ORIGINAL ARTICLE

# *Candidatus* 'Rickettsia senegalensis' in cat fleas in Senegal

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## Abstract

Epidemiological studies of *Rickettsia felis* and related bacteria are very important, because the natural cycle of this important infection has not yet been established. The recent emergence of *R. felis*-associated febrile diseases in West and East Africa demands insightful epidemiological studies of the vectors and reservoirs of this bacterium in Africa. Twenty-nine cat fleas, *Ctenocephalides felis*, were tested for the presence of rickettsiae, including *R. felis*, bartonellae, and borreliae, with specific quantitative real-time PCR assays. Supporting our previous studies, *R. felis* was not detected in the fleas collected. In addition, neither *Bartonella* nor *Borrelia* was found. In five (17%) examined fleas, we found another species of rickettsia. We isolated three rickettsial strains, and genetic analysis demonstrated that these strains represent a probable new species, provisionally called *Candidatus* Rickettsia senegalensis here.



**Keywords:** Africa, *Candidatus* Rickettsia senegalensis, flea, *Rickettsia felis*, *Rickettsia*, Senegal
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## Introduction

Recently, several papers have highlighted an emerging problem in tropical medicine in Africa, namely, acute rickettsioses [1], including those caused by *Rickettsia felis* [2] and related rickettsiae. Rickettsioses appear to be some of the most important and often neglected causes of febrile diseases in tropical countries [3–5]. In Laos, rickettsioses may be responsible for 7% of the cases of patients hospitalized with acute fevers [4]. In Senegal, our previous studies have shown that up to 15% of the cases of fever encountered in rural dispensaries may be caused by *R. felis* [2]. Since 2010, two teams, one based in Senegal [2] and another in Kenya [6,7], have been working independently on the detection of the causes of febrile illnesses, including *R. felis*-associated illness, in Africa. However, the natural reservoirs of *R. felis* have not yet been completely described. Since the first clinical descriptions of *R. felis*-associated fever, the cat and dog fleas, *Ctenocephalides felis* and *Ctenocephalides canis*, respectively, have been implicated as the most probable vectors [8]. However, *R. felis* has been identified by molecular methods in >20 different species of fleas, soft and hard ticks, mites and booklice [9], and mosquitoes, including *Anopheles* species [10,11]. Multiple reported cases of *R. felis*-associated fever in Senegal [2,12] may not be explained by transmission of *R. felis* by flea bites, because of its actual absence (or rarity) in fleas in Senegal [13]. In this article, we report the results of a study aimed at finding rickettsiae in fleas in Senegal.

## Materials and methods

Cat fleas were collected manually from two cats in Dakar, Senegal. Twenty-four fleas were placed directly into 70% ethanol, and five were kept alive. Fleas were identified with a standard taxonomic key [14]. Five live fleas were homogenized and blindly inoculated into a cell culture; DNA was extracted from the supernatant and tested together with the DNA of the



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fleas initially placed into alcohol. DNA was extracted from fleas and the cell culture suspension with a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions, and stored at 4°C until being used in PCR amplifications. Rickettsial DNA was detected by performing quantitative real-time PCR (qPCR) assays with a BIORAD CFX96 system and software (Bio-Rad Life Science, Marnes-la-Coquette, France). Master mixtures were prepared according to the instructions of the manufacturer, by using *Rickettsia*-specific primers and probes that target a *gltA* gene and the *bioB* gene specific to *R. felis* [12]. The samples were screened for the presence of *Borrelia* species and *Bartonella* species by qPCR with primers and probes that target the 16S rRNA gene of *Borrelia* [15] and the ITS gene of *Bartonella* [16]. Amplifications of almost the entire *rrs*, *sca4*, *ompB*, *ompA* and *gltA* genes of the new isolates of *Rickettsia* were performed as previously described [17]. The isolation of rickettsial strains was performed in an XTC-2 cell line with a shell-vial technique [18] at 28°C. Rickettsiae were detected by Gimenez staining followed by *Rickettsia*-specific qPCR. The *gltA* sequences and concatenated *rrs*, *gltA*, *sca4* and *ompB* sequences were aligned by the use of CLUSTALW, and phylogenetic inferences were obtained with Bayesian phylogenetic analysis [19] with TOPALi 2.5 software (Biomathematics and Statistics Scotland, Edinburgh, UK) by use of the integrated MrBayes application (http://mrbayes.csit.fsu.edu/).

