FIRST MOLECULAR DETECTION OF RICKETTSIA FELIS IN FLEAS
FROM ALGERIA

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Abstract. Fleas collected in Algeria in the district of Oran between July and September 2003 were tested by
polymerase chain reaction for the presence of Rickettsia spp. DNA using primers amplifying gltA and OmpA genes. Two
gltA sequences identical to those of an emerging pathogen, Rickettsia felis, were detected including i) R. felis California
2 in Ctenocephalides canis from rodents and ii) R. felis RF2125 in Archeopsylla erinacei from hedgehogs.

INTRODUCTION

With the exception of the rat flea Xenopsylla cheopis and Yersinia pestis, the agent of plague, fleas and human flea-
borne infections have been scarcely studied in Algeria.1 Muri-
tine typhus, a typhus group rickettsiosis induced by Rickettsia typhi and transmitted by X. cheopis, is also known to occur in
Algeria but cases are poorly documented.2,3 However, in re-
cent years, fleas have been associated worldwide with emerg-
ing human infections including flea-borne spotted fever (also
called cat flea typhus) caused by a spotted fever rickettsia,
Rickettsia felis,2 and cat scratch disease in connection with
Bartonella henselae.5 In June 2003, the Ministry of Health,
Algeria, reported an outbreak of plague in the Oran district.6
Investigations including an entomological survey were con-
ducted to identify and describe a possible natural focus as well
as the mechanisms of spread of Y. pestis in this area. During
the same period, clinicians working in the Infectious Diseases
Unit at the Oran Hospital claimed of also having admitted
several patients with spotted fever in the past month, leading
to suspect a potential Rickettsia infection. The entiological
agents responsible for the disease were unknown, but they
may be associated with enzootic vectors, such as fleas or ticks.
The aim of the current work was to subsequently survey fleas
collected in the District of Oran and detect rickettsial DNA
by means of polymerase chain reaction (PCR) involving gltA
and OmpA rickettsial genes.

MATERIALS AND METHODS

Sampling area and hosts. The sites that represented the
original focus of the reported plague cases were Kehailia and
Tafaraouï, both located in the District of Oran, ca. 450 km
west of the capital Algiers. This settlement area is situated at
an altitude of approximately 208 m above sea level and has
around 1,400 inhabitants. The predominant occupation is ag-
riculture, and a large number of domestic and peridomestic
animals (dogs, goats) populate the area. Fleas were collected
on rodents and hedgehogs trapped inside the houses and in
the peridomestic areas of the cities of Kehailia and Tafaraouï.
The sampling comprised 57 specimens collected on 3 hedge-
hogs (Atelœrix algirus) in Tafaraouï and 86 specimens col-
lected in Kehailia on rodents including 11 Meriones shawii, 8
Rattus rattus, and 15 Rattus norvegicus.

Collection and identification of fleas. Most of the fleas were
used for a survey on plague. However, a total of 4 fleas col-
clected on hedgehogs and 11 fleas collected on rodents were
randomly chosen, stored in 70% alcohol, and sent to the
WHO Collaborative Center for Rickettsial Reference and
Research in Marseille, France, where the molecular studies
were performed in February 2004. Alcohol-remained fleas
were rinsed with distilled water for 10 minutes and dried on
sterile filter paper in a laminar flow hood. Preliminary enzo-
teological identification was done by an entomologist (I.B.),
using reference taxonomic keys.7 Voucher specimen could
not be retained, as whole specimen extractions were per-
formed.

