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***Rickettsia felis* Infections and Comorbid Conditions, Laos, 2003–2011**

To the Editor: Fleaborne disease is highly prevalent in Laos, mainly attributed to murine typhus (*Rickettsia typhi* infection), transmitted by *Xenopsylla cheopis* fleas, but data on other fleaborne diseases are limited (1). We screened blood and cerebrospinal fluid (CSF) from participants in 2 large prospective studies in Laos for *Rickettsia* spp. using a genus-specific 17-kDa-based *Rickettsia* real-time quantitative PCR assay, and positive results were confirmed by DNA sequencing (2,3). In samples from >2,500 patients (2,540 blood and 1,112 CSF), we

detected 3 cases of sequence-confirmed *R. felis* infections.

A 50-year-old man, an official in Vientiane City, was admitted to a hospital with fever and headache in October, 2008. HIV infection and cryptococcal meningitis were diagnosed. Treatment with intravenous amphotericin B, then oral fluconazole, was successful; antiretroviral treatment was initiated 1 month after diagnosis. Among a panel of diagnostic PCRs, the CSF sample specimen tested positive for genus-specific 17-kDa-*Rickettsia* quantitative PCR, but was negative for *Orientia tsutsugamushi* and *R. typhi*. DNA sequencing of 434 bp of the 17-kDa gene (Macrogen, Seoul, South Korea) revealed a 100% homology to the *R. felis* URRWXCal2 strain (Table).

R. felis positivity in CSF is rare; 4 cases have been reported (3). The combined findings of *R. felis* infection and severe immunodeficiency in this patient led to a reevaluation of the 2 reported *R. felis* infections in Laos (2). Before this study, *R. felis* DNA or culture had not been handled in our facility. The interval between processing positive samples, dedicated separate areas for samples before and after PCR, and the low positivity rate make DNA contamination highly unlikely.

A 39-year-old housewife from Luang Namtha in northern Laos had a history of diabetes mellitus, which had been treated with glibenclamide. On arrival at the hospital in November, 2008, she had fever, headache, myalgia, and an eschar. She was empirically treated with doxycycline (Table). An eschar biopsy specimen was PCR-positive for *Rickettsia* spp. and *O. tsutsugamushi*; PCR of buffy coat detected *O. tsutsugamushi* DNA only (2). Molecular characterization included 17-kDa and *sca4* gene sequencing, which both revealed amplicons of 100% identity to the *R. felis* URRWXCal2 strain. Serologic evidence for *O. tsutsugamushi* infection (scrub typhus) included a 4-fold rise in IgM and IgG titers, and IgM and IgG

titers against typhus group rickettsiae, spotted fever group rickettsiae, and *R. felis* (isolate B377 in XTC-2 cells, Australian Rickettsial Reference Laboratory) were negative in admission and convalescent-phase samples (6-day interval) (Table).

A 13-year-old boy from Salavan, in southern Laos, had fever, headache, and nonspecific symptoms in July, 2009. *P. falciparum* malaria and dengue were diagnosed, both confirmed by PCR (Table). PCR results for the buffy coat specimen were positive for the 17-kDa gene; subsequent sequencing confirmed *R. felis* with 100% identity to the URRWX-Cal2 strain. The fever resolved after treatment with antimalarial drugs and ceftriaxone; neither would be expected to be efficacious for *R. felis* infection.

These data suggest that *R. felis* occurs in Laos, and is possibly emerging, but whether it results in clinical disease or commonly causes subclinical infection is unknown. The screened cohorts of consecutively enrolled patients with febrile illnesses across 3 diverse geographic regions are representative of etiologic agents of fever across Laos. PCR has previously been used for detection of *R. felis* and resulted in the discovery of a new *R. felis*-like organism in fleas in Kenya, *Candidatus Rickettsia asemboensis* (4). Reports from Southeast Asia suggest that *R. felis* is not a common cause of febrile illness (1,2), which contrasts with findings in Kenya, where *R. felis* was found in ≈7% of febrile patients (4,5), and also in ≈3% of afebrile patients (5).

The high *R. felis* carriage rate in fleas found in Laos (77% overall; 53% in *Ctenocephalides felis felis*, 89% in *C. f. orientis*) contrasts strongly with the apparent low incidence of *R. felis* human infections (6). Among febrile hospitalized patients in Vientiane, 1 case of *R. felis* infection was serologically diagnosed by using species-specific cross-absorption (1). Seroprevalence studies in the region could

Table: Clinical and laboratory findings of 3 patients with *Rickettsia felis* infections, Laos*

