Presence of *Rickettsia felis* in the Cat Flea from Southwestern Europe

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*Rickettsia felis*, formerly called ELB agent, was identified by using molecular biology techniques in the cat flea (*Ctenocephalides felis felis*) from southwestern Spain. For the first time this flea-transmitted rickettsia has been detected within its vector in Eurasia.

Members of the genus *Rickettsia* are commonly associated with hematophagous arthropods such as ticks, fleas, or lice. *Rickettsia felis*, formerly ELB agent, was detected in 1990 when tissues from the cat flea, *Ctenocephalides felis*, were examined under electron microscopy. After this, several antigenic and molecular studies concerning this rickettsia were developed (1). *R. felis* is maintained in cat fleas by transovarian transmission (2). Infection in humans has been described in the USA (3), Mexico (4), and Brazil (1) by polymerase chain reaction (PCR) amplification and recently in France by serologic tests (1).

During a study concerning rickettsial organisms transmitted by ticks in southwest Spain, using molecular tools for diagnosis, a rickettsial microorganism was detected in some cat fleas on domestic cats and dogs from different counties of the Cadiz Province.

The Study

The fleas used in this study (60 females and 11 males) were collected, together with ticks, from 2 cats and 12 dogs from eight localities of Cadiz Province in southwestern Spain from May to August of 1999 (Figure, Table). The hosts were domestic and peridomesticated dogs and cats living in a range of health-care conditions. Collected fleas were fixed in 70% ethyl alcohol and stored at 4°C until they were processed. Taxonomic determination was made by using current taxonomic keys (5,6). All specimens subjected to analysis were *C. felis felis* (Bouché, 1835).

DNA was extracted from 14 lots of fleas (ranging from 1 to 11 specimens per lot) by using the DNeasy_Tissue kit (Qiagen GmbH, Hilden, Germany) (7). Elution of DNA was made in 100 µL of TE buffer (1 mM Tris HCl, 0.1 mM EDTA). Extraction blanks, consisting of water processed along with flea samples, were also included as controls.

A Biometra DNA Thermalcycler (Gottingen, Germany) was used for all PCR amplification. Three microliters of each DNA extraction were added to 27 µL of master mixture for each reaction. Final reagent concentration was 0.2 µM for each primer, 200 µM for each deoxynucleotide triphosphate (Promega Corp., Madison, WI), 2 U of Biotaq polymerase (BioLine, London, UK), and 1x Bioline buffer. The following thermal cycler parameters were used with the primer pairs for citrate synthase (*gltA* RpCS.877p and RpCS1258n (8), 120-kDa genus common antigen (*ompB*) (rfompbf: 5’–GAC AAT TAA TAT CGG TGA CGG, and rfompbr: 5’-TGC ATC AGC ATT ACC GCT TGC), 190-kDa protein antigen (*ompA*) Rr190.70p, and Rr190.602n (8): 96°C (90 sec), followed by 35 cycles of 94°C (30 sec), 50°C (30 sec), and 72°C (45 sec), followed by an extension period (72°C, 7 min). For the amplification of a 426-base pair fragment of 16S rRNA gene, we used the primers fD1 (9) and Rc16S.452n (10) and 59°C as annealing temperature.

Seven lots from five localities around Cadiz and Gibraltar bays were positive to amplification of fragments of 16S rRNA, *gltA*, *ompA* and *ompB* genes.

In brief, after amplification, primers and nucleotides were removed from 300 µL of PCR products by purification on the Wizard PCR prep system (Promega, Madison, USA). The purified products were used as templates for sequencing reactions. The sequences were compared to the GenBank database.

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Identical to previously reported sequence for 16S rRNA, (National Center for Biotechnology Information, Bethesda, Rickettsia near primers, we used a 10% polyacrylamide 7M urea electro-
in scanning the gel. To determine the sequence of positions
the standard Promega protocol. A permanent record was made
and 2 hr 30 min, respectively. Gel was silver stained by using
constant electrophoresis (55°C) and separated for 4 hr 30 min.
Acid Sequencing System (BioRad, Hercules, CA) at 55 W of
mide 7M urea gels by electrophoresis in the Sequi-Gen Nucleic
reaction products were loaded twice on 40 cm 6% polyacryla-
ver sequence DNA Sequencing System (Promega). Sequencing
con by using the correct forward or reverse primers and the Sil-
µL) were used directly in the sequencing reaction.
Approximately 100 fmol of the purified PCR product (4-5
 WI). Approximately 100 fmol of the purified PCR product (4-5 µL) were used directly in the sequencing reaction.

The PCR cycle sequencing was performed for each ampli-
con by using the correct forward or reverse primers and the Sil-
er sequence DNA Sequencing System (Promega). Sequencing
reaction products were loaded twice on 40 cm 6% polyacryla-
mide 7M urea gels by electrophoresis in the Sequi-Gen Nucleic
Acid Sequencing System (BioRad, Hercules, CA) at 55 W of
constant electrophoresis (55°C) and separated for 4 hr 30 min.
and 2 hr 30 min, respectively. Gel was silver stained by using
the standard Promega protocol. A permanent record was made
in scanning the gel. To determine the sequence of positions
near primers, we used a 10% polyacrylamide 7M urea electrophoresis gel. The sequence of both strings was determined
twice for each fragment.

Sequences obtained were compared with those from other
Rickettsia species in GenBank by using the BLAST utility
(National Center for Biotechnology Information, Bethesda, MD) and FASTA routine from GCg environment. Fragment
sequence for 16S rRNA, gltA, ompA, and ompB sequence were
identical to previously reported sequence for R. felis. The 16S
rRNA amplified fragment was identical to previously reported
sequence (GenBank L28944) between positions 1 and 410 (3).
The fragment sequenced for citrate synthase corresponded to
positions 757 and 1138 in GenBank accession AF210692 (1).
The fragment amplified for ompB corresponded to positions 478 to 987 in GenBank accession AF191026 (11). The frag-
ment amplified for ompB corresponded to positions 599 to
1259 in GenBank accession AF210695 (1). Amplification was
unsuccessful in all negative controls.

**Conclusions**

*R. felis* has been found extensively in commercial colonies
and natural cat fleas, parasitizing a large range of mammalian
hosts in several states of the United States (12,13).

For the first time *R. felis* was detected in Eurasia, by means
of PCR and partial sequencing of genes classically used in
rickettsial molecular characterization and phylogeny. The sequences of gltA, ompA, ompB, and 16S rRNA from Cadiz cat
fleas were identical to the homologous sequences previously
reported for *R. felis* obtained from fleas reared in EL Laborato-
ries (Soquel, CA) (3) and Louisiana State University (11) and
isolated by Flea Data Inc. (Freeville, NY) (1).

In humans, *R. felis* may produce a clinical syndrome similar
to murine typhus (3). Thus, *R. felis* could be implicated in
murine typhus-compatible cases detected in southwest Spain
(14), especially since the oriental rat flea, * Xenopsylla cheopis*
(Rothschild, 1903), is absent from this area.

Thirteen species of flea belonging to the genus *Ctenocephalides*
have been described to date (15), mainly distributed in
continental Africa (16), with a worldwide contemporary distribu-
tion in a large range of hosts, mainly anthropic species of the
group (*C. felis*), which has a large potential host range. The
primary source of the bacterium might be Africa, where this flea
genus apparently originated.

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