12-2012

Rickettsia felis in Cat Fleas, *Ctenocephalides felis* Parasitizing Opossums, San Bernardino County, California

Kyle F. Abramowicz
Centers for Disease Control and Prevention

J. W. Wekesa

C. N. Nwadike

Maria L. Zambrano
Centers for Disease Control and Prevention

Sandor E. Karpathy
Centers for Disease Control and Prevention

See next page for additional authors

Recommended Citation

Abramowicz, Kyle F., J. W. Wekesa, C. N. Nwadike, Maria L. Zambrano, Sandor E. Karpathy, D. Cecil, J. Burns, R. Hu, Marina E. Eremeeva. 2012. "*Rickettsia felis* in Cat Fleas, *Ctenocephalides felis* Parasitizing Opossums, San Bernardino County, California." *Medical and Veterinary Entomology*, 26 (4): 458-462. doi: 10.1111/j.1365-2915.2012.01017.x source: https://doi.org/10.1111/j.1365-2915.2012.01017.x https://digitalcommons.georgiasouthern.edu/environ-health-facpubs/56

Medical and Veterinary Entomology

Medical and Veterinary Entomology (2012) **26**, 458–462 doi: 10.1111/j.1365-2915.2012.01017.x

SHORT COMMUNICATION

Rickettsia felis in cat fleas, *Ctenocephalides felis* parasitizing opossums, San Bernardino County, California

K. F. ABRAMOWICZ[1*], J. W. WEKESA[2], C. N. NWADIKE[2], M. L. ZAMBRANO[1*], S. E. KARPATHY[1*], D. CECIL[3], J. BURNS[4], R. HU[4] and M. E. EREMEEVA[1*]

[1]Rickettsial Zoonoses Branch, Division of Vector-Borne Diseases, Centers for Disease Control and Prevention, Atlanta, GA, U.S.A., [2]Division of Environmental Health Services, Department of Public Health, Mosquito and Vector Control Program, San Bernardino, CA, U.S.A. and [3]Division of Geographic Information, Department of Information Services, San Bernardino, CA, U.S.A. and [4]California Department of Public Health, Center for Infectious Diseases, Vector-Borne Disease Section, Ontario, CA, U.S.A.

Abstract. Los Angeles and Orange Counties are known endemic areas for murine typhus in California; however, no recent reports of flea-borne rickettsioses are known from adjacent San Bernardino County. Sixty-five opossums (*Didelphis virginiana*) were trapped in the suburban residential and industrial zones of the southwestern part of San Bernardino County in 2007. Sixty out of 65 opossums were infested with fleas, primarily cat fleas, *Ctenocephalides felis* (Bouché, 1835) . The flea minimum infection rate with *Rickettsia felis* was 13.3% in pooled samples and the prevalence was 23.7% in single fleas, with two *glt*A genotypes detected. In spite of historic records of murine typhus in this area, no evidence for circulation of *R. typhi* in fleas was found during the present study. Factors contributing to the absence of *R. typhi* in these cat fleas in contrast to its presence in cat fleas from Orange and Los Angeles Counties are unknown and need to be investigated further in San Bernardino County.

Key words. *Rickettsia felis*, cat flea, genotyping, opossums.

Introduction

Rickettsia felis is an obligate intracellular bacterium that causes cat flea rickettsiosis (Reif & Macaluso, 2009). While most commonly associated with the cat flea, *Ctenocephalides felis* (Bouché), *R. felis* has been detected in other flea species, ticks, mites and even a non-hematophagous arthropod, the book louse, *Liposcelis bostrychophila* (Adams *et al.*, 1990; Reif & Macaluso, 2009; Thepparit *et al.*, 2011). Whether arthropods other than *C. felis* are competent vectors for *R. felis* or the PCR detection just reflects rickettsial DNA passively acquired with a bloodmeal from a rickettsiemic host is unknown. Infected fleas are most frequently found on cats, dogs and peridomestic opossums (*Didelphis* spp.); however, fleas containing *R. felis* DNA have also been collected from rodents, hedgehogs, horses, sheep, goats, gerbils and monkeys (Perez-Osorio *et al.*, 2008; Reif & Macaluso, 2009). *Rickettsia felis* maintains a persistent infection in *C. felis* through transovarial and transstadial transmission (Azad *et al.*, 1992; Hirunkanokpun *et al.*, 2011). Cats exposed to *R. felis*-positive *C. felis* could both seroconvert and may exhibit PCR positive blood, suggesting that horizontal transmission can also occur (Wedincamp & Foil, 2000), but whether cats or other animals serve as a reservoir of *R. felis* needs investigation.