Polymerase chain reaction and sequencing. Fleas were
crushed individually in sterile Eppendorf tubes with the tip of
a sterile pipette. DNA was extracted by using the QIAamp
Tissue Kit (Qiagen, Hilden, Germany) according to the
manufacturer’s instructions. Rickettsial DNA was detected
by PCR using primers RpCS.877p (5’-GGGGACCTGCT-
CACGGCGG-3’) and RpCS.1258n (5’-ATTCGAAAAAG-
TACAGTGAAAC-3’) (Eurogentec, Seraing, Belgium), which
amplify a 396-bp fragment of the citrate synthase gene (gltA)
of Rickettsia as previously described.8,9 Additionally, a 629–
632 base-pair fragment of the ompA gene was amplified using
Rr190.70p (5’-ATGGCGAATATTTCCTCAAAA-3’) and
Rr190. 701n (5’-GTTCCGTFATGTGGCAGCATCT-3’).9
Negative controls consisted of DNA extracted (in the same
biosafety cabinet) of live uninfected lice from colonies of our
laboratories (1 louse for 5 tested Siphonaptera). A positive
control (Rickettsia montanensis) was included in each test. To
identify the detected Rickettsia sp., PCR products were puri-
fied and DNA sequencing was performed as previously de-
scribed.8,9 Additionally, we sequenced species-level informa-
tive regions of siphonapteran 18S rDNA to ensure correct
detection of the etiological agent. The identified Rickettsia
isolates were compared in phylogenetic (rodent fleas) and sta-
tistical frameworks (Maximum parsimony [MP], and pairwise base
aligned using Sequencher 4.2 (GeneCodes 2003). The data
were compared in phylogenetic (rodent fleas) and statistical
frameworks (Maximum parsimony [MP], and pairwise base
changes; PAUP*) to flea sequences available in the 18S
rDNA database of the Whiting laboratory (Ctenocephalides
canis 2; Ctenocephalides felis felis; not yet published) and

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Ctenocephalides canis [AF423914]; Ophthalmosthysylla volgensis [AF423895]; Pulex irritans [AF423915]; Ctenocephalides pseudoaugyrtes [AF423892]; and Panorpa communis [AF423900, outgroup]. Nonparametric boot-strap values (10,000 replicates, 100 random additions per re-plicate) were calculated for data sets to assess confidence in the resulting relationships. Independently, all obtained sequences were compared with those available in GenBank by using the nucleotide-nucleotide BLAST (blastn) program (see http://www.ncbi.nlm.nih.gov/BLAST/).

**RESULTS**

Using morphologic taxonomic keys, the four fleas that had been collected on hedgehogs Atelerix algirus were identified as Archaeopsylla erinacei. This was unambiguously confirmed when a 331 fragment of the 18S RNA showed 100% sequence similarity with a previously deposited sequence of this flea in GenBank (X89486). The morphologic identification of the fleas collected on rodents however remained doubtful. A 918 base pair 18S rRNA was shown to have 99.89% similarity (but one gap) with the sequence of *Ctenocephalides canis* deposited in GenBank (AF423914). Comparisons of pairwise base differences with 177 entries in the database of one of us (K.D.D.L.C.) covering all known flea families showed a 0.4% sequence difference to the closely related C. felis felis, and between 0.5% and 4.8% difference to the rest of the taxa. Additionally, MP analysis under 1,000 replicates of heuristic search with random addition sequence and tree bisection and reconnection branch swapping (TBR) revealed the grouping of the sampled sequence, the previously deposited Genbank sequence, and the yet unpublished sequence from the database (C. canis) 2 as a monophyletic polytomy. C. felis felis forms a sister group to all C. canis (Figure 1). All relationships are recovered with high nodal support values. Therefore, fleas collected on the Algerian rodents were ultimately identified as C. canis.

Using the *glt A* primers, PCR products of rickettsial DNA were detected in all of the 15 studied fleas and from the positive controls. No nucleic acids were amplified from the negative controls. All fleas were also shown to be positive with the second PCR screening using *OmpA* primers. By sequencing the *gltA* amplified fragments, two sequences of the expected size were identified. They were shown to be respectively 100% identical to two sequences deposited in GenBank originating from *R. felis* including i) *R. felis* California 212 (AF210692) for those detected in the 11 *C. canis* fleas and ii) *Rickettsia* RF2125, considered to be *R. felis* (AF516333), from those detected in the 4 *A. erinacei* fleas.

**FIGURE 1.** Maximum parsimony topology, tree score: 158, out-group: *Panorpa communis*. Numbers above branches denote boot-strap support values.

**DISCUSSION**

We present here the first molecular detection of *R. felis* in Algeria. By using primers targeting two different rickettsial genes and appropriate positive and negative controls, measurements were taken to detect genuine *Rickettsia* infestation of the fleas under study. No nucleic acids were amplified from the negative controls. Furthermore, two different sequences were identified from the PCR products that were amplified during the same experiment, thus significantly reducing the possibility of potential contamination with DNA products. Additionally, the ethanol in which the fleas were stored in Algeria originated from a new bottle and had never been used before. However, fleas of the same species collected for this work were stored in the same vial. Also, only a small number of specimens were tested in this work. Thus, we cannot speculate about the high infestation rate of *R. felis* infection of the fleas tested here.

