

SHORT REPORT: FIRST DETECTION OF *RICKETTSIA FELIS* IN *CTENOCEPHALIDES FELIS* FLEAS PARASITIZING RATS IN CYPRUS

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Abstract. *Rickettsia felis* was identified by polymerase chain reaction amplification and DNA sequencing analysis in *Ctenocephalides felis* fleas parasitizing rats in Cyprus. Murine typhus caused by *R. typhi* was believed to be the only flea-transmitted rickettsiosis on the island. This is the first report of this pathogen in southeastern Europe.

Fleas are hematophagous arthropods and serve as vectors for microbial pathogens of medical and veterinary importance. There is increasing interest in the role of *Ctenocephalides felis* fleas in the epidemiology of flea-transmitted pathogens, and recent studies have implicated this flea as a vector of *Rickettsia felis*.¹ This new pathogenic agent, which is the cause of flea-borne spotted fever, is a recently described flea-transmitted rickettsia that was first detected in 1990 as the ELB agent from midgut epithelial cells of *C. felis* cat fleas.² It has since been documented in patients in various countries, including the United States, Mexico, Brazil, Germany, France, and Thailand.^{3–7} To date, its presence has been demonstrated in cat, dog, and opossum *C. felis* flea populations in the United States, Brazil, Mexico, Spain, France, the United Kingdom, Ethiopia, New Zealand, and Thailand.^{7–12}

Rickettsia felis is not lethal to fleas^{1,8} and it is transmitted transovarially in the arthropod host; thus, it can be maintained naturally within a flea population, making *C. felis* an important reservoir and amplifying host of this microorganism, and allowing its persistence in a niche during adverse conditions. *Ctenocephalides felis* has a broad host range (cats, dogs, and other mammals of similar size) and can readily switch to different hosts if needed. It has been found parasitizing peri-urban wildlife species such as opossums, rats, and mice, as well as humans.¹³ We report the detection of *R. felis* in Cyprus in *C. felis* fleas collected from rats (*Rattus norvegicus* and *R. rattus*) and analyzed by polymerase chain reaction (PCR) amplification and DNA sequencing.

Six hundred twenty-two wild rats (220 *Rattus rattus frugivorus* and 402 *R. norvegicus*) were captured at 51 different localities during a survey conducted on the island of Cyprus in 2001–2003 that investigated pathogens transmitted by rodents and their arthropod ectoparasites. Fleas were collected by combing the trapped rodents; 1,035 fleas were obtained from 40.6% of the rats. Fleas were identified using morphologic criteria and stored in 70% ethanol until tested. Of these, 250 (24.2%) were *C. felis* from 21% of the flea-infested rats (Table 1). All *C. felis* fleas were tested for the presence of *R. felis*.

Each flea sample was immersed for five minutes in a solution of 70% ethanol/0.2% iodine, washed three times (five minutes per wash) in sterile distilled water, and crushed in-

dividually in sterile Eppendorf (Hamburg, Germany) tubes using the tip of a sterile pipette. DNA was extracted by using the QIAamp Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. A PCR was conducted using the primer pairs Rr 190.70p/Rr 190-701 and CS.Rp877p/CS.Rp1258n derived from genes encoding the outer membrane protein A (*ompA*) and citrate synthase (*gltA*), respectively, as previously described.^{6,7,13} All recommended measures were taken to avoid PCR contamination. After purification of the obtained PCR amplicons (QIAquick Spin PCR purification kit; Qiagen), sequencing analysis was conducted and DNA sequences obtained were compared for similarity with sequences in GenBank using the Basic Local Alignment Search Tool program (National Center for Biotechnology Information, National Institutes of Health (Bethesda, MD) (<http://www.ncbi.nlm.nih.gov>).

The PCR amplicons were obtained from 14 fleas (5.6%) obtained from 4 *R. rattus* and 10 *R. norvegicus* from 8 localities. The *ompA* and *gltA* DNA sequences of all PCR products were identical to those for *R. felis*. Three ticks were also found on the rats examined. All were tested by a PCR and were negative for *Rickettsia* species.

Murine typhus caused by *R. typhi* was considered to be the only flea-transmitted rickettsiosis present in Cyprus. This is the first report of a second flea-borne rickettsia on the island and the first report of *R. felis* in southeastern Europe. Our study adds to the accumulating data on *R. felis*, and supports the belief that this pathogen has a worldwide distribution.

