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RICKETTSIA FELIS INFECTION IN CAT FLEAS *CTENOCEPHALIDES FELIS FELIS*

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ABSTRACT

The present study evaluated the rickettsial infection in a laboratory colony of cat fleas, *Ctenocephalides felis felis* (Bouche) in Brazil. All flea samples (30 eggs, 30 larvae, 30 cocoons, 30 males, and 30 females) tested by polymerase chain reaction (PCR) were shown to contain rickettsial DNA. PCR products, corresponding to the rickettsial *gltA*, *htrA*, *ompA* and *ompB* gene partial sequences were sequenced and showed to correspond to *Rickettsia felis,* indicating that the flea colony was 100% infected by *R. felis*. The immunofluorescence assay (IFA) showed the presence of *R. felis*-reactive antibodies in blood sera of 7 (87.5%) out of 8 cats that were regularly used to feed the flea colony. From 15 humans that used to work with the flea colony in the laboratory, 6 (40.0%) reacted positively to *R. felis* by IFA. Reactive feline and human sera showed low endpoint titers against *R. felis,* varying from 64 to 256. With the exception of one human serum, all *R. felis*-reactive sera were also reactive to *Rickettsia rickettsii* and/or *Rickettsia parkeri* antigens at similar titers to *R. felis*. The single human serum that was reactive solely to *R. felis* had an endpoint titer of 256, indicating that this person was infected by *R. felis*.

Key words: *Rickettsia felis*, flea, *Ctenocephalides felis felis*, cats, humans

Rickettsia felis, a bacterium formerly belonging to the spotted fever group (SFG) rickettsiae has been recently reclassified into the transitional group (TRG) rickettsiae (9). The organism has been detected infecting fleas (mostly *Ctenocephalides* spp) in various countries among all continents of the world, except Antarctica (19). *R. felis* has been reported as the causative agent of flea-borne spotted fever, an emerging human rickettsiosis that has been diagnosed in the United States (27), Mexico (33, 34), Brazil, France (22), Germany (24), Thailand (18), South Korea (7), Tunisia (35), Laos (20), Spain (5), and Taiwan (29). To date, all cases of human or animal infection by *R. felis* have been confirmed solely by serological and molecular tests; isolation of *R. felis* from humans or any other vertebrate has never been reported.

R. felis was first observed within midgut cells of cat fleas, *Ctenocephalides felis felis*, from a commercial colony maintained by El Labs, Soquel, CA (ELB) in the United Sates (1), which showed > 90% infection rate, with successful demonstration of transovarial and transstadial transmission as shown by occurrence of infected eggs and unfed larvae (4).

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Higgins *et al*. (10) evaluated the *R. felis* infection in eight laboratory colonies of cat fleas from different areas of USA by molecular analysis, with infection rates varying from 43 to 93% (mean: 72%). Wedincamp & Foil (30) found ≈65% infection rate in a colony kept at the Lousiania State Agricultural Center St. Gabriel Research Station (LSUSG). In two more recent studies, cats naturally exposed to *R. felis*-infected fleas demonstrated anti-*R. felis* antibodies (6, 17).

The present study aimed to verify *Rickettsia felis* infection in all developmental stages of the life cycle of *C. felis felis* from a laboratory colony, and to determine the presence of *Rickettsia*-reactive antibodies in cats and humans in direct contact with the flea colony.

In July 2004, larvae, cocoons, and adults of *C. felis felis* were obtained from the laboratory flea colony of the Department of Parasitology of the Federal Rural University of Rio de Janeiro, in Seropédica Municipality, Rio de Janeiro, Brazil. In July 2006, flea eggs were also obtained from the same colony. The flea colony has been maintained in the laboratory since 1996. Eight cats were used for feeding adult fleas inside individual cages, in an isolated room. Three times a week, cats were infested with 50 male and 50 female fleas. For colony maintenance, falling eggs from cat fur were collected below the cages, and held inside tubes with artificial diet in an incubator at 27°C and 75% RH (8).

Samples of 30 eggs, 30 larvae, 30 cocoons, 30 males, and 30 females of *C. felis felis* colony were washed in absolute ethanol and individually submitted to DNA extraction. Each specimen was air dried and subsequently triturated in 50 µl of TE buffer [10mM Tris HCl; 1 mM ethylene diamine tetracetic acid (EDTA) pH 8.0] in sterile micro tubes. The final suspension was boiled at 100ºC for 20 minutes and held at -20ºC (11). For every 10 individual flea samples, a blank tube was included in the DNA extraction. Before being triturating for boiling, flea cocoons were individually opened with sterile blades and needles, and only the adult flea present inside the cocoon was used for DNA extraction. This procedure was adopted because in a preliminary assay, whole cocoons from this same flea colony failed to yield PCR products.

