Rickettsia typhi and *Rickettsia felis* in *Xenopsylla cheopis* and *Leptopsylla segnis* Parasitizing Rats in Cyprus

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Abstract. Fleas collected from rats during a three-year period (2000–2003) in 51 areas of all provinces of Cyprus were tested by molecular analysis to characterize the prevalence and identity of fleaborne rickettsiae. *Rickettsia typhi*, the causative agent of murine typhus, was detected in *Xenopsylla cheopis* (4%) and in *Leptopsylla segnis* (6.6%). *Rickettsia felis* was detected in *X. cheopis* (1%). This is the first report of *R. typhi* in *X. cheopis* and *L. segnis* from rats, in Cyprus, and the first report of *R. felis* in *X. cheopis* in Europe. The role of fleas (mainly *X. cheopis*) was confirmed in the epidemiologic cycle of murine typhus in Cyprus by interrelation of current results with those of previous studies. The geographic distribution of fleas coincided with the geographic distribution of the pathogen they can harbor, which emphasizes the potential risk of flea-transmitted infections in Cyprus.

INTRODUCTION

Murine typhus, sometimes referred to as endemic typhus, is a zoonotic infectious disease caused by *Rickettsia typhi* (*R. mooseri*), an obligate intracellular bacterium. *Rickettsia typhi* is a member of the typhus group rickettsiae. The disease is characterized by headache, rash, and fever and occurs worldwide in a variety of environments.¹

The pathogen is maintained in nature by a cycle involving vertebrate hosts and their ectoparasites. The classic cycle of murine typhus involves rats (*Rattus norvegicus* and *R. rattus*) as reservoirs, and their fleas; the oriental rat flea *Xenopsylla cheopis* is the main vector.^{1,2} However, other vertebrate hosts, such as house mice, shrews, opossums, skunks, and cats, which live in or enter rat-infested buildings and human habitations, may be involved in the epidemiology of murine typhus.^{3,4} Infection of the vertebrate host presumably results from contamination of broken skin, the respiratory tract, or the conjunctivae of the host with infected flea feces or flea tissues.¹ Although *X. cheopis* is considered the major vector of murine typhus, natural infection with *R. typhi* has been reported for nine flea species.⁵

Rickettsia felis is the causative agent of an emerging disease known as fleaborne spotted fever. This pathogenic agent is a recently described flea-transmitted *Rickettsia* that was first detected in 1990 in *Ctenocephalides felis* and described as the ELB agent.^{6,7} To date, *R. felis* has been identified worldwide, mainly in the cat flea *C. felis*, which is regarded as its main vector. However, evidence of *R. felis* in other flea species and in ticks and mites suggests a variety of arthropod hosts.^{8,9} Nevertheless, the occurrence of *R. felis* infections in humans is relatively rare: 69 human cases had been reported worldwide during 1994–2009.^{9,10} Because clinical signs of the disease are similar to those of murine typhus and other febrile illnesses, the infection in humans may easily be underestimated and remain poorly characterized.

The prevalence of *R. typhi* infections among humans in Cyprus was unknown until 1996, when an outbreak of 35 clinical

cases of murine typhus occurred in an area near Nicosia, the capital of Cyprus. Murine typhus was diagnosed by immunofluorescent antibody testing. The predominant clinical manifestations of patients were fever, headache, and rash; acute renal failure also developed in two patients. During the summer of 1997 24 additional cases were reported, most from the same area. After this outbreak, a serologic survey was conducted in 1997, in which a prevalence of 47.3% for IgG and 14.4% for IgM to *R. typhi* was found (Psaroulaki A and others, unpublished data). Recently, 21 pediatric cases of murine typhus were reported.¹¹ However, the actual incidence of murine typhus and the prevalence of *R. felis* infections among humans in Cyprus are unknown.

The current study is part of a survey that involved capturing rats and their fleas and using them as indicators of the presence and dispersal of zoonotic agents in Cyprus by using geographic information system technology (Psaroulaki A and others, unpublished data).¹² Fleas collected from 622 captured rats were tested by molecular means for *Rickettsia* spp. The results were compared with those of previous studies for human cases of murine typhus in Cyprus.

