

The complete NSP4 nucleotide sequence of PoRV CMP034 strain was 750 bp and contained a single long open reading frame coding for a protein of 175 aa. Comparative analysis of the CMP034 NSP4 sequence with those of the 5 representative established genetic groups (A–E) showed the highest sequence identity, at 92.6% nt and 96.9% aa levels, with 1 PoRV strain, P21–5 (9). However, CMP034 and P21–5 shared a low degree of sequence identity with other NSP4 genetic groups. The NSP4 sequence identities of the CMP034 and P21–5 strains ranged from 74% to 78% nt and 75%–79% aa levels with those of genetic group A; 77%–86% nt and 79%–86% aa levels with genetic group B; 69%–73% nt and 75%–78% aa levels with genetic group C; 62%–65% nt and 55%–60% aa levels with genetic group D; and only 43%–50% nt and 29%–33% aa levels with genetic group E. The phylogenetic tree confirmed that PoRV strains CMP034 and P21–5 were located exclusively in a separated branch, which was distantly related to the other 5 known NSP4 genetic groups (Figure). However, a bootstrap support for the separation of

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References


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