

***Rickettsia felis* (Rickettsiales: Rickettsiaceae) Discovered in Cat Fleas (Siphonaptera: Pulicidae) in the Philippines¹**

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Dengue and dengue hemorrhagic fevers are significant diseases in the tropics. In the Philippines, annual infection rates vary depending on a myriad of environmental and host factors, but annual rates sometimes exceed 30,000 cases with over 500 deaths (Mahilum et al. 2005, J. Vector Ecol. 30: 277 - 283). Physicians and medical personnel should be aware of other infectious diseases resembling dengue. Measles and rubella are sometimes mistaken as dengue. Arboviruses, such as chikungunya virus, cause similar symptoms and share the same mosquito vectors as dengue. Some rickettsial agents cause symptoms similar to dengue. Scrub typhus and rickettsial spotted fevers can be mistaken for dengue (Watt et al. 2003, Am. J. Trop. Med. Hyg. 68: 536 - 538; Spicer et al. 2007 Papua New Guinea Med. J. 50:172 - 183). An epidemic caused by *Rickettsia felis* in Mexico was clinically mistaken for dengue (Zavala-Velazquez et al. 1996, Am. J. Trop. Med. Hyg. 55: 157 - 159). Clinical symptoms of *R. felis* infections in that outbreak included fever, headache, myalgia, and rash, which are similar to those of dengue fever.

Arthropod vectors of rickettsial agents were opportunistically collected from animals and people in the City of San Pablo, Laguna province, Philippines from 16 - 20 February 2010 during a U.S. Air Force humanitarian mission. Collections included 105 brown dog ticks (*Rhipicephalus sanguineus* Latreille), 4 cattle ticks (*Rhipicephalus* [*Boophilus*] *microplus* [Canestrini]), 31 water buffalo lice (*Haematopinus tuberculatus* [Burmeister]), and 2 cat fleas (*Ctenocephalides felis* Bouché). Arthropods were preserved in 95% ethanol until they were identified and processed in the laboratory. Arthropods were tested individually or those from the same host were in pools of 5 or less. DNA was extracted from arthropods using a MagNAPure LC robot (Roche Diagnostics, Mannheim, Germany) as previously described by McAvinet et al. (2005, Mil. Med. 170: 1060 - 1065). Individual extracts were screened by real time PCR for

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Rickettsia following previously described protocols (Loftis et al. 2006, Am. J. Trop. Med. Hyg. 75: 41 - 48). Negative controls consisted of DNA extracts from uninfected humans; positive controls consisted of a ~200 bp synthetic oligonucleotide that corresponded to the PCR primers and probes but did not match known agents. Extracts from 2 individual cat fleas collected on 16 February 2010 in Barangay Mangata and 20 February 2010 in Barangay San Lorenzo tested positive for *Rickettsia*. Other tests were negative, except the positive controls. We verified the positive results by amplifying the 17 kD antigen gene from the 2 *Rickettsia* positive fleas using previously published PCR primers (Webb et al. 1990, J. Clin. Microbiol. 28: 530 - 534). Products were separated by 4% agarose gel electrophoresis and visualized under UV light with ethidium bromide. PCR products were purified with a QIAquick PCR Purification Kit (Qiagen, Valencia, CA) and sequencing reactions were performed with a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) using PCR primers. Sequences were determined using an ABI 3,100 capillary sequencer (Applied Biosystems, Foster City, CA). Primers sequences were removed and sequences were assembled with Chromas Lite 2.01 (Technelysium, Tewantin, Australia) and ClustalW (Kyoto University, Kyoto, Japan), and compared with each other and sequences in GenBank using the BLAST 2.0 program (NCBI, Bethesda, MD). Both sequences were 100% identical to the *R. felis* complete genome (Accession# CP000053).

Dengue is a continuous threat to people in the Philippines. However, our data indicate that *R. felis* is present in the same region. This should be taken into account if a patient with dengue-like symptoms does not test positive for virus or a recent infection. *Rickettsia felis* was not previously reported in the Philippines. However, this pathogen is being reported from countries throughout the world and the lack of reporting from the Philippines is likely an oversight rather than a new introduction. *Rickettsia felis* can cause serious or life-threatening disease in humans, and it should be considered when a patient meets the case definition.

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