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Presence or absence of plasmid in *Rickettsia felis* depending on the source of fleas

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INTRODUCTION

Rickettsiae are obligate intracellular small gram-negative bacteria associated with different arthropod hosts. *Rickettsia felis*, the agent of the flea-borne spotted fever rickettsiosis, has been found worldwide in flea species such as *Ctenocephalides felis* and *C. canis*, parasitising cats and dogs, *Archeopsylla erinacei* and *Pulex irritans* [1,2]. The genome of *R. felis* has been recently sequenced and revealed that besides a circular chromosome of 1 485 148 bp, it exhibits a conjugative plasmid in two forms, a short form (39 263 bp named pRFδ) and a long one (62 829 bp named pRF) [3]. The objective of this study was to evaluate the detection of *R. felis* in several flea species from different hosts worldwide by the use of real-time PCR (RT-PCR) with Taqman® (Applied Biosystems, Courtaboeuf, France) probes targeting either specific chromosomic genes (*gltA* and *bioB*) or plasmidic open reading frames (ORFs).

MATERIALS AND METHODS

A total of 34 fleas collected in dogs from Gabon, stray cats from Lebanon, and foxes from Corsica, France, were included in the study. These fleas were tested by RT-PCR in a Lightcycler® (Roche Diagnostics, Meylan, France) instrument for the presence of *Rickettsia* spp. DNA using primers and Taqman® probes targeting *gltA* (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 *bioB* (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) chromosomic genes and three different ORFs from the *R. felis* strain California 2 plasmids. Three set of primers and probes target the plasmid pRF and pRFδ (R_felPl45000F : 5′- TTG-CTG-AAG-CAC-CTC-CCA-AG –3′; R_felPl45000R : 5′- TGC-AGT-TTA-AAG-ATG-CGG-TGA –3′; R_felPl45000Probe : 6-FAM-CCG-AAA-GCA-TTG-AAA-CCA-ACG-CTA-GC-TAMRA; R_felPlD13000F : 5′-TGA-TTT-TAC-ACA-AAA-GCA-AGG-AGT-GA–3′; R_felPlD13000R : 5′-CTT-GCT-TCT-GCT-CCG-TTC-CA-3′; R_felPlD13000Probe : 6-FAM-GGC-TTT-GAA-GAC-GCT-GCA-TGG-C-TAMRA; R_felPlD20000F : 5′-CCA-TGC-CTC-TTA-ATT-TCT-GAC-TGC-3′; R_felPlD20000R : 5′-AGC-TTC-GGT-TCT-TGG-CTT-GC-3′; R_felPlD20000Probe : 6-FAM-CAA-GTA-CTT-CAA-ATG-CAG-CGG-AGC-CG-TAMRA). Positive fleas were also confirmed by standard PCR amplification and sequencing of a fragment of the citrate synthase gene *gltA* as previously described [2].

RESULTS

Using morphologic taxonomic keys, the 18 fleas that had been collected on stray cats from Lebanon were identified as *C. felis*, the 12 fleas from dogs from Gabon as *C. canis*, and the four fleas from foxes from Corsica, France, as *A. erinacei*. Eight out of the 18 *C. felis* fleas from stray cats (Lebanon), 12/12 *C. canis* fleas (Gabon) and 2/4 *A. erinacei* fleas from foxes (Corsica, France) were found to be infected with *R. felis* as demonstrated using standard PCR amplification and sequencing of partial *gltA* gene as well as RT-PCR targeting chromosomic genes (Table 1). Partial *gltA* sequences obtained from *C. felis* fleas were 100% identical to *R. felis* strain California 2 (isolated from *C. felis* fleas) whereas those obtained from *C. canis* and *A. erinacei* fleas were 100% identical to that of *Rickettsia* sp. RF2125 (detected in *C. canis* fleas from Thailand). The percentage of homology of the partial *gltA* sequences obtained from *C. canis* and *A. erinacei*

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Table 1. Results of RT-PCR and partial *gltA* sequences of positive fleas