Correspondence: Marina Eremeeva, Jiann-Ping Hsu College of Public Health, Georgia Southern University, Statesboro, GA, U.S.A. Tel.: +1 912 478 0504; Fax: +1 912 478 0171; E-mail: meremeeva@georgiasouthern.edu

*The findings and conclusions are those of the authors and do not necessarily reflect the views of the U.S. Department of Health and Human Services.

At the time of its original discovery, *R. felis* was thought to be another pathogen, similar to *Rickettsia typhi*, which causes murine typhus, and circulates in cat fleas and is associated with opossums (Adams *et al.*, 1970, 1990; Sorvillo *et al.*, 1993). However, data based on molecular detection and identification of *R. felis* worldwide indicate that its circulation does not necessarily coincide with that of *R. typhi*, which is found far less frequently (Eremeeva *et al.*, 2008; Perez-Osorio *et al.*, 2008; Sackal *et al.*, 2008; Reif & Macaluso, 2009; Richards *et al.*, 2010). The first and only US association of *R. felis* with human illness was documented in 1991 when *R. felis* DNA was detected in the blood of a patient from Texas suspected to have murine typhus (Schriefer *et al.*, 1994). As of 2011, other human cases as a consequence of *R. felis* infection were reported from 15 other countries (Perez-Osorio *et al.*, 2008; Richards *et al.*, 2010; Socolovschi *et al.*, 2010). These cases manifested with non-specific symptoms of varying incidences of rash, fatigue, fever, headache, arthralgia and myalgia, indicating that disease as a result of *R. felis* may be significantly under-recognized and under-diagnosed in the U.S.A., as is true of other rickettsioses.

In the present study, we describe extensive surveillance for flea-borne pathogens in cat fleas infesting opossums in San Bernardino County, CA, and discuss the public health relevance of these findings.

Materials and methods

Site description and sampling method

The study was conducted in the southwestern corner of San Bernardino County, CA. This mostly suburban residential and industrial area is bounded by Riverside, Los Angeles and Orange Counties to the south, west and southwest, respectively; and by the San Bernardino Mountains to the north. Twenty-seven sites were selected based on past reports of opossum presence. Two to three large live-capture wire traps per site were baited with cat food and fruit, and set in late afternoon every other week from March to October 2007. Traps were checked the following morning for animal capture, with non-target species released immediately. Trapped opossums were anaesthetized and the ectoparasites were removed, identified to species using standard taxonomic keys, frozen at −70 °C and shipped on dry ice to the Centers for Disease Control and Prevention (CDC, Atlanta, GA, U.S.A,) for testing.

Preparation of DNA and testing for flea-borne rickettsiae

Fleas were processed individually if 10 or fewer fleas were found or in pools of 2 fleas if 11 or more fleas per opossum were collected. A maximum of 50 fleas per animal were tested for the latter group. Fleas were surface disinfected, frozen in liquid nitrogen and pulverized using Konte pestles (Kimble-Kontes, Vineland, NJ, U.S.A.) (Abramowicz *et al.*, 2011). The flea powder was re-suspended in 160 μL Nuclei lysis solution (Promega, Madison, WI, U.S.A.) supplemented with 40 μL 0.5 M EDTA and 20 μL of 20 mg/mL Proteinase K (Qiagen, Valencia, CA, U.S.A.), and incubated overnight at 56 °C. DNA was extracted using the Wizard SV 96 Genomic DNA purification system (Promega) and a Biomek 2000 Robotic Workstation (Beckman Coulter, Fullerton, CA, U.S.A.), and was eluted with 120 μL of sterile nuclease-free water and stored at 4 °C.