*R. felis* is an obligate intracellular Gram-negative bacteria belonging to the spotted fever group of genus *Rickettsia* within the order Rickettsiales. It is known as the agent of flea-borne spotted fever, an emerging disease, which is to date only incompletely described. *R. felis* was probably first detected in cat fleas *Ctenocephalides felis* in 1918 and rediscovered in 1990. This bacterium was characterized by molecular biology techniques and was initially named the ELB agent (EL Laboratory, Soquel, CA). In 1994, DNA fragments of this agent were detected in blood samples obtained from a patient from Texas in 1991. The name *R. felis* was proposed after the first attempts to cultivate and characterize the *in vitro* and *in vivo* antibiotic susceptibilities of the ELB agent were reported in 1995 and 1996. Nevertheless, the first definitive cultivation of this low temperature growing *R. felis* was only achieved in 2001.

More arguments on the pathogenicity of *R. felis* for humans were provided in 2000 in Mexico, when three patients with fever rash were diagnosed with *R. felis* infection by specific PCR of blood or skin and a seroconversion to rickettsial antigens. Serological evidence of *R. felis* infection has also been shown in patients from France and Brazil. Moreover, molecular documentation was obtained in the serum of one Brazilian patient. In 2002, two cases of typical rickettsial spotted fever including generalized maculopapular rash and a black eschar were reported in an adult couple in Germany. *R. felis* infections were documented by serology for both patients and by detection of *R. felis* DNA in the woman’s sera. Finally, the first case of *R. felis* infection was recently documented by serology in Asia. Rash and/or eschar (6 of 8) have been reported in the few cases documented. Interestingly, these clinical features are known in the Mediterranean area including Algeria as typical signs of the Mediterranean spotted fever (MSF), a tick-borne spotted fever rickettsiosis...
due to *R. conorii* and transmitted by the brown dog tick, *Rhipicephalus sanguineus*.

In recent years, *R. felis* has been associated with fleas throughout the world. It has been detected in fleas in Brazil,

Ethiopia,

Europe including Spain,

France,

and the United Kingdom,

in Thailand,

and in New Zealand.

To date, three species of fleas have been associated with *R. felis*, namely *C. felis*,

*Pulex irritans*.

Interestingly, transovarial transmission of *R. felis* in fleas has also been reported, suggesting that fleas could act as reservoirs of the rickettsia.

As taxonomical identification of fleas to the species level is difficult and can only be undertaken by experts, which are ever more scarce, and given the fact of a new flea association for *R. felis*, we used 18S rRNA sequence analysis to confirm identification of the fleas. Although rodents are not a common host for *C. canis*, many dogs (the usual hosts) were around the places where the rodents were trapped. To our knowledge, this is the first time that *A. erinacei* is associated with *R. felis*, but some collaborators in Portugal have also recently detected *R. felis* in this species of fleas collected on hedgehogs in Portugal. (R. Sousa and others, unpublished data). Although these data suggest that *R. felis* infection may be prevalent worldwide, the role of mammals, including rodents, hedgehogs, cats, and dogs, in the life cycle and circulation of *R. felis* is still unclear.

Our results suggest that *R. felis* infection is prevalent in Algeria and that fleas are potential vectors of human rickettsioses in this country. This is the second time that this emerging pathogen was detected in Africa. Further studies are needed to elucidate and describe the epidemiology of *R. felis* infection. Clinicians, however, in Algeria or those elsewhere who may see patients returning from this country, must now be aware of possible *R. felis* infection in patients presenting signs of spotted fever and/or an eschar. Although it may be misdiagnosed as and treated similar to the tick-borne Mediterranean spotted fever, the epidemiologic aspects of flea-transmitted diseases regarding the risk of exposure as well as prevention aspects are different and need consideration. To date, few confirmed cases of *R. felis* infections have been described throughout the world, including none in Algeria. A better description of this emerging disease may allow recognizing subtle clinical differences to MSF.

Received October 14, 2004. Accepted for publication March 21, 2005.

Acknowledgments: We are grateful to Dr. Razik, Dr. Mofok, and Dr. Blel from the Infectious Diseases Unit at the Oran Hospital and to Dr. Hamou from the Ministry of Health, Algeria. The American Society of Tropical Medicine and Hygiene (ASTMH) assisted with publication expenses.

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