	Flea species Target gene	RKND03 *gltA*	R_fel0527 *bioB*	R_felPl45000 plasmid	R_felPlD13000 plasmid	R_felPlD2000 plasmid	gltA sequence identification – Accession number
		Cycle threshold (Ct) obtained using the Lightcycler® instrument					
Stray cats from Lebanon							
1	*Ctenocephalides felis*	24.49	24.29	24.09	21.14	20.83	*Rickettsia felis* URRWXCal2 (100%) – CP000053
2	*Ctenocephalides felis*	22.99	22.67	21.26	20.2	19.59	*Rickettsia felis* URRWXCal2 (100%) – CP000053
3	*Ctenocephalides felis*	22.2	22.17	20.91	19.75	13.05	*Rickettsia felis* URRWXCal2 (100%) – CP000053
4	*Ctenocephalides felis*	22.61	22.25	20.41	19.79	19.34	*Rickettsia felis* URRWXCal2 (100%) – CP000053
5	*Ctenocephalides felis*	24.14	23.18	27.08	21.05	20.55	*Rickettsia felis* URRWXCal2 (100%) – CP000053
6	*Ctenocephalides felis*	35.51	34.1	34.14	32.01	31.66	*Rickettsia felis* URRWXCal2 (100%) – CP000053
7	*Ctenocephalides felis*	21.99	21.81	20.36	19.28	17.43	*Rickettsia felis* URRWXCal2 (100%) – CP000053
8	*Ctenocephalides felis*	23.14	22.67	21.78	13.79	18.76	*Rickettsia felis* URRWXCal2 (100%) – CP000053
Dogs from Gabon							
1	*Ctenocephalides canis*	28.95	28.16	NEG	NEG	NEG	*Rickettsia* sp. RF2125 (100%) – AF516333
2	*Ctenocephalides canis*	24.64	25.31	NEG	NEG	NEG	*Rickettsia* sp. RF2125 (100%) – AF516333
3	*Ctenocephalides canis*	25.94	26.13	NEG	NEG	NEG	*Rickettsia* sp. RF2125 (100%) – AF516333
4	*Ctenocephalides canis*	25.52	26.25	NEG	NEG	NEG	*Rickettsia* sp. RF2125 (100%) – AF516333
5	*Ctenocephalides canis*	25.22	26.89	NEG	NEG	NEG	*Rickettsia* sp. RF2125 (100%) – AF516333
6	*Ctenocephalides canis*	25.26	25.24	NEG	NEG	NEG	*Rickettsia* sp. RF2125 (100%) – AF516333
7	*Ctenocephalides canis*	27.28	29.07	NEG	NEG	NEG	*Rickettsia* sp. RF2125 (100%) – AF516333
8	*Ctenocephalides canis*	31.7	32.48	NEG	NEG	NEG	*Rickettsia* sp. RF2125 (100%) – AF516333
9	*Ctenocephalides canis*	32.28	30.96	NEG	NEG	NEG	*Rickettsia* sp. RF2125 (100%) – AF516333
10	*Ctenocephalides canis*	34.54	33.96	NEG	NEG	NEG	*Rickettsia* sp. RF2125 (100%) – AF516333
11	*Ctenocephalides canis*	36.61	NEG	NEG	NEG	NEG	*Rickettsia* sp. RF2125 (100%) – AF516333
12	*Ctenocephalides canis*	29.05	31.05	NEG	NEG	NEG	*Rickettsia* sp. RF2125 (100%) – AF516333
Foxes from Corsica, France							
1	*Archeopsylla erinacei*	28.2	25.96	NEG	NEG	NEG	*Rickettsia* sp. RF2125 (100%) – AF516333
2	*Archeopsylla erinacei*	26.89	24.58	NEG	NEG	NEG	*Rickettsia* sp. RF2125 (100%) – AF516333

fleas with *R. felis* strain California 2 ranged from 97.1 to 97.4%. Interestingly, RT-PCR with primers and probes targeting the three specific ORFs of the plasmids of *R. felis* strain California 2 were positive only for *C. felis* fleas from cats (Table 1).

CONCLUSIONS

We present here the first molecular detection of *R. felis* in *C. felis* fleas from stray cats from Lebanon, in *A. erinacei* fleas from foxes from Corsica (France) and in *C. canis* fleas from dogs from Gabon. *Rickettsia felis* is an obligate intracellular gram-negative bacteria belonging to the spotted fever group of genus *Rickettsia* within the order Rickettsiales, and is the agent of flea-borne spotted fever, an emerging disease. In recent years, *R. felis* has been associated with fleas throughout the world in several flea species, including *C. felis*, *C. canis*, *Pulex irritans* and *A. erinacei* [2,4]. Two genotypes of this *Rickettsia* have been recently detected in fleas from Algeria, including *R. felis* RF2125 and *R. felis* California 2 [2]. *Rickettsia felis* strain California 2 is the first obligate intracellular bacterium exhibiting a conjugative plasmid as demonstrated by whole genome sequencing [3]. Recently, the presence of two plasmid forms in *R. felis* strain California 2 has been unambiguously confirmed by PCR, but it has been observed that the plasmid content of this species, from none to two plasmid forms, may depend on the culture passage history of the studied strain [5]. In this same study, only the pRF plasmid form was detected from *R. felis* strain RF2125 in 64 *A. erinacei* fleas from Algeria [2,5]. Herein, we failed to detect any plasmid form in another batch of *A. erinacei* fleas from France. This is the second evidence that the plasmid content may vary within a given strain. Thus, in addition to culture conditions, the plasmid content of *R. felis* may vary from one strain to another. Moreover, our data may indicate that the two genotypes may differ by the presence or the absence of such conjugative plasmids depending on the source of fleas and on the area of collection.

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