Although *R. felis* infections in humans have been reported in three continents (America, Europe, and Asia), only 11 human cases have been studied; therefore, the prevalence of this infection is not known. *Rickettsia felis* infections in humans could easily be misdiagnosed. The clinical symptoms are not specific and include fever, headache, and rash. Moreover, specific laboratory diagnostic tests are not commercially available. Human cases of flea-borne spotted fevers with symptoms such as fever, rash, or both occur in Cyprus. In a previous study conducted in 1996–1997, a significant seroprevalence of IgG antibodies against *R. typhi* (45%) and *R. conorii* (40%) were demonstrated in a healthy population (Psaroulaki A and others, unpublished data). At the time of this study (2001–2003), 195 of 313 patients with suspected rickettsiosis had antibodies against both *R. conorii* and *R. typhi* (Psaroulaki A. and others, unpublished data).

Since the only known rickettsioses in Cyprus are Murine typhus and Mediterranean spotted fever, *R. conorii* and *R. typhi* antigens were the only antigens used for laboratory diagnosis and in epidemiologic studies. Serologic cross-

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TABLE 1
Distribution of rodents, fleas, and *Rickettsia felis* in the five districts of the government-controlled area of Cyprus

District	No. of rodents	No. of <i>Rattus rattus</i>	No. of <i>Rattus norvegicus</i>	No. of <i>Mus musculus</i>	No. of rats infested by fleas	No. of rats infested by <i>Ctenocephalides felis</i>	No. of <i>C. felis</i> collected	No. of <i>C. felis</i> positive for <i>R. felis</i> (%)
Nicosia	210	35	172	3	60	9	64	9 (14)
Famagusta	23	0	23	0	16	7	61	2 (3.27)
Larnaka	191	5	186	0	117	19	77	2 (2.59)
Limassol	138	119	19	0	49	16	48	1 (2.08)
Paphos	63	61	2	0	1	0	0	0
Total	625	220	402	3	243	51	250	14 (5.6)

reactivity between rickettsial species is not easy to identify. In some cases, differences in titers among tested antigens help identify the causative rickettsia.¹⁴

Since the clinical symptoms for infection with *R. felis* are similar to those for other rickettsioses,¹³ and antibodies to *R. felis* show cross-reactivity with *R. conorii* and *R. typhi* antigens, cases of infections with *R. felis* in Cyprus may have been misdiagnosed as Mediterranean spotted fever or murine typhus. This is further supported by the findings of a seroepidemiologic study of pathogens transmitted by wild rodents conducted in Cyprus in 2001–2003, which showed a high seroprevalence of antibodies against rickettsiae (38.4% against

R. typhi and 32.9% against *R. conorii*) (Psaroulaki A. and others, unpublished data). Again, only commercial antigens were used in the study and the presence of antibodies against *R. felis* in rats could not be confirmed. However, analysis of the results by a geographic information system showed that the distribution of *C. felis* corresponded to the distribution of rats seropositive for rickettsiae. (Figure 1).

Rickettsia felis has been detected in several countries in *C. felis* fleas parasitizing cats, dogs, and opossums. There is only one report of rats hosting *C. felis*.¹⁵ Since 5.6% of the fleas removed from rats were infected with *R. felis*, contact with *C. felis* fleas carried by rats would account for *R. felis*

