

Rickettsia felis and *Rickettsia massiliae* in Ivory Coast, Africa

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INTRODUCTION

Spotted fever group (SFG) rickettsioses are caused by obligate intracellular Gram-negative bacteria belonging to the SFG of the genus *Rickettsia*. These zoonoses are now recognized as important emerging vector-borne infections of humans worldwide, and share characteristic clinical features, including fever, headache, rash and sometimes eschar formation at the site of the bite. They are transmitted by arthropods, mainly ticks [1]. SFG rickettsioses have been poorly studied in sub-Saharan Africa. In this work, we analysed, for evidence of rickettsial infection, fleas and ticks collected from rodents and dogs near Bouaké in Ivory Coast.

MATERIALS AND METHODS

In 2007, ticks and fleas were collected from dogs and rodents near Bouaké in Ivory Coast. Arthropods were identified by using morphological criteria. They were transported to France and identified by one of us (PP). DNA was extracted from each arthropod [2]. Each sample was tested by real-time (RT)-PCR in a Lightcycler instrument for the presence of *Rickettsia* spp. DNA, using primers and Taqman probes targeting a partial sequence of the citrate synthase *gltA* gene (RKND03F, 5'-GTG AAT GAA AGA TTA CAC TAT TTA T-3'; RKND03R, 5'-GTA TCT TAG CAA TCA TTC TAA TAG C-3'; RKND03Probe, 6-FAM-CTA TTA TGC TTG CGG CTG TCG GTT C-TAMRA) and, for DNA fleas, with primers and probe targeting a chromosomal gene specific for the *Rickettsia felis* *bioB* gene (R_fel0527_F, 5'-ATG TTC GGG CTT CCG GTA TG-3'; R_fel0527_R, 5'-CCG ATT CAG CAG GTT CTT CAA-3'; R_fel0527_Probe, 6-FAM-GCT GCG GCG GTA TTT TAG GAA TGG G-TAMRA). A negative control composed of DNA extracted from non-infected laboratory

ticks, and positive controls of *R. felis* DNA, were included in each test. Tick DNA samples that tested positive by RT-PCR were confirmed using two standard PCRs. The first PCR was performed using CS.409p and CS.1258n, which amplify a 750-bp fragment of the *gltA* gene of *Rickettsia*, and the second PCR was performed using primers 190.70, 190.180 and 190.701 for the *OmpA* gene, which amplify a 629–632-bp fragment [2]. Then, *gltA* amplified products were sequenced and compared to GenBank sequences. Negative controls were similar to those described above. Positive controls of *Rickettsia montanensis* DNA were included in each test.

RESULTS

In total, 25 ticks (ten *Rhipicephalus sanguineus*, four *Rhipicephalus guilhoni*, six *Rhipicephalus* sp., one *Amblyomma variegatum*, four *Haemaphysalis leachi*) and 13 fleas (11 *Xenopsylla cheopis*, collected on rodents, and two *Ctenocephalides canis*, collected on dogs) were tested. Positive and negative controls tested were as expected in all tests. RT-PCR and both *OmpA* and *gltA* PCR were positive for a single tick (engorged damaged female of *Rhipicephalus* sp.). By sequencing of the *gltA* amplified fragment, a sequence of the expected size was obtained, and was shown to correspond to *Rickettsia massiliae* (100% similarity with GenBank accession number U59720). Using the *R. felis* RT-PCR, *R. felis* DNA were detected in one specimen of *C. canis* collected on a dog.

DISCUSSION

Until recently, the sole SFG rickettsia known to be prevalent in Ivory Coast was *Rickettsia africae*, the agent of African tick bite fever [3]. Our results have shown for the first time the prevalence of two more human pathogens, *R. massiliae* and *R. felis*, in this country (Fig. 1).

R. massiliae is a worldwide rickettsia that was isolated in 1992 and thereafter detected in *Rhipicephalus* spp. in Europe, Africa, Argentina and, recently, Arizona, USA [3]. The recognition of the pathogenicity of *R. massiliae* occurred in 2005,

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Fig. 1. Site of tick and flea collection in Ivory Coast, Africa.

when molecular tools were used to identify a rickettsial isolate obtained 20 years before from a man hospitalized in Italy with fever, an eschar, and a maculopapular rash [4]. In fact, *R. massiliae* is the sole pathogenic tick-borne rickettsia known to occur in America, Africa and Europe.

R. felis is the recently recognized agent of the so-called flea-borne spotted fever [1,5]. It has been detected in several species of fleas throughout the world, and few cases have been diagnosed. Although further studies are needed to describe the epidemiology of *R. massiliae* and *R. felis* infections, clinicians in Ivory Coast and those who may see patients returning from this country should be aware that many species of rickettsiae exist in this region, and should consider a range of spotted fever rickettsial diseases

in the differential diagnosis of those patients with febrile illnesses.

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