Samples were individually tested by polymerase chain reaction (PCR) with a primer pair (CS78 F and CS323 R) targeting a 401-bp fragment of the rickettsial gene *gltA* (Table 1). Random samples of 2-5 eggs, larvae, cocoons, adult males, and females were submitted to a battery of PCR using all primer pairs described in Table 1, targeting fragments of the rickettsial genes *gltA, htrA*, *ompA* and *ompB*, followed by DNA sequencing the PCR products. For each reaction, 5 µl of the DNA template from each individual sample (egg, larva, cocoon, male or female) was added to 5 µl of PCR buffer (10X), 8 µl of deoxynucleotide triphosphates mixture (1.25mM), 1.5 $MgCl_2$ (50mM), 25 pmol of each primer, 0.25 µl of *Taq* polymerase (5,000 U/ml) and bi-distilled water to a final volume of 50 µl. For each reaction, DNA extracted from *Rickettsia parkeri* strain At24 was used as positive control (28) and bi-distilled water samples were used as negative controls. PCR thermal conditions were used as previously described (3, 15, 22, 25, 26). PCR products were stained by ethidium bromide and visualized by electrophoresis in 1.5% agarose gel. For DNA sequencing, PCR products were purified using ExoSap (USB, Cleveland, USA) and sequenced in an automatic sequencer (ABI Prism 3100 Genetic – Applied Biosystems/Perking Elmer, California, USA). All DNA sequences generated in the present study were submitted to BLAST (Basic Local Alignment Search Tool) analysis (2) to determine similarities to other *Rickettsia* species.

In February 2006, blood serum samples were collected from 8 cats that were used to feed the adult flea colony, and from 15 immune competent humans (researchers, technicians, and students) that had direct contact with these animals and fleas. Sera were tested by Indirect Immunofluorescence Assay (IFA) to detect antibodies reactive to *Rickettsia* spp. All cats had been continually used for feeding the colony for at least 3 years, while all 15 humans, who had worked with the flea colony for at least 2 years, reported multiple flea bites during this period in the laboratory. IFA was performed in glass slides according to previously described methods (13, 32), employing three rickettsial antigens: *R. felis* strain Pedreira (12), *Rickettsia rickettsii* strain Taiaçú (21), and *R. parkeri* strain At24 (28).

Human and animal sera were diluted in two-fold increments with PBS starting from a 1:64 dilution. Twenty microliters of diluted sera were added to each well of the antigen slides. The slides were incubated at 37°C for 30 minutes in a humid chamber. The slides were rinsed once, and then washed twice for 15 minutes per wash in PBS. The slides were incubated with fluorescein isothiocyanate-labeled goat anti-human IgG or goat anti-cat IgG (Sigma, St Louis, USA) and washed as described earlier. The slides were mounted with buffered glycerin under coverslips, and read using an ultraviolet microscope (Olympus, Tokyo, Japan) at 400x magnification. Serum was considered to contain antibodies against rickettsiae if it displayed a reaction at the 1:64 dilution. Reactive sera were tested in two or three replications before determining the endpoint titer by serial two-fold serum dilution. In each slide, a serum previously shown to be non-reactive (negative control) and a known reactive serum (positive control) were tested.

All the 150 (100%) flea samples (30 eggs, 30 larvae, 30 cocoons, 30 males, and 30 females) yielded amplicon of the expected length by PCR targeting a 401-bp fragment of the rickettsial *gltA* gene. PCR product was never visualized in neither blank samples that were submitted to DNA extraction nor PCR negative control samples. Fragments of 1089, 394, 575 and 676-bp of the genes *gltA*, *htrA*, *ompA* and *ompB*, respectively, were sequenced from each of 2 eggs, 5 larvae, 5 cocoons, 5 adult males, and 5 females. Partial sequences of the *gltA*, *htrA*, and *ompB* genes were 100% identical to the corresponding sequence of *R. felis* in GenBank (CP000053). Partial sequence of the *ompA* gene showed a single nucleotide polymorphism (99.8% of identity) in comparison with *R. felis* in GenBank (CP000053), which resulted in an amino acid change (aspartic acid to tyrosine). Partial *ompA* sequence of *R. felis* generated in the present study has been submitted to GenBank (accession number FJ425917)