Flea DNA was tested for the presence of *R. typhi* and *R. felis* DNA using the Brilliant QPCR reagent kit (Stratagene, Cedar Creek, TX, U.S.A.) and a species-specific TaqMan for the citrate synthase (*glt*A) gene of *Rickettsia* as described previously (Karpathy *et al.*, 2009). Positive control plasmids were constructed by cloning target fragments from *R. typhi* strain Wilmington and *R. felis* strain LSU into pCR2.1 (Invitrogen, Carlsbad, CA, U.S.A.), according to the manufacturer's instructions. All reactions were run with a non-template control in which sterile water was substituted for the DNA sample. Amplicons were sequenced using an Applied Biosystems Big Dye Terminator v3.1 Cycle Sequencing Kit and purified using the ABI BigDye XTerminator kit (Applied Biosystems, Carlsbad, CA, U.S.A.). Assembly and sequence analysis was performed using the Sequencher 4.8 software (Gene Codes, Ann Arbor, MI, U.S.A.) and analysed using MEGA4 (Tamura *et al.*, 2007).

Data analysis

The flea index was calculated as an arithmetic average of flea number per opossum. Prevalence was estimated as percentage of the total number of samples in which *R. felis* was detected. Minimum infection rates (MIR) were calculated as percentage of a ratio between the total number of flea pools positive for *R. felis* and the total number of fleas tested. Statistical assessment was done using Student's *t*-test. Confidence intervals (CI) for MIR and prevalence rates were calculated using the Wilson score method without continuity correction (Newcombe, 1998).

Results

During the study period, 65 opossums were trapped at the 27 sites (Fig. 1A). Among them, 34 (26 adults and 8 subadults) were females and 31 (8 adults and 23 subadults) were males. The largest collections of 17 and 12 opossums were trapped in May and September, respectively. Four to eight animals were trapped at 4 sites, whereas one to three opossums were collected from the other 23 sites during the same trapping period (Fig. 1A).

A total of 1870 fleas were collected from 60 (92.3%) of the 65 opossums trapped (1–289 fleas per animal, flea index 31.2). Male opossums had a higher although not statistically significant flea index of 34.4 than females (flea index 24.3, *t*-test $P = 0.56$). Among the fleas collected, 1867 were *C. felis*, 2 were human flea, *Pulex irritans* Linnaeus, 1758 and 1 was sticktight flea, *Echidnophaga gallinacea* Westwood, 1875. In total, 1258 fleas were tested, including 570 pools of 2 fleas from 33 opossums, and 118 samples of 1 flea from 27 opossums. One hundred and fifty-two