MATERIALS AND METHODS

Study area and rats and their fleas. During a three-year survey (2001–2003), fleas were collected from 622 wild rats (220 *R. rattus frugivorus* and 402 *R. norvegicus*) captured at 51 localities in all five prefectures of Cyprus.

Fleas were counted and identified by using accepted morphologic criteria and were kept frozen at –80°C in sterile tubes until they were sent to the Laboratory of Clinical Bacteriology, Parasitology, Zoonoses and Geographical Medicine, Faculty of Medicine, University of Crete, Heraklion, Greece, for further analysis.

A total of 1,035 fleas were collected after combing the animals and identified as *X. cheopis* (70.34%, *n* = 728), *C. felis* (24.22%, *n* = 250), *C. canis* (0.48%, *n* = 5), *Leptopsylla segnis* (4.36%, *n* = 45), and *Nosophylla fasciatus* (0.67%, *n* = 7). All *C. felis* collected, had already been tested in a previous study.¹³ In the present study, 457 fleas were tested: 400 *X. cheopis* randomly selected from 728 animals, 45 *L. segnis*, 7 *N. fasciatus*, and 5 *C. canis*.

DNA extraction. Each flea was immersed for 5 minutes in a solution of 70% ethanol/0.2% iodine, washed 3 times

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(5 minutes/wash) in sterile distilled water, and dried on sterile filter paper before being crushed in a sterile plastic tube. DNA was extracted by using the QIAamp Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. A sample of sterile water was included as negative control for every 10 flea samples. DNA extracts were stored at -20°C until further processed.

Polymerase chain reaction amplifications and sequence analysis. DNA extracts were tested by PCR with the primer pair CS.Rp877p/CS.Rp1258n, which amplifies a 381-basepair fragment of the citrate synthase gene (*gltA*) of rickettsiae, as described.¹⁴ To confirm positive PCR results, a second PCR assay was conducted with the primer pair 120-M59/120-807, which amplifies a 833-bp fragment of the *ompB* protein gene (*ompB*) of *Rickettsia* species, as described.¹⁵

The PCR amplicons were purified (QIAquick Spin PCR Purification Kit; Qiagen) and sequenced (sequencer CEQ 8000; Beckman Coulter, Athens, Greece). BLASTn (<http://www.ncbi.nlm.nih.gov/BLAST>), Chromas version 1.49 (Technelysium Pty. Ltd., Holland Park, Queensland, Australia), ClustalW37, and Lasergene version 7.1 (DNASTAR Inc., Madison, WI) software was used for processing identified sequences.

Statistical analysis. Statistical analysis was performed by using SPSS version 16 (SPSS Inc., Chicago, IL). Analysis of variance and chi-square tests were performed at a significance level of $P < 0.05$. The geographical distribution of fleas and of the PCR-positive fleas was investigated by using geographic information system (GIS) technology (ArcGIS 8.1; Environmental Systems Research Institute, Redlands, CA) and Microsoft (Redmond, WA) Access. Coordinates (provided by the Veterinary Services of Cyprus) of each sampling site were entered on a map of the island that was placed into a GIS database. The map was linked to a database that enabled detailed epidemiologic studies. A software program was developed that allowed contemporary visualization of the name of the village, its population, altitude, the number of animals, and other relative epidemiologic information.

Correlation of results with previous data. During the same study period (2001–2003), human cases of murine typhus were recorded by using data from the reporting system established in the Department of Medical and Public Health Services of the Ministry of Health. The geographic distribution of the murine typhus cases was investigated by using the GIS technology and areas were correlated with area where positive fleas were detected.