Serum samples from 8 cats and 15 humans were tested against *R. felis*, *R. rickettsii* and *R. parkeri* antigens by IFA. Six humans (40.0%) and 7 cats (87.5%) showed titers ≥64 to at least one *Rickettsia* species (Table 2). All samples reactive to *R. rickettsii* or *R. parkeri* were also reactive to *R. felis.* One human serum was reactive solely to *R. felis.*

Table 1. Primers used for amplification of rickettsial genes in both PCR and DNA sequencing.

Primer Pairs	Gene	Nucleotide sequence (5'-3')	Expected amplicon length*	Reference
CS78 F	*gltA*	GCAAGTATCGGTGAGGATGTAAT	401 bp	15
CS323 R		GCTTCCTTAAAATTCAATAAATCAGGAT		15
CS239 F	*gltA*	GCTCTTCTCATCCTATGGCTATTAT	834 bp	15
CS1069 R		CAGGGTCTTCGTGCATTTCTT		15
17K1 F	*htrA*	GCTCTTGCAACTTCTATGTT	434 bp	3
17K2 R		CATTGTTCGTCAGGTTGGCG		3
190.70 F	*ompA*	ATGGCGAATATTTCTCCAAAA	617 bp	26
190.701 R		GTTCCGTTAATGGCAGCATCT		25
190.59 F	*ompB*	CCGCAGGGTTGGTAACTGC	862 bp	22
190.807 R		CCTTTTAGATTACCGCCTAA		22

* according *R. felis* (strain URRWXCal2) sequence in GenBank (CP000053)

Table 2. Endpoint titers of indirect immunofluorescence assay (IFA) for *Rickettsia felis*, *Rickettsia rickettsii* and *Rickettsia parkeri* in cats and humans with direct contact with a *Rickettsia felis*-infected laboratory flea colony.

Sample	*Rickettsia* antigens used for IFA		
	R. felis	***R. rickettsii***	***R. parkeri***
Cat 1	64	64	64
Cat 2	64	-	64
Cat 3	128	128	128
Cat 4	64	-	64
Cat 5	64	64	64
Cat 6	64	64	128
Cat 7	128	64	64
Cat 8	-	-	-
Human 1	-	-	-
Human 2	-	-	-
Human 3	-	-	-
Human 4	64	128	64
Human 5	-	-	-
Human 6	-	-	-
Human 7	-	-	-
Human 8	128	256	-
Human 9	-	-	-
Human 10	64	128	128
Human 11	256	-	-
Human 12	-	-	-
Human 13	128	-	128
Human 14	-	-	-
Human 15	128	256	256

- : non reactive at titer ≥ 64

In the present study, DNA sequencing of PCR products from random samples indicate that all individuals of the flea colony were infected by *R. felis.* High infection rates, varying from 43 to 100%, were also reported for laboratory cat flea colonies in the United States (23). Previous studies with wild cat flea populations in South America have reported *R. felis*-infection rates varying from 13.5 to 90% (17). Since we found *R. felis* DNA in eggs, and all post-embryonic developmental stages of cat fleas, our results corroborate previous studies showing that *R. felis* is transmitted transovarially and transstadially in cat fleas (4, 31).

In a recent study (17) with cats naturally infested by *R. felis*-infected cat fleas (70% infection rate) in Chile, 16 (72.7%) out of 22 cats had *R. felis*-reactive antibodies (titer ≥64). Among those, 3 (13.6%) also reacted with *R. parkeri* and 2 (9.1%) with *R. rickettsii*. Like in the present study, no Chilean cat serum reacted with *R. rickettsii* or *R. parkeri* without reacting with *R. felis*. *R. felis*-endpoint titers (128 to 512) in four of these cats were at least 4-fold higher than that to any of the other *Rickettsia* species, suggesting homologous reaction to *R. felis* infection (17). In the present study, 7 out of 8 cats used to feed a *R. felis*-infected cat flea colony (100% infection rate) showed antibodies reactive to both *R. felis* and *R. parkeri* antigens, and 5 cats were also seroreactive to *R. rickettsii* antigens. Endpoint titers to the three rickettsial antigens were always low, varying from 64 to 128 (Table 2). In this case, no cat showed endpoint titer 4-fold higher for a *Rickettsia* species than for the other species. However, since these cats have been continually infested by *R. felis*-infected cat fleas in the laboratory, under no contact with ticks or lice (potential vectors of other *Rickettsia* species) and fed exclusively with commercial pellets and water (cats were not allowed to go out hunting), it is likely that their serological reactiveness to SFG *Rickettsia* was elicited by *R. felis* during

previous contacts with infected cat fleas from the colony.