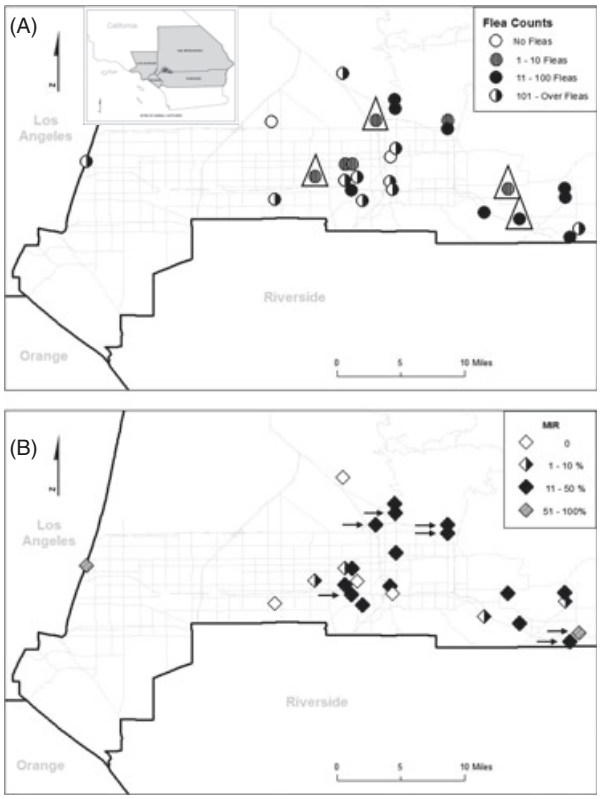

Fig. 1. Geographical map of the area studied. (A) Distribution of the trapping sites, opossum collection data and total number of fleas collected at each site. Triangles indicate collection sites where four to eight animals were trapped per site; one to three animals were trapped in other locations. Inset illustrates the location of the studied area relative to Los Angeles and Orange Counties of California; (B) the distribution of fleas and prevalence and/or average minimum infection rates of *R. felis* in cat fleas per animal collected at each site with fleas. Arrows indicate sites of fleas infected with *R. felis* genotype RF2125.

out of 570 pools (minimum infection rate 13.3%, 95% CI 11.48–15.43) and 28 out of 118 single flea samples (prevalence 23.7%, 95% CI 16.96–32.16) were tested positive for *R. felis* DNA by *glt*A TaqMan. The distribution of tested fleas with their average MIR or prevalence is displayed in Fig. 1B. *Rickettsia felis*-infected fleas were present on 44 out of 60 flea-infested opossums (73%); *C. felis* from 13 of the 27 opossums infested with < 10 fleas were not infected whereas only 4 of the 33 heavily infested animals did not have infected flea pools. The *E. gallinacea* and one of the two *P. irritans* also tested positive for *R. felis* DNA. None of the fleas tested positive for *R. typhi* DNA.

All PCR amplicons were sequenced and two genotypes of *R. felis* were identified based on their *glt*A nucleotide sequences. One was identical to that of *R. felis* URRWXCal2 (NC_007109), whereas the other type had four mutations at the nucleotide positions 26(C/T), 83(T/G), 170(C/T) and 179(G/A) and was identical to RF2125 (AF516333). Genotype URRWXCal2 was dominant and found in 93.4% of 180 samples sequenced. The RF2125 genotype was found in 12 *C. felis* from 9 opossums trapped in 8 sites from 5 different administrative affiliations (Fig. 1B). Fleas carrying both genotypes of *R. felis* were collected from the same animal, including two adult females, three adult males and four young males.

Discussion

Murine typhus is endemic in the state of California with human cases primarily reported from Los Angeles and Orange Counties (Civen & Ngo, 2008). San Bernardino County is of particular interest as it is directly adjacent to these counties. It has similar ecological characteristics; however, no recent reports of human exposure are known despite historic records of both murine typhus and *R. typhi* in rats from San Bernardino (Beck *et al.*, 1944; Beck & Van Allen, 1950). We evaluated the presence and distribution of opossums and their associated ectoparasites, and determined the prevalence of the flea-borne rickettsiae, *R. typhi* and *R. felis* in San Bernardino County. Sixty-five *D. virginiana* were trapped in the southwestern portion of the county where they live in relatively close contact with humans in a predominantly urban and suburban environment in the most densely inhabited section of the largest county in the U.S.

The prevalence of *R. felis* in cat fleas ranges from 1% to 45.8% and has been reported as high as 100% in small batches of samples tested (Reif & Macaluso, 2009). The prevalence determined in the present study is within the same range; however, we observed a significant difference between MIR of pooled fleas and prevalence determined by testing individual samples. Therefore, it may be that the MIR underestimates the prevalence of *R. felis* in pools, possibly because the greater amount of DNA in pools or other contaminants may inhibit the TaqMan assay to some degree (Demeke & Jenkins, 2010). If one corrects for a possible underestimation of prevalence of infection in flea pools from the 9 animals with greater than 40% of their pools infected (both fleas are actually infected rather than only one) and the remaining 24 animals with less than 33% infected pools (one of two fleas actually infected), the prevalence of *R. felis* in pooled fleas is 20.2%. This is within the CI estimated from fleas that were tested individually.