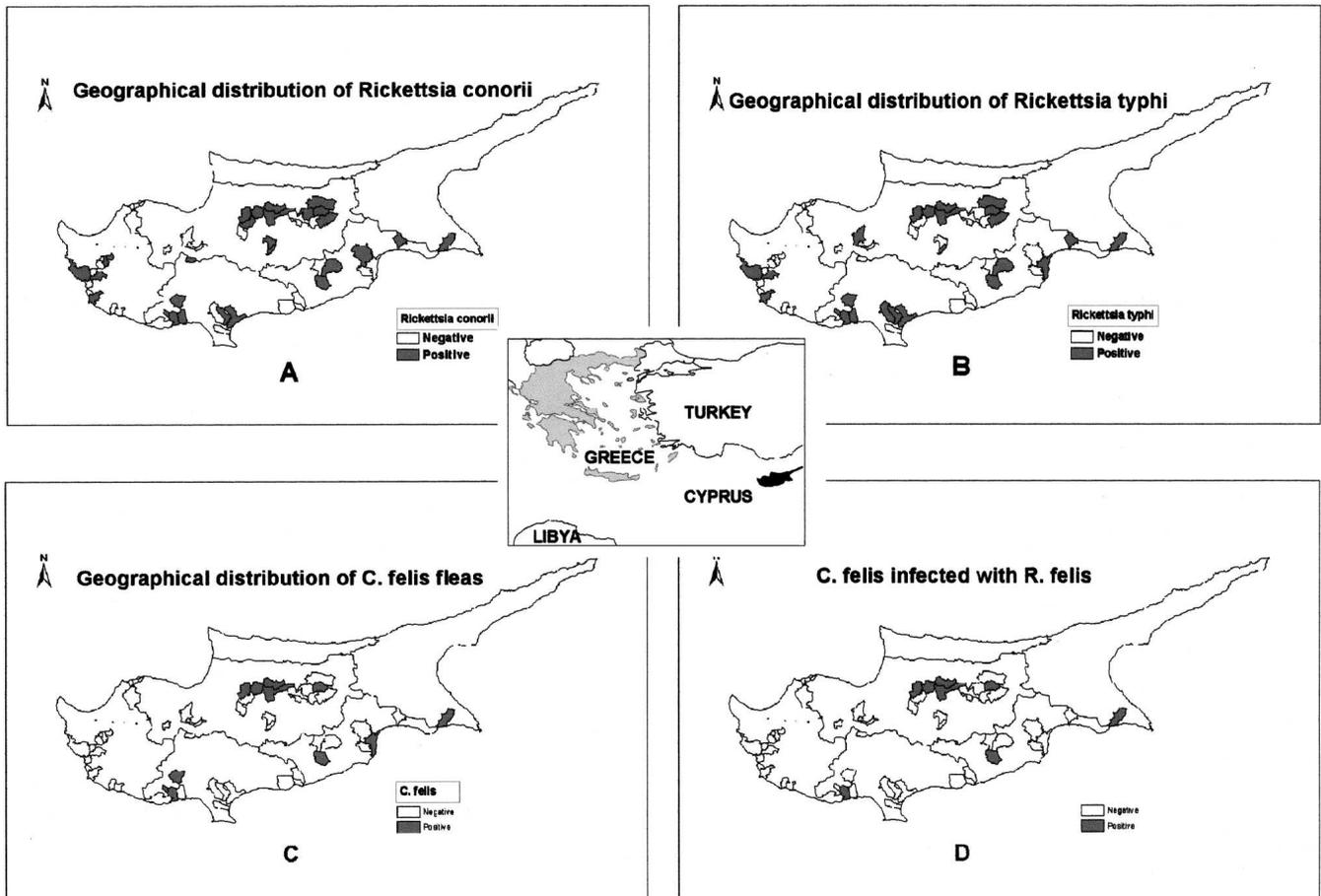


FIGURE 1. Geographic distribution of A, Rats seropositive to *Rickettsia conorii*; B, rats seropositive to *Rickettsia typhi*; C, Rats infested with *Ctenocephalides felis* fleas; and D, *C. felis* infected with *Rickettsia felis* in Cyprus, as determined using geographic information system technology, Microsoft Access® (Microsoft, Redmond, WA), and ArcView version 8.1 (Environmental Systems Research Institute, Redlands, CA) The shaded areas indicate regions with infected rats and fleas.

infection in humans and animals. If one considers that *C. felis* is a common ectoparasite of cats and dogs in Cyprus and that this flea, which often feeds on humans, was found infected with *R. felis* in eight different regions of the island, we believe that this pathogen poses a high risk to human and animal health.

The results of this study suggest that *R. felis* may be prevalent in clinical cases in humans in Cyprus and should be considered in the differential diagnosis of typhus-like illnesses, especially following a flea bite. Further studies should be conducted to identify *R. felis* infections in human and mammalian hosts in Cyprus and to determine the prevalence and the clinical spectrum of this infection on the island by using more specific diagnostic tests. The role of rats and *C. felis* fleas in the epidemiology of *R. felis* needs further investigation.

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