## Results

All collected fleas were morphologically identified as *C. felis*. In total, five of the 29 fleas (17.2%) tested positive by qPCR with the *Rickettsia*-specific primers and probe, including three fleas inoculated in XTC-2 cells. All five positive fleas were among 12 collected from one cat; 17 fleas collected from the second cat were negative. However, none tested positive for *R. felis*. Over a period of 2 weeks, we succeeded in isolating three rickettsial strains, namely, PU01-02, PUX1-X2, and PU03-04, from three of five fleas inoculated into the XTC-2 cell cultures. All three strains were visualized by Gimenez staining, and their appearance was typical of cell cultures infected with rickettsiae: Gimenez-positive intracellular bacteria accompanied by a moderate cytopathic effect (Fig. 1).

We amplified almost the entire lengths of the *gltA* (for all strains and positive fleas), *rrs*, *ompB* and *sca4* genes (for the PU01-02 strain only), and sequenced the amplicons. All attempts to amplify the *ompA* gene typical for the spotted fever group of rickettsiae [20] produced negative results. The *gltA* gene sequences were identical among all three isolates. BLAST searches of the sequences of all four sequenced genes demonstrated that the isolated PU01-02 strain does not

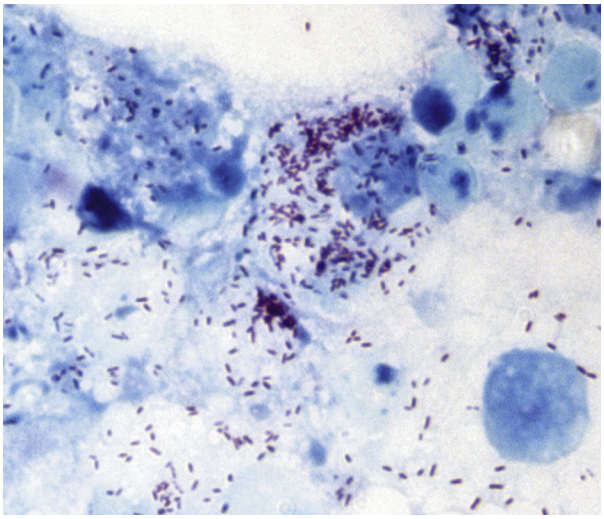

**FIG. 1.** XTC-2 cell line infected with *Candidatus* Rickettsia senegalensis, strain PU01-02, seventh day post-inoculation, Gimenez staining, 1500×.

completely share identity with any other known rickettsial strain. The closest officially validated species was *R. felis* strain California 2 (CP000053); the PU01-02 strain showed 99.65%, 97.68%, 95.24% and 97.2% sequence identity of its *rrs*, *gltA*, *ompB* and *sca4* genes, respectively, with *R. felis*. A phylogenetic tree constructed on the basis of the *gltA* sequences (Fig. 2) and concatenated *rrs*, *gltA*, *sca4* and *ompB* gene sequences (Fig. 3) also indicated a distinct position for the PU01-02 strain. The PU01-02 strain grouped together with *R. felis* and *Rickettsia hoogstraalii* and a number of sequences of unisolated rickettsiae that were amplified from fleas, *Ornithodoros* ticks, and ladybird beetles. A detailed BLAST search found that a very similar rickettsia ('*Rickettsia* sp. Rf31', AF516331, 1149/1150 (99%) for the *gltA* gene) was identified in a *C. canis* flea collected from a dog in Thailand [21]. Moreover, other genetically similar rickettsiae were identified in human blood in south-eastern Senegal (D. Raoult, personal communication; GenBank accession number JQ674485) and in the mosquito malaria vectors *Anopheles gambiae* from Côte d'Ivoire (JN620082) and *Anopheles melas* from Gabon (JQ354961) [10,11]. Overall, *R. felis*, *R. hoogstraalii*, *Rickettsia* sp. PU01-02 and other genetically related rickettsiae that have been identified in fleas, soft ticks, ladybird beetles and tsetse flies form a well-supported (99/100 bootstrap support) clade, here provisionally called the '*R. felis* group' (Fig. 2).

The sequences were deposited in GenBank under the following accession numbers: KF666476, KF666472, KF666470 and KF666474 for the *rrs*, *gltA*, *ompB* and *sca4* genes of the PU01-02 strain, respectively.

The PU01-02 strain (provisional name, '*Rickettsia senegalensis*') was deposited in two international collections of bacterial strains under the numbers CSUR R184 and DSM2850.