Since all 15 humans sampled in the present study had recent history of multiple flea bites while handling the flea colony in the laboratory, we expected most, or even all, of them to show anti- *R. felis* antibodies, since it has been shown that *R. felis* is a human pathogen (19). However, only 6 (40%) human sera were reactive to *R. felis* antigens. Five of these sera also showed similar antibody titers to *R. rickettsii* and/or *R. parkeri,* precluding any inference of the rickettsial agent responsible for eliciting serological response, because they all lived outside the laboratory and had different histories of exposure to additional rickettsial vectors, especially ticks (data not shown). On the other hand, one human serum (human 11, Table 2) was reactive to *R. felis* (titer 256) without reacting with the remaining rickettsial antigens. According to serological interpretations used in previous studies (14, 16, 18, 29), this human 11 was infected by *R. felis* or a very closely related genotype because its endpoint titer to *R. felis* was at least 4-fold higher than that to any of the other *Rickettsia* species. Although recent febrile illness compatible with classic spotted fever (fever, headache, and rash) was not recalled by any of the 15 humans (data not shown), rickettsiosis can manifest with a multiplicity of general symptoms (19), making it difficult to recall previous clinical rickettsiosis with certainty.

R. felis is currently recognized as an emerging human pathogen of worldwide distribution However, the global scarcity of *R. felis* clinical reports deserves further attention. As the disease is considered to be rare, its confirmatory diagnostic testing is difficult because very few laboratories are equipped with specific diagnostic assays. These facts should be related to the low number of flea-borne rickettsioses cases that have been confirmed in the world.

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REFERENCES

1. Adams, J.R.; Schmidtmann, E.T.; Azad, A.F. (1990). Infection of colonized cat fleas, *Ctenocephalides felis* (Bouché), with a rickettsia-like microorganism. *Am. J. Trop. Med. Hyg*. 43, 400-409.
2. Altschul, S.F.; Gish, W.; Miller, W.; Myers, E.W.; Lipman, D.J. (1990). Basic local alignment search tool. *J. Mol. Biol.* 215, 403-410.
3. Azad, A.F.; Webb, L.; Carl, M.; Dasch, G.A. (1990). Detection of *Rickettsiae* in arthropod vectors by DNA amplification using the polymerase chain reaction. *Ann. N. Y. Acad. Sci.* 590, 557-563.
4. Azad, A.F.; Sacci Jr, J.B.; Nelson, W.M.; Dasch, G.A.; Schmidtmann, E.T.; Carl, M. (1992). Genetic characterization and transovarial transmission of a typhus-like rickettsia found in cat fleas. *Proc. Natl. Acad. Sci. USA* 89, 43-46.
5. Bernabeu-Wittel, M.; Del Toro, M.D.; Nogueras, M.M.; Muniain, M.A.; Cardenosa, N.; Márquez, F.J.; Segura, F.; Pachón, J. (2006). Seroepidemiological study of *Rickettsia felis*, *Rickettsia typhi*, and *Rickettsia conorii* infection among the population of southern Spain. *Eur. J. Clin. Microbiol. Infect. Dis.* 25, 375-381.
6. Case, J.B.; Chomel, B.; Nicholson, W.; Foley, J.E. (2006). Serological survey of vector-borne zoonotic pathogens in pet cats and cats from animal shelters and feral colonies. *J. Feline Med. Surg.* 8, 111-117.
7. Choi, Y.J.; Jang, W.J.; Ryu, J.S.; Lee, S.H.; Park, K.H.; Paik, H.S.; Koh, Y.S.; Choi, M.S.; Kim, I.S. (2005). Spotted fever group and typhus group rickettsioses in humans, South Korea. *Emerg. Infect. Dis.* 11, 237-244.
8. Correia, T.R.; Souza, C.P.; Fernandes, J.I.; Martins, I.V.F.; Santos, H.D.; Scott, F.B. (2003). Ciclo biológico de *Ctenocephalides felis felis* (Bouché, 1835) (Shiphonaptera:Pulididae) a partir de diferentes dietas artificiais. *Rev. Bras. Zoocien., Juiz de Fora* 5, 153-160.
9. Gillespie, J.J.; Beier; M.S.; Rahman, M.S.; Ammerman, N.C.; Shallom, J.M.; Purkayastha, A.; Sobral, B.S.; Azad, A.F. (2007). Plasmids and rickettsial evolution: insight from *Rickettsia felis*. *PLoS ONE* 2, e266.
10. Higgins, J.A.; Sacci Jr, J.B.; Schriefer, M.E.; Endris, R.G.; Azad, A.F. (1994). Molecular identification of rickettsia-like microorganisms associated with colonized cat fleas (*Ctenocephalides felis*). *Insect Molecular Biol.* 3, 27-33.
11. Horta, M.C.; Pinter, A.; Cortez, A.; Soares, R.M.; Gennari, S.M.; Schumaker, T.T.S.; Labruna, M.B. (2005). *Rickettsia felis* (Rickettsiales:Rickettsiaceae) in *Ctenocephalides felis felis* (Siphonaptera: Pulicidae) in the State of São Paulo, Brazil. *Arq. Bras. Méd. Vet. Zootec*. 57, 321-325.
12. Horta, M.C.; Labruna, M.B.; Durigon, E.L.; Schumaker, T.T.S. (2006). Isolation and Cultivation of *Rickettsia felis* in the mosquito cell line C6/36. *Appl. Environ. Microbiol.* 72, 1705-1707.
13. Horta, M.C.; Labruna, M.B.; Pinter, A.; Linardi, P.M.; Schumaker, T.T.S. (2007). *Rickettsia* infection in five areas of the State of São Paulo, Brazil.

