VECTOR-BORNE AND ZOONOTIC DISEASES
Volume 19, Number 11, 2019

DOI: 10.1089/vbz.2018.2396

Arthropod-Borne Bacteria Cause Nonmalarial Fever in Rural Ethiopia: A Cross-Sectional Study in 394 Patients

Jose M. Ramos,[1,2] Ramón Pérez-Tanoira,[1,3] Inés Martín-Martín,[1,4] Laura Prieto-Pérez,[1,3] Abraham Tefasmariam,[1] Gebre Tiziano,[1] Raquel Escudero,[5] Judit Gil-Zamorano,[5] Horacio Gil-Gil,[5] Miguel Górgolas,[1,3,6] and Isabel Jado[5]

Abstract

Bacterial arthropod-borne pathogens are a common cause of fever in Africa, but their precise impact is unknown and usually underdiagnosed in the basic rural laboratories of low-resourced African countries. Our aim was to determine the prevalence of arthropod-borne bacterial diseases causing fever among malaria smear-negative patients in a rural hospital located in Ethiopia. The study population included patients aged 2 years or older; referred to Gambo Rural General Hospital (West Arsi, Ethiopia), between July and November 2013, for fever or report of fever in the previous 48 h; attending the outpatient department; and testing negative for malaria by Giemsa-stained thin blood smears. We extracted DNA from 394 whole blood samples, using reverse line blot assays of amplicons to look for bacteria from the genera: *Anaplasma*, *Bartonella*, *Borrelia*, *Coxiella*, *Ehrlichia*, *Francisella*, and *Rickettsia*. Thirteen patients showed presence of DNA for these pathogens: three each by *Borrelia* spp., the *Francisella* group (*F. tularensis tularensis*, *F. tularensis holartica*, and *F. novicia)*, *Rickettsia bellii*, and *Rickettsia Felis*, and one by *Bartonella rochalimae*. Thus, in this rural area of Africa, febrile symptoms could be due to bacteria transmitted by arthropods. Further studies are needed to evaluate the pathogenic role of *R. bellii*.

Keywords: *Rickettsia*, *Bartonella*, *Borrelia*, *Francisella*, fever, malaria

Introduction

After malaria, rickettsial diseases are widely considered to be one of the most important causes of systemic febrile illness in sub-Saharan Africa; however, the precise disease burden is largely unquantified (Freedman et al. 2006, Jensenius et al. 2009). The incidence and prevalence of rickettsioses diagnosis by DNA amplification in nonmalaria febrile illness (NMFI) in indigenous populations in sub-Saharan Africa are poorly studied, with most investigations taking place in Western Africa and Kenya. The prevalence of *Rickettsia* spp. in NMFI ranges from 1.5% to 10.5%, with *R. felis* predominating (Mourembou et al. 2015, Sothmann et al. 2017).

Since reclassifying the genus *Bartonella* in 1993, the number of species has ballooned to the current 45 members (Okaro et al. 2017). Infection by *Bartonella* may be associated with a wide range of clinical presentations, including chronic bacteremia, trench fever, bacillary angiomatosis, and endocarditis. Despite the evident interest of bartonellosis, there is still a need to know its prevalence in sub-Saharan Africa. In Senegal and South Africa, it was present in 2.5% of the NMFIs studied (Trataris et al. 2012, Diatta et al. 2014).

Borrelia causes fever in sub-Saharan Africa and *B. recurrentis*, louse-borne relapsing fever, produces sporadic illness and outbreaks especially in Ethiopia, Eritrea, Somalia, and Sudan. This bacterium has also been described in Europe among immigrants from sub-Saharan Africa (Hoch et al. 2015,

[1]Department of Medicine and Laboratory, Gambo Rural General Hospital, Kore, Ethiopia.
[2]Department of Internal Medicine, Hospital General Universitario de Alicante, Universidad Miguel Hernandez, Alicante, Spain.
[3]Division of Infectious Diseases, IIS-Fundación Jiménez Díaz, Madrid, Spain.
[4]Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, Maryland.
[5]Laboratory of Special Pathogens of the National Microbiology Center of Spain, Institute of Health Carlos III, Madrid, Spain.
[6]Department of Medicine, Universidad Autónoma de Madrid, Madrid, Spain.

Zammarchi et al. 2016). The genus *Francisella* is a highly virulent intracellular Gram-negative bacterium, classified into different species, mainly *F. turalensis* and *F. novicida*. The associated infections are transmitted by direct contact with animals and their products. Also, the lice can transmit them. *F. tularensis* has been classified as a biothreat; of its four subspecies, *F. tularensis* subsp. *tularensis* and *F. tularensis* subsp. *holarctica* are responsible for tularemia in humans (Njeru et al. 2017).

The role of this microorganism in infection in Africa is poorly understood and usually underdiagnosed in the basic rural laboratories of low-resourced African countries. We performed this study to fill some of the large gaps in knowledge of arthropod-borne pathogens as a cause of nonmalarial fever in sub-Saharan Africa. We aimed to determine the bacterial species causing zoonoses and belonging to the genera *Anaplasma*, *Bartonella*, *Borrelia*, *Coxiella*, *Ehrlichia*, *Francisella*, and *Rickettsia* in NMFI in rural Ethiopia.

Materials and Methods

The study took place in Gambo Rural General Hospital (GRH), situated in the province of West Arsi, Ethiopia, 245 km southeast of the capital, Addis Ababa, at an altitude of 2250 meters (~7382 feet) above sea level (7°18′22.4″N+ 38°48′54.7″E). GRH serves 11 municipalities (or ''kebeles''), whose population is estimated to be 100,000 inhabitants. The temperature varies between 13°C and 30°C, with varying levels of precipitation, concentrated from June to October. Subsistence farming and animal husbandry are the residents' major occupations.