Patient characteristics	Signs and symptoms	Molecular findings	Serologic findings	Other laboratory findings
Male, 50 y, Vientiane City, central Laos	Fever, severe headache × 7 d; contact with cats and dogs 14 d before admission; HIV/AIDS (CD4-count: 34 cell/μL)	qPCR: <i>Orientia tsutsugamushi</i> (CSF, blood): negative; <i>Rickettsia</i> spp. (CSF): positive; <i>Rickettsia</i> spp. (blood): negative; <i>R. typhi</i> (CSF, blood): negative <u>Conventional PCR and sequencing (CSF):</u> <i>Rickettsia</i> spp. 17 kDa; GenBank accession no: KF489454	<u>Scrub/murine typhus:</u> IgM/IgG static titers (<1:100; negative) <u>Spotted fever group:</u> IgM/IgG static titers (1:200; negative) <i>R. felis:</i> IgM/IgG static titers (<1:128; negative)	<u>Increased intracranial pressure</u> (>40 cm H ₂ O) <u>CSF:</u> clear <u>CSF cellularity:</u> 5 leukocytes/μL (100% lymphocytes) <u>CSF glucose:</u> 1.1 mmol/L; <u>CSF/blood glucose ratio:</u> 1:5 <u>CSF protein:</u> 80 mg/L; <u>CSF <i>Cryptococcus</i> culture:</u> positive/serotyping (PCR/RFLP) <i>C. neoformans</i> var. <i>grubii</i> <u>HIV rapid tests:</u> † positive
Female, 39 y, Luang Namtha, northern Laos	Fever × 7 d; diabetes mellitus, treated with glibenclamide; HIV status: unknown	qPCR: <i>O. tsutsugamushi</i> (eschar, blood): positive; <i>Rickettsia</i> spp. (eschar): positive; <i>R. typhi</i> (eschar): negative <u>Conventional PCR and sequencing (eschar):</u> <i>Rickettsia</i> spp. 17kDa and <i>sca4</i> ; GenBank accession no: KF489455, KF489457	<u>Scrub typhus:</u> dynamic IgM/IgG 4-fold rise (1:3,200/1:12,800) <u>Murine typhus:</u> IgM/IgG static titers (<1:100; negative) <u>Spotted fever group:</u> IgM/IgG static titers (1:200; negative); <i>R. felis:</i> IgM/IgG static titers (<1:128; negative)	None
Male, 13 y, Salavan, southern Laos	Fever × 7 d, contact with cat, rat, and fleas 14 d before admission; HIV status: unknown	qPCR: <i>O. tsutsugamushi</i> (blood): negative; <i>Rickettsia</i> spp. (blood): positive <i>R. typhi</i> (blood): negative <u>Conventional PCR and sequencing (blood):</u> <i>Rickettsia</i> spp. 17 kDa GenBank accession no: KF489456	Data not available	<u>Malaria microscopy:</u> <i>P. falciparum</i> ; <u>Malaria rapid test:</u> <i>P. falciparum</i> <u>ICT Malaria Combo Cassette Test†:</u> <i>P. falciparum</i> PCR: positive; <u>Dengue fever reverse transcription qPCR:</u> positive <u>Dengue genotyping PCR:</u> serotype 4

*qPCR, quantitative PCR; CSF, cerebrospinal fluid; leukocyte; RFLP, restriction fragment length polymorphism; ICT, immunochromatographic test.

†Uni-Gold™ HIV, Trinity Biotech, Ireland; Alere Determine HIV-1/2 Ag/Ab Combo, Alere Medical, Japan.

‡ICT Diagnostics, Cape Town, South Africa.

elucidate exposure to this pathogen and unmask subclinical infections missed in fever etiology studies.

The 3 patients from Laos described herein had comorbidities associated with variable degrees of immunodeficiency (HIV infection and malaria with cellular and humoral deficiencies, diabetes with functional neutrophil/macrophage impairment) (7,8). *R. felis* infections have not been associated with immunosuppression, but few investigations of this possible association have been published. Of the 3 patients, the woman and the boy had other vectorborne infections: scrub typhus, transmitted by *Lep-totrombidium* mites, and *P. falciparum* malaria and dengue, transmitted by *Anopheles* and *Aedes* mosquitoes, respectively. Recent reports described *R. felis* within a great diversity of

vectors, including mites in South Korea and *Anopheles* and *Aedes* mosquitoes in Africa (9,10).

More work is needed on the role of non-flea vectors in transmission of *R. felis* and the consequences this may have in terms of mixed infections of diverse vectorborne pathogens. The rare detection of *R. felis* in patients, combined with high flea carriage rates, unusual signs and symptoms linked to immunodeficiencies or multiple infections, and reports from Africa describing *R. felis* in asymptomatic patients, underscore the need for further investigations into the organism's natural history and its uncertain role as a pathogen.

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Chikungunya Outbreak in Bueng Kan Province, Thailand, 2013

To the Editor: Chikungunya fever is a dengue-like syndrome characterized by acute fever, arthralgia, and maculopapular rash. The causative agent is chikungunya virus (CHIKV), which is transmitted by *Aedes aegypti* and *Aedes albopictus* mosquitoes (1). Based on the genome and the viral envelope E1 sequences, CHIKV is classified into 3 genetic lineages: Asian,

West African, and East/Central/South African (ECSA) genotypes (2).

In Thailand, the first report of CHIKV infection occurred in Bangkok in 1958 (3); later, sporadic cases of chikungunya fever occurred in many provinces during 1976–1995 (4). All of the CHIKV strains found in Thailand at that time were of the Asian genotype. The virus has since reemerged during 2008–2009 and caused large outbreaks in southern Thailand, affecting >50,000 persons (5). These outbreaks were attributed to the ECSA genotype. We report an outbreak of CHIKV infection in the northeastern province of Bueng Kan in 2013.

Bueng Kan Province is located on the Mekong River on the foothills of the mountainous region of Laos to the north. An outbreak of suspected dengue cases was reported during the rainy season during April–September 2013(6). Beginning in September, however, hospital physicians noticed that patients were reporting fever with moderate to severe joint pain resulting in limitation of movement that lasted for weeks. Serum samples were collected from 109 persons (hospitalized and outpatient) in October. Clinical data showed that 38 (34.9%) had moderate to severe joint pain; median duration of illness was 4 days (range 1–7). Median timing of sample collection from the onset of illness was 8 days (range 1–21).

Samples were sent to Chulalongkorn University Hospital in Bangkok to screen for mosquito-borne viruses. The study protocol was approved by the Institutional Review Board of Chulalongkorn University and consents were waived because all samples were stored as anonymous. Viral genomic RNA was assayed by using seminested reverse transcription PCR (RT-PCR) for CHIKV nucleic acid (7). Serum samples were tested for IgM against CHIKV by using SD BIO-LINE Chikungunya IgM Test (Standard Diagnostics Inc., Kyonggi-do,