It is also unknown if the determined prevalence and infection rate of *R. felis* in cat fleas parasitizing opossums in San Bernardino County is sufficient for efficient transmission to humans. Clearly, opossums which are highly flea infested are more likely to carry infected fleas than poorly infested animals. Furthermore, the significance of co-circulation of two genetic types of *R. felis*, URRWXCal2 (or Cal2) and RF2125 reported here for the first time in cat fleas from California is not well understood. The RF2125 type was first found in 2 dog flea, *C. canis* (prevalence 5.9%, $n = 34$) from Thai-Myanmar border and then was detected in other flea species from Europe, Northern Africa, Uruguay and Eastern U.S. (Parola *et al.*, 2003; Bitam *et al.*, 2006; Loftis *et al.*, 2006; Venzal *et al.*, 2006; Nelder *et al.*, 2009). Beside cat and dog fleas, the RF2125 genotype of *R. felis* was detected in *E. gallinacea* (100%, $n = 12$), hedgehog flea, *Archeopsylla erinacei* (100%, $n = 4$) and *P. irritans* (Bitam *et al.*, 2006; Loftis *et al.*, 2006; Hornok *et al.*, 2010).

In our previous studies *R. felis* was shown to co-circulate with *R. typhi* (Eremeeva *et al.*, 2008; Karpathy *et al.*, 2009; Abramowicz *et al.*, 2011), similarly to when it was first discovered in its sylvatic cycle in CA (Williams *et al.*, 1992). However, in those sites *R. felis* infection was not easily recognized as a separate flea-borne cause of human illness as current diagnosis relies on serological testing with cross-reactive *R. typhi* antigen, and is therefore reported as murine typhus (Green *et al.*, 2011). Whether *R. typhi* also circulates in a classic rat flea, *Rattus norvegicus–Xenopsylla cheopis* cycle in San Bernardino County is unknown as only opossums and their ectoparasites were available for this study. The roof rat, *Rattus rattus*, appears to be more common in this region; however, they are most frequently infested with mouse flea, *Leptosylla segnis* (Schoenherr) and Northern rat flea, *Nosopsylla fasciatus* (Bosc, 1800) with seasonal variations (Schwan *et al.*, 1985). Although *L. segnis* can be infected with *R. typhi,* it is unlikely to cause human infection as it rarely bites people; the potential of *N. fasciatus* for transmission of *R. typhi* needs further evaluation (Traub *et al.*, 1978). Further study focusing on rats and rat fleas may provide more insight into the dynamics of rickettsial circulation and the occurrence of rickettsial diseases in San Bernardino County. By contrast, suggestions that opossums and associated cat fleas can serve as an alternative animal host for *R. typhi* and sylvatic reservoir of murine typhus (Civen & Ngo, 2008; Adjemian *et al.*, 2010) may not be completely justified as reliable findings of those associations in the absence of active rodent cycles of *R. typhi* are infrequent (Williams *et al.*, 1992; Boostrom *et al.*, 2002; Karpathy *et al.*, 2009) and need further evaluation.

Ctenocephalides felis is a very common flea in pets, and cats can easily acquire them from opossums entering urban and suburban properties. As about 32.4% of U.S. households have one or more cats (AVMA, 2007) awareness of the potential risk of flea-borne rickettsial pathogens should be an important component in the disease prevention programme.

Acknowledgements

We thank Filomeno Gasmen and Tam Nguyen for their assistance with trapping and bleeding the opossums, and Gregory A. Dasch for his helpful comments on the manuscript. Kyle F. Abramowicz was supported by a fellowship from the Oak Ridge Institute for Science and Education (ORISE).

References

Abramowicz, K.F., Rood, M.P., Krueger, L. & Eremeeva, M.E. (2011) Urban focus of *Rickettsia typhi* and *Rickettsia felis* in Los Angeles, California. *Vector-Borne Zoonotic Diseases*, **11**, 979–984.

