Molecular detection of *Rickettsia typhi* and *Rickettsia felis* in fleas from Algeria

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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]. Murine typhus, a typhus group rickettsiosis caused by *Rickettsia typhi* and transmitted by *X. cheopis*, is also known to occur in Algeria, but cases are poorly documented [2,3]. However, recently, fleas collected in Algeria, in the district of Oran, between July and September 2003 (*Ctenocephalides canis* from rodents, and *Archeopsylla erinacei* from hedgehogs) were tested by PCR for the presence of *Rickettsia* spp., and were shown to harbour an emerging pathogen, *Rickettsia felis* [4]. In this work, in an effort to identify the possible aetiological agents and vectors for rickettsiosis affecting humans in Algeria, we analysed more fleas collected from rodents trapped in peridomestic areas for evidence of rickettsial infection.

MATERIALS AND METHODS

From May 2005 to September 2006, fleas were collected in two areas of Algeria, including Tafaraoui and Benouali, west of Algiers. Fleas were collected from peridomestic rodents (*Rattus rattus* (*n* = 6); *Rattus norvegicus* (*n* = 5); *Mus musculus* (*n* = 1); and *Mus spretus* (*n* = 1)). Fleas were identified by one of us (I.B.) and stored in absolute ethanol. DNA was extracted from each flea [4], and rickettsial DNA was detected by PCR using primers R1258–409D, which amplify a 396-bp fragment of the *gltA* gene encoding the citrate synthase gene of *R. typhi* [4]. The PCR products were purified, and DNA sequencing was performed [4]. The obtained *gltA* and *ompA* sequences were assembled, edited, and compared with sequences in Genbank [4]. All fleas that tested positive for rickettsial DNA were molecularly identified by amplifying, sequencing and comparing an 1867-bp informative region of siphonapteran 18S rDNA [4].

RESULTS

Two species of fleas have been morphologically identified, including *X. cheopis* and *Leptopsylla segnis*. Identification was unambiguously confirmed with molecular tools. Using the *gltA* primers, PCR products of rickettsial DNA were detected in 14 of the 69 studied fleas and from the positive controls. All these positive fleas also tested positive with the second PCR using *ompA* primers. By sequencing of the *gltA* amplified fragments, three sequences of the expected size were identified. They were shown to be identical to the two sequences deposited in GenBank originating from *R. felis*, for eight specimens of *X. cheopis*, and for two specimens of *L. segnis*. Finally, *R. typhi* was detected in four *X. cheopis* fleas.

DISCUSSION

In this study, we present the first molecular detection of *R. typhi* in fleas from Algeria. Murine typhus is most often a relatively mild disease with non-specific signs [3]. Fewer than half of the patients report exposure to fleas or flea hosts. Diagnosis may be missed, because the rash, the hallmark of rickettsial diseases, is present in fewer than 50% of the patients and is often transient or difficult to observe. Basically, clinicians have to be aware of the presence of
murine typhus in Algeria. Many patients can present with non-specific signs such as ‘fever of unknown origin’.

We have also confirmed the prevalence of *R. felis* in Algeria, and we have described a new flea (*X. cheopis*) as a potential vector of this bacterium in Algeria. *R. felis* is the recently recognized agent of the so-called flea-borne spotted fever. It has been detected in several species of fleas throughout the world, and a few cases have been diagnosed [4]. Further studies are needed to elucidate and describe the epidemiology of *R. felis* infection. However, clinicians in Algeria or elsewhere who may see patients returning from this country have now to 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 in a similar way to the tick-borne Mediterranean spotted fever, the epidemiological aspects of flea-transmitted diseases regarding the risk of exposure as well as prevention aspects are different and need consideration. To date, two cases only have been diagnosed in Algeria (Mokrani N, Dib L, Tebba S, Dalichaouche M, Parola P, Raoult D. First serologic evidence of *Rickettsia felis* infection in Algeria. Presented as Poster 151 at the 5th International Congress of Rickettsiae and Rickettsial Diseases, Marseille, France, 2008).

REFERENCES