Clinical sample collection

The study was prospective and clinical sample collection was performed between July 1 and November 30, 2013. The study population included patients aged 2 years or older; referred to the hospital laboratory for fever (axillary temperature >37.5°C) or report of fever in the previous 48 h; attending the outpatient department; and testing negative for malaria by Giemsa-stained thin blood smears, with no malaria trophozoites or gametocytes identified by microscopic analysis. The differential diagnosis in study participants with microscopy-negative malaria considered short febrile illnesses, including a relapsing fever, typhus, typhoid fever, and meningitis. These were treated with tetracycline hydrochloride or chloramphenicol in accordance with Ethiopian national guidelines. We collected the main symptoms, signs, and analytical variables from patients with NMFI.

A medical attendant examined each individual with fever, collecting whole blood (three to four drops) from the fingertip by lancet stick and preserving samples on filter paper (Whatman 3 MM) for molecular assay.

DNA extraction

Samples included in the study were received by the Laboratory of Special Pathogens of the National Microbiology Center of Spain (Institute of Health Carlos III, Madrid, Spain) for diagnostic purposes. DNA was extracted from blood using the QIAamp Tissue Kit (QIAGEN, Hilden, Germany), according to the manufacturer's instructions. A previous overnight treatment with a proteinase K solution (20 mg/mL) and a final step of 15 min at 100°C for inactivation of the protease were included. The DNA concentration was measured using the NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE).

Molecular detection

Around 200 ng of DNA from each sample was analyzed with routine in-house single PCRs with specific biotinylated primers followed by a RLB for the individual detection of *Anaplasma* spp., *Bartonella* spp., *Borrelia* spp., *Coxiella burnetii*, *Francisella* spp., and *Rickettsia* spp.

In brief, PCR amplifications for each bacteria genus were carried out individually using biotinylated primers in the MJ Research PCT-200 (Ecogen, Barcelona, Spain) as described previously (Jado et al. 2006). Subsequently, an RLB was performed hybridizing PCR products to specific probes with a C6 amino-link modification and detected using immunoenzymatic chemiluminescence (Super Signal West Dura Extended Duration Substrate; Pierce Biotechnology, Rockford, IL) as previously described (Jado et al. 2006). The sensitivity of the PCR-RLBs was assessed by amplifying 10^2, 10, and 1 genome equivalents for *Bartonella*, *Borrelia*, *C. burnetii*, and *Francisella* or plasmid copies with inserts of synthetic DNA for the detection of *Anaplasma* and *Rickettsia* in spiked distilled water (Jado et al. 2006, Toledo et al. 2009). After multiplex PCR combined with RLB, a conventional nested PCR and sequencing of at least one informative standard amplicon for each genus-specific were performed. These amplicons can be found in previous studies performed by Garcia-Esteban et al. (2008) and Jado et al. (2006). In summary, for the detection of *Bartonella* spp., a multiplex PCR to amplify the 16S rRNA and the hypervariable intergenic transcribed spacer 16S-23S rRNA (ITS) was carried out. The probe designed for the 16S rRNA is generic and allows the detection of *Bartonella* spp., whereas for species identification, 17 species-specific probes were designed for the 16S-23S rRNA (Garcia-Esteban et al. 2008).

For the detection of *Borrelia* spp., a generic probe and primers were designed to target the 16S rRNA. Subsequently, positive samples were subjected to a second PCR to amplify the 5S (rrf)–23S (rrl) intergenic spacer, genospecies were determined by using specific probes (Rijpkema et al. 1995). For the identification of *C. burnetii*, the transposase *IS1111* was targeted (Toledo et al. 2009). To identify *Francisella* spp., the *lpnA* gene was amplified (Escudero et al. 2008). Lastly, *Rickettsia* was detected by amplifying a fragment of the 23S-5S rRNA intergenic spacer that allocates generic and specific probes, allowing the differentiation of the spotted fever and typhus groups, as well as the identification of *Rickettsia* at the species level (Jado et al. 2006).

Statistical analysis

We entered data into a spreadsheet using Microsoft Excel 2011 and analyzed the data using IBM SPSS statistical software, version 22.0 (SPSS, Inc., Chicago, IL). To analyze the association of categorical variables and the presence of pathogen DNA, we used either the chi-square test with Yates' correction or Fisher's exact test, where appropriate. To analyze continuous variables (age and white blood cell counts), we used the Mann–Whitney *U*-test, as data did not follow a normal distribution. We considered p values of <0.05 to be statistically significant. We obtained the estimates of prevalence along with their 95% CIs.

Ethics approval and consent to participate

The Research and Publication Committee of the GRH, and the Health Unit and Ethical Review Committee of the Ethiopian Catholic Secretary (GH/MSMHF/709), approved the study protocol, which conformed to the ethical guidelines of the Declaration of Helsinki. Given the high rates of illiteracy in the population, we deemed written consent to be unfeasible. Therefore, we obtained oral informed consent from each participant, who took part on a voluntary basis and freely provided information to complete a comprehensive epidemiological and clinical questionnaire. The next of kin, caretaker, or guardians provided oral consent on behalf of participating minors/children enrolled in the study, and this was recorded on the questionnaire as well. Participants received no economic compensation for their role in the study. All the data were treated confidentially and anonymized.

Availability of data and material

The data sets used and/or analyzed during this study are available from the corresponding author on reasonable request.

Results

Among the 394 patient samples studied, 13 (3.3%, 95% confidence interval [CI]: 1.8–5.7) were positive for arthropod-borne disease. We isolated three cases each of *Borrelia* spp., the *Francisella* group (*F. turalensis tularensis*, *F. tularensis