Adams, W.H., Emmons, R.W. & Brooks, J.E. (1970) The changing ecology of murine (endemic) typhus in Southern California. *The American Journal of Tropical Medicine and Hygiene*, **19**, 311–318.

Adams, J., Schmidtmann, E. & Azad, A. (1990) Infection of colonized cat fleas, *Ctenocephalides felis* (Bouche) with a rickettsia-like microorganism. *The American Journal of Tropical Medicine and Hygiene*, **43**, 400–409.

Adjemian, J., Parks, S., McElroy, K. *et al.* (2010) Murine typhus in Austin, Texas, USA, 2008. *Emerging Infectious Diseases*, **16**, 412–417.

American Veterinary Medical Association (AVMA) (2007) Market research statistics. U.S. pet ownership—(2007). www.avma.org/reference/marketstats/ownership.asp [Accessed 7 December 2011]. U.S. Pet Ownership & Demographic Sourcebook.

Azad, A.F., Sacci, J.B.J., 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. *Proceedings of the National Academy of Sciences*, **89**, 43–46.

Beck, M. & Van Allen, A. (1950) *Typhus Fever in California, 1916–1948, Inclusive.* State of California Department of Public Health, San Francisco, CA.

Beck, M.D., Bodily, H.L. & O'Donnell, R. (1944) A strain of typhus rickettsiae isolated from the brain of a wild rat in California. *Public Health Reports*, **59**, 701–712.

Bitam, I., Parola, P., De La Cruz, K.D. *et al.* (2006) First molecular detection of *Rickettsia felis* in fleas from Algeria. *The American Journal of Tropical Medicine and Hygiene*, **74**, 532–535.

Boostrom, A., Beier, M.S., Macaluso, J.A. *et al.* (2002) Geographic association of *Rickettsia felis*-infected opossums with human murine typhus, Texas. *Emerging Infectious Diseases*, **8**, 549–554.

Civen, R. & Ngo, V. (2008) Murine typhus: an unrecognized suburban vectorborne disease. *Clinical Infectious Diseases*, **46**, 913–918.

Demeke, T. & Jenkins, G.R. (2010) Influence of DNA extraction methods, PCR inhibitors and quantification methods on real-time PCR assay of biotechnology derived traits. *Analytical and Bioanalytical Chemistry*, **396**, 1977–1990.

Eremeeva, M.E., Warashina, W.R., Sturgeon, M.M. *et al.* (2008) *Rickettsia typhi* and *R. felis* in rat fleas (*Xenopsylla cheopis*), Oahu, Hawaii. *Emerging Infectious Diseases*, **14**, 1613–1615.

Green, J.S., Singh, J., Cheung, M., Adler-Shohet, F.C. & Ashouri, N. (2011) A cluster of pediatric endemic typhus cases in Orange County, California. *The Pediatric Infectious Disease Journal*, **30**, 163–165.

Hirunkanokpun, S., Thepparit, C., Foil, L.D. & Macaluso, K.R. (2011) Horizontal transmission of *Rickettsia felis* between cat fleas, *Ctenocephalides felis*. *Molecular Ecology*, **20**, 4577–4586.

Hornok, S., Meli, M.L., Perreten, A. *et al.* (2010) Molecular investigation of hard ticks (Acari: Ixodidae) and fleas (Siphonaptera: Pulicidae) as potential vectors of rickettsial and mycoplasmal agents. *Veterinary Microbiology*, **140**, 98–104.

Karpathy, S.E., Hayes, E.K., Williams, A.M. *et al.* (2009) Detection of *Rickettsia felis* and *Rickettsia typhi* in an area of California endemic for murine typhus. *Clinical Microbiology and Infection*, **15** (Suppl. 2), 218–219.

