Rickettsia felis in Fleas, Southern Ethiopia, 2010

To the Editor: Fleas (order Siphonaptera) are obligate hematophagous insects. They are laterally flattened, holometabolous, and wingless ectoparasites. More than 2,500 species of flea, belonging to 16 families and 238 genera, have been described. A minority of these genera live in close association with humans (synanthropic), including genera that live in close association with other species of arthropods, including Siphonaptera) are obligate vectors of protozoa (e.g., Yersinia pestis, Bartonella spp. [qPCR] that were specific for 2 species of flea (P. irritans and C. felis) based on the sequences of mitochondrial cytochrome oxidase gene published in GenBank (Table). All of these identifications made by morphologic appearance were confirmed by qPCR because some specimens were damaged and difficult to identify. We found that most (52/55) of the flea species Forward primer, 3′→5′ Reverse primer, 3′→5′ Fluorescent probe

| Species | Forward primer, 3′→5′ | Reverse primer, 3′→5′ | Fluorescent probe |
|---------|----------------------|----------------------|-------------------|
| P. irritans | CGGAATCTTTTTAGGGGAAAAACA | CATTCTTACTGGAGATTTTAGAGTGG | TTGCTTTACCTTCTTCTCCGACTTT |
| C. felis | TGTTATTTTTACTGAAAGACAAAAATG | TCATTCTTACTGGAGATTTTAGAGTGG | TTGCTTTACCTTCTTCTCCGACTTT |

*P. Pulex; C. Ctenocephalides.

In Africa, the presence of R. felis in fleas has been documented in Algeria, Tunisia, Egypt, Ethiopia, Gabon, Côte d’Ivoire, and the Democratic Republic of Congo. Recent studies conducted in Senegal (3) and Kenya (4) have shown that as much as 4.4% and 3.7%, respectively, of acute febrile diseases in these regions may be caused by R. felis infections. We conducted a study to determine the distribution and prevalence of R. felis in fleas in Ethiopia.

In our study, 55 fleas were collected in 2010 in 2 villages in Ethiopia; 25 fleas were collected from Tikemit Eshet (6°59′51″N and 35°35′07″E; altitude 1,700 m), and 30 fleas were collected from Mizan Teferi (6°59′64″N and 35°35′50″E; altitude 1,700 m). The specimens were collected by using a plate filled with soapy water and a candle in the middle of the plate. Because fleas are thermotrophic, they jumped toward the candle and fell onto the plate, where they rapidly drowned in the soapy water. The fleas were identified by morphologic features and stored in 90% ethanol until DNA extraction.

To confirm the phenotypic identification, we designed primers and probes for quantitative real-time PCR (qPCR) that were specific for 2 species of flea (P. irritans and C. felis) based on the sequences of mitochondrial cytochrome oxidase gene published in GenBank (Table). All of the identifications made by morphologic appearance were confirmed by qPCR because some specimens were damaged and difficult to identify. We found that most (52/55) of the fleas collected in human dwellings were P. irritans, and 3 specimens were C. felis. A screening by amplification using primers and probes specific for the 16S–23S internal transcribed spacer of Bartonella spp. (7) produced no positive results.

We screened rickettsial DNA by using qPCR with a Rickettsia-specific, gltA gene-based RKND03 system (8) and a bioB-based qPCR system specific for R. felis. We found that the 3 specimens of C. felis fleas contained the DNA of R. felis; however, 23 (43%) of 53 P. irritans specimens also contained DNA of R. felis. We amplified and sequenced nearly the entire rickettsial gltA gene from 3 C. felis and 10 P. irritans specimens and found that the sequence was identical to that of R. felis (GenBank accession no. NC_007111).

During the field collection of the fleas, the conservation of specimens may be difficult. Degradation of specimens may pose a problem for the ensuing morphologic identification. For fleas, a specific preparation is required that destroys internal organs and produces a chitin complex of the insect. This type of preparation makes it difficult, and sometimes impossible, to use the insect later for molecular studies. The development of qPCR specific for P. irritans and C. felis fleas facilitated the identification of damaged samples and also precluded the laborious and time-consuming procedure of identification by morphologic features.

We conclude that the reservoirs of R. felis in Ethiopia include both C. felis and P. irritans fleas. In Ethiopia, P. irritans fleas have been reported to be prevalent (9). P. irritans fleas have been shown to be infected by R. felis in several locations, notably in the Democratic Republic of the Congo. A screening by amplification using primers and probes specific for the 16S–23S internal transcribed spacer of Bartonella spp. (7) produced no positive results.

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Congo and in the United States, and another rickettsia phylogenetically similar to *R. felis* has been detected in *P. irritans* fleas in Hungary (10). Reports attributing substantial numbers of acute febrile illnesses to fleaborne rickettsioses caused by *R. felis* in Senegal and Kenya (3,4) place fleaborne rickettsiosis among emerging diseases with the potential for adverse public health effects. Furthermore, the identification of the vectors of *R. felis* in Ethiopia reveals the epidemiologic background for the fleaborne spotted fever in this region. We speculate that the elucidation of the full range of possible vectors of *R. felis* may facilitate the development of prevention measures that will help control this disease.

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Identification of Cause of Posttransplant Cachexia by PCR

To the Editor: A man, 56 years of age, was admitted to the hospital for epigastric pain, fever, and fatigue 8 years after a cardiac transplant. His immunosuppressive regimen consisted of cyclosporine A, mycophenolate mofetil, and steroids. Clinical examination revealed a 4-kg weight loss within 3 months without peripheral lymph node enlargement.

Laboratory test results showed moderate anemia, severe lymphopenia, and moderately increased C-reactive protein. Serologic results for HIV, *Brucella* spp., *Coxiella burnetii*, and *Francisella tularensis* were negative. Whole-body computed tomography scanning showed enlarged mediastinal and abdominal lymph nodes. Bone marrow histopathologic results ruled out lymphoma or granuloma but showed a histiocytic infiltrate and intracellular acid-fast bacilli (AFB) with positive Ziehl–Neelsen staining. Sputum, urine, gastric aspirates, and bronchoalveolar lavage specimens revealed no AFB. A mediastinal lymph node biopsy showed few AFB, suggesting *M. tuberculosis* or nontuberculous mycobacteria. Isoniazid, rifampin, ethambutol, and clarithromycin were prescribed for 2 months, followed by rifampin, ethambutol, and clarithromycin. Cultures for mycobacteria remained negative.

Five months after treatment initiation, the patient experienced severe abdominal pain, diarrhea, and continued weight loss. Lymph node biopsy was repeated; results showed intramacrophagic coccobacilli tinted with Ziehl-Neelsen, Gram, and periodic acid–Schiff (PAS) stains. Two diagnoses were considered: malakoplakia and Whipple disease (WD). Screening results from quantitative real-time PCR (qPCR) for *Tropheryma whipplei* were negative for blood, saliva, stools, urine, and lymph nodes.

Although no characteristic Michaelis–Gutmann bodies were seen, the staining characteristics of the intracellular coccobacilli were compatible with *Rhodococcus equi*, a pathogen associated with malakoplakia. Combined treatment with ertapenem, teicoplanin, and amikacin was implemented but failed to induce clinical improvement. Culture of the biopsy specimen failed to grow *R. equi* or mycobacteria, and...