Mem. Inst. Oswaldo Cruz 102, 793-801.

14. Jansen, A.; La Scola, B.; Raoult, D.; Lierz, M.; Wichmann, O.; Stark, K.; Schneider, T. (2008). Antibodies against *Rickettsia* spp. in hunters, Germany. *Emerg. Infect. Dis.* 14, 1961-1963.
15. Labruna, M.B.; Whitworth, T.; Horta, M.C.; Bouyer, D.H.; McBride, J.W.; Pinter, A.; Popov, V.; Gennari, S.M.; Walker, D.H. (2004). *Rickettsia* species infecting *Amblyomma cooperi* ticks from an area in the State of São Paulo, Brazil, where Brazilian spotted fever is endemic. *J. Clin. Microbiol.* 42, 90-98.
16. Labruna, M.B.; Horta, M.C.; Aguiar, D.M.; Cavalcante, G.T.; Pinter, A.; Gennari, S.M.; Aranha, L.M.A. (2007). Prevalence of *Rickettsia* infection in dogs from the urban and rural areas of Mone Negro Municipality, Western Amazon, Brazil. *Vector-Borne Zoonotic Dis.* 7, 249-255.
17. Labruna, M.B.; Ogrzewalska, M.; Moraes-Filho, J.; Lepe, P.; Gallegos, J.L.; López, J. (2007). *Rickettsia felis* in Chile. *Emerg Infect Dis.* 13, 1794-1795.
18. Parola, P.; Miller, R.S.; McDaniel, P.; Telforf III, S.R.; Rolain, J.M.; Wongsrichanalai, C.; Raoult, D. (2003). Emerging rickettsioses of the Thai-Myanmar Border. *Emerg. Infect. Dis.* 9, 592-595.
19. Pérez-Osorio, C.E.; Zavala-Velazquez, J.E.; León, J.J.A.; Zavala-Castro, J.E. (2008). *Rickettsia felis* as emergent global threat for humans. *Emerg. Infect. Dis.* 14, 1019-1023.
20. Phongmany, S.; Rolain, J.M.; Phetsouvanh, R.; Blacksell, S.D.; Soukkhaseum, V.; Rasachack, B.; Phiasakha, K.; Soukkhaseum, S.; Frichithavong, K.; Chu, V.; Keolouangkhot, V.; Martinez-Aussel, B.; Chang, K.; Darasavath, C.; Rattanavong, O.; Sisouphone, S.; Mayxay, M.; Vidamaly, S.; Parola, P.; Thammavong, C.; Heuangvongsy, M.; Syhavong, B.; Raoult, D.; White, N.J.; Newton, P.N. (2006). Rickettsial infections and fever, Vientiane, Laos. *Emerg. Infect. Dis.* 12, 256-262.
21. Pinter, A.; Labruna, M.B. (2006). Isolation of *Rickettsia rickettsii* and *Rickettsia bellii* in cell culture from the tick *Amblyomma aureolatum* in Brazil. *Ann. N. Y. Acad. Sci.* 1078, 523-529.
22. Raoult, D.; La Scola, B.; Enea, M.; Fournier, P.E.; Roux, V.; Fenollar, F.; Galvão, M.A.M.; Lamballerie, X. (2001). A flea-associated *Rickettsia* pathogenic for humans. *Emerg. Infec. Dis.* 7, 73-81
23. Reif, K.E.; Stout, R.W.; Henry, G.C.; Foil, L.D.; Macaluso, K.R. (2008). Prevalence and infection load dynamics of *Rickettsia felis* in actively feeding cat fleas. *PLoS ONE* 3, e2805.