RESULTS

Twenty-three (5%) of 457 fleas tested were positive by both PCRs. Of these 23 fleas, 20 were *X. cheopis* and 3 were *L. segnis*. A sequence with 100% similarity to the *gltA* (AE017197) and

ompB (AE017197) genes of *R. typhi* was detected in 16 (4%) of 400 *X. cheopis* and in 3 (6.6%) of 45 *L. segnis*. A sequence with 100% similarity to the *gltA* (CP000053) and *ompB* (AF182279) genes of *R. felis* was identified in 4 (1%) of 400 *X. cheopis* tested.

The proportion of fleas infected by *R. typhi* or *R. felis* was significantly higher ($P < 0.001$) in the Nicosia Prefecture, where 13 *X. cheopis* and 3 *L. segnis* harbored *R. typhi* and 2 of 4 *X. cheopis* harbored *R. felis* (Table 1).

Distribution of tested and positive fleas and human cases of murine typhus is shown in Figure 1. Areas with *R. typhi*-infected fleas and human cases of murine typhus were identified and characterized as high-risk areas for *R. typhi* (Figure 2).

DISCUSSION

Cyprus is an island ecosystem located in the southeastern part of the Mediterranean Sea in which climatic and ecologic conditions are favorable for maintaining reservoirs and vectors of fleaborne rickettsiae. Nevertheless, little data are available on the presence and distribution of fleaborne rickettsiae in ectoparasitic fleas that might transmit the disease to humans. Murine typhus caused by *R. typhi* had been considered to be the only fleaborne rickettsiosis present in Cyprus until 2006, when *R. felis* was detected in *C. felis*.¹⁶ We report molecular detection and identification of *R. typhi* in *X. cheopis* and *L. segnis* collected from rats in Cyprus and the occurrence of *R. felis* in *X. cheopis*.

Rickettsia felis has primarily been associated with *C. felis* parasitizing cats, dogs, or opossums.⁴ However, the only recognized vector is the cat flea.⁸ Other flea species such as *C. canis*,^{17–19} *Anomopsyllus nudata*,²⁰ *Archaeopsylla erinacei*,^{16,21,22} *Ctenophthalmus* sp.,²¹ *X. cheopis*,^{22,23} *Pulex irritans*,¹⁸ and ticks and mites⁸ have been implicated as alternative competent vectors of *R. felis*.

Animal hosts of infected ectoparasites include cats, dogs, rodents, opossums, hedgehogs, horses, sheep, goats, gerbils, and monkeys.⁸ However, the role of vertebrates as reservoirs of this emerging pathogen has not been determined.

The presence of *R. felis* in *X. cheopis*-infested rats (*R. rattus* and *R. norvegicus*), as demonstrated in the present study, corroborates results of other studies.^{23,24} The same rat species also harbored *R. felis*-infected *C. felis* as reported.¹⁷ These data highlight two rodent flea species that can harbor *R. felis* in Cyprus. Other investigators also reported rodent-associated *R. felis*-infected flea species, such as *A. nudata*,²⁰ *A. erinacei maura*,^{17,21} *Ctenophthalmus* sp.,²¹ which suggests a role of rats and their fleas in the epidemiology of *R. felis*.

Rickettsia typhi was identified in *L. segnis* (collected from *R. rattus* and *R. norvegicus*) in Cyprus. *Leptopsylla segnis*

TABLE 1
Fleas collected from rats and tested for *Rickettsia* spp. by PCR, Cyprus*

Flea species	No. fleas tested	No. (%) PCR positive	<i>Rickettsia</i> spp. (no. fleas)	Host (no.)	Province localities
<i>Xenopsylla cheopis</i>	400	20 (5)	<i>R. typhi</i> (16) <i>R. felis</i> (4) <i>R. typhi</i> (3)	<i>Rattus rattus</i> (2), <i>Rattus norvegicus</i> (12) <i>R. rattus</i> (1), <i>R. norvegicus</i> (2) <i>R. rattus</i> (2)	10 3 2
<i>Leptopsylla segnis</i>	45	3 (6.7)			
<i>Ctenocephalides canis</i>	5	–			
<i>Nosphylla fasciatus</i>	7	–			
Total	457	23 (5)			

* PCR = polymerase chain reaction.