Loftis, A.D., Reeves, W.K., Szumlas, D.E., Abbassy, M.M., Helmy, I.M., Moriarity, J.R. & Dasch, G.A. (2006) Surveillance of Egyptian fleas for agents of public health significance: *Anaplasma*, *Bartonella*, *Coxiella*, *Ehrlichia*, *Rickettsia*, and *Yersinia pestis*. *The American Journal of Tropical Medicine and Hygiene*, **75**, 41–48.

Nelder, M.P., Reeves, W.K., Adler, P.H., Wozniak, A. & Wills, W. (2009) Ectoparasites and associated pathogens of free-roaming and captive animals in zoos of South Carolina. *Vector-Borne Zoonotic Diseases*, **9**, 469–477.

Newcombe, R.G. (1998) Two-sided confidence intervals for the single proportion: comparison of seven methods. *Statistics in Medicine*, **17**, 857–872.

Parola, P., Sanogo, Y.O., Lerdthusnee, K. *et al.* (2003) Identification of *Rickettsia* spp. and *Bartonella* spp. in fleas from the Thai-Myanmar border. *Annals of the New York Academy of Sciences*, **990**, 173–181.

Perez-Osorio, C., Zavala-Velasques, J., Leon, J. & Zavala-Castro, J. (2008) *Rickettsia felis* as emergent global threat for humans. *Emerging Infectious Diseases*, **14**, 1019–1022.

Reif, K. & Macaluso, K. (2009) Ecology of *Rickettsia felis*: a review. *Journal of Medical Entomology*, **46**, 723–736.

Richards, A., Jiang, J., Omulo, S. *et al.* (2010) Human Infection with *Rickettsia felis*, Kenya. *Emerging Infectious Diseases*, **16**, 1081–1086.

Sackal, C., Laudisoit, A., Kosoy, M. *et al.* (2008) *Bartonella* spp. and *Rickettsia felis* in fleas, Democratic Republic of Congo. *Emerging Infectious Diseases*, **14**, 1972–1974.

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. *Journal of Clinical Microbiology*, **32**, 949–954.

Schwan, T.G., Thompson, D. & Nelson, B.C. (1985) Fleas on roof rats in six areas of Los Angeles County, California: their potential role in the transmission of plague and murine typhus to humans. *The American Journal of Tropical Medicine and Hygiene*, **34**, 372–379.

Socolovschi, C., Mediannikov, O., Sokhna, C. *et al.* (2010) *Rickettsia felis*-associated uneruptive fever, Senegal. *Emerging Infectious Diseases*, **16**, 1140–1142.

Sorvillo, F.J., Gondo, B., Emmons, R. *et al*. (1993) A suburban focus of endemic typhus in Los Angeles County: association with seropositive domestic cats and opossums. *The American Journal of Tropical Medicine and Hygiene*, **48**, 269–273.

Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution*, **24**, 1596–1599.

Thepparit, C., Sunyakumthorn, P., Guillotte, M., Popov, V., Foil, L. & Macaluso, K. (2011) Isolation of a rickettsial pathogen from a non-hematophagous arthropod. *PLoS ONE*, **6**, e16396.

Traub, R., Wisseman, C.L. & Farhang-Azad, A. (1978) The ecology of murine typhus-a critical review. *Tropical Diseases Bulletin*, **75**, 237–317.

Venzal, J.M., Perez-Martinez, L., Felix, M.L., Portillo, A., Blanco, J.R. & Oteo, J.A. (2006) Prevalence of *Rickettsia felis* in *Ctenocephalides felis* and *Ctenocephalides canis* from Uruguay. *Annals of the New York Academy of Sciences*, **1078**, 305–308.

Wedincamp, J. & Foil, L. (2000) Infection and seroconversion of cats (*Ctenocephalides felis* Bouche) infected with *Rickettsia felis*. *Journal of Vector Ecology*, **25**, 123–126.

Williams, S.G., Sacci, J.B. Jr, Schriefer, M.E. *et al.* (1992) Typhus and typhuslike rickettsiae associated with opossums and their fleas in Los Angeles County, California. *Journal of Clinical Microbiology*, **30**, 1758–1762.

Accepted 25 January 2012
First published online 20 June 2012