24. Richter, J.; Fournier, P.E.; Petridou, J.; Häussinger, D.; Raoult, D. (2002). *Rickettsia felis* infection acquired in Europe and documented by by polymerase chain reaction. *Emerg. Infect. Dis.* 8, 207-208.
25. Roux, V.; Fournier, P.E.; Raoult, D. (1996). Differentiation of spotted fever group rickettsiae by sequencing and analysis of restriction fragment length polymorphism of PCR amplified DNA of the gene encoding the protein rOmpA. *J. Clin. Microbiol.* 34, 2058-2065.
26. Roux, V. (1999). Plylogenetic analysis and taxonomic relationship among the genus *Rickettsia*. *In*: Raoult, D.; Brouqui, P. (eds). *Rickettsiae and rickettsial diseases at the turn of the third millennium.* Elsevier, Marseille, France, p. 52-66.
27. Schriefer, M.E.; Sacci, J.B.; Dumler, J.S.; Bullen, M.G.; Azad, A.F. (1994). Identification of a novel rickettsial infection in a patient diagnosed with murine typhus. *J. Clin. Microbiol.* 32, 949-954.
28. Silveira, I.; Pacheco, R.C.; Szabó, M.P.J.; Ramos, H.G.C.; Labruna, M.B. (2007). First report of *Rickettsia parkeri* in Brazil. *Emerg. Infect. Dis*. 13, 1111-1113.
29. Tsai, K.H.; Lu, H.Y.; Tsai, J.J.; Yu, S.K.; Huang, J.H.; Shu, P.Y. (2008). Human case of *Rickettsia felis* infection, Taiwan. *Emerg. Infect. Dis.* 14, 1970-1972.
30. Wedincamp, J.; Foil, L.D. (2000). Infection and seroconversion of cats exposed to cat fleas (*Ctenocephalides felis* Bouché) infected with *Rickettsia felis*. *J. Vector Ecology* 25, 123-126.
31. Wedincamp, J.; Foil, L.D. (2002). Vertical transmission of *Rickettsia felis* in the cat flea (*Ctenocephalides felis* Bouché). *J. Vector Ecology* 27, 96-101.
32. Zavala-Velazquez, J.E.; Yu, X.J.; Walter, D.H. (1996). Unrecognized spotted fever group rickettsiosis masquerading as dengue fever in Mexico. *Am. J. Trop. Med. Hyg.* 55, 157-159.
33. Zavala-Velazquez, J.E.; Ruiz-Sosa, J.A.; Sánchez-Elias, R.A.; Becerra-Carmona, G.; Walker, D.H. (2000). *Ricketsia felis* rickettsiosis in Yucatán. *Lancet* 356, 1079-1080.
34. Zavala-Velazquez, J.E.; Laviada-Molina, H.; Zavala-Castro, J.; Perez-Osorio, C.; Becerra-Carmona, G.; Ruiz-Sosa, J.A.; Bouyer, D.H.; Walker, D.H. (2006). *Rickettsia felis*, the agent of an emerging infectious disease: report of a new case in Mexico. *Arch. Med. Research* 37, 419-422.
35. Znazen, A.; Rolain, J.M.; Hammami, N.; Hammami, A.; Jemaa, M.B.; Raoult, D. (2006). *Rickettsia felis* infection, Tunisia. *Emerg. Infect. Dis.* 12, 138-140.