FIGURE 1. Scattering of *Xenopsylla cheopis* (A) and *Leptopsylla segnis* (B) collected fleas from Cyprus. *Rickettsia typhi*- and *R. felis*-positive fleas are also demonstrated. This figure appears in color at www.ajtmh.org.

fleas harboring *R. typhi* have been reported in Egypt (2.7%)²⁴ and Portugal (23.8%).²¹ Laboratory studies have shown that *L. segnis* is more effective than *X. cheopis* in transmitting *R. typhi* to rats.²⁵ However, *L. segnis* has not been considered as an important vector of *R. typhi* regarding the transmission of murine typhus humans because it does not bite humans. Nevertheless, the dense accumulation of infective feces on certain sites of the fur of the host raises the possibility of air-borne transmission to humans or rodents.²⁶

Rickettsia typhi was also identified in *X. cheopis* collected from *R. rattus* and *R. norvegicus* throughout Cyprus. The high abundance of *X. cheopis* and the high infection rate (4%), similar to that reported in Greece²⁷ and Egypt,²⁴ indicate that this flea species plays a key role in the epidemiology of murine typhus in Cyprus.

The results of the present study showed a correlation with data from previous studies regarding murine typhus and

other zoonoses in Cyprus (Psaroulaki A and others, unpublished data),¹² in which 48.6% of rats (the same rats from which the fleas in this study were removed) were seropositive for *R. typhi*. The seropositivity was strongly associated with region (the highest number of seropositive cases was found in the Larnaka area), season (more seropositive cases in autumn), and the presence of fleas. Seropositivity against *R. typhi* was strongly associated with *X. cheopis* ($P < 0.0001$). The geographic distribution of *X. cheopis* appeared to coincide with the geographic distribution of *R. typhi*-seropositive rats (mainly *R. norvegicus*) (Psaroulaki A and others, unpublished data). Moreover, the geographic distribution of *R. typhi*-positive *X. cheopis* correlated with the geographic distribution of the recorded human cases of murine typhus during the same study period (2001–2003) (Figure 2).

Our results showed that two fleaborne rickettsiae, *R. felis*, and *R. typhi*, circulate in Cyprus. *Rickettsia felis* in two flea

FIGURE 2. Incidence map showing human *Rickettsia typhi* cases in Cyprus during 2000–2003. This figure appears in color at www.ajtmh.org.

species, *C. felis*,¹⁶ which often feed on humans and animals, and *X. cheopis*, which are highly abundant on rats, amplifies the risk for rickettsial transmission in Cyprus. However, to date, no *R. felis* infections in humans have been documented, and the prevalence of *R. felis* infections among humans in Cyprus is unknown. Because the clinical symptoms of *R. felis* infection (fever, headache, and rash) are similar to those of other rickettsioses and antibodies against *R. felis* cross-react with those against *R. typhi* and other spotted fever group *Rickettsia* antigens,²⁷ *R. felis* infection in humans may be misdiagnosed or missed. When cross-reactivity between *R. felis*, *R. conorii*, and *R. typhi* is observed, the infection is probably with *R. felis*, whereas when cross-reactivity between *R. felis* and *R. typhi* is observed, the causative agent likely belongs to the typhus group.²⁸ Because all cases of murine typhus in Cyprus were diagnosed only by serologic tests, it is difficult to exclude or confirm the cause of infection as *R. felis* or *R. typhi*.

Additional studies are needed to investigate the clinical manifestations of these two flea-borne infections, their epidemiology, and their natural maintenance and transmission in mammals to assess the possible role of *X. cheopis* in the epidemiology of *R. felis* and to determine whether humans and animals are exposed to the pathogen. *Rickettsia felis* infection should be considered an emergent threat to human health.

Received February 23, 2010. Accepted for publication July 14, 2010.

Acknowledgments: We thank the technical staff of the Veterinary Services and the Department of Agriculture of Cyprus and Papadopoulos Byron and Christofi Nikos for valuable assistance.

Financial support: This study was partially supported by the Cyprus Research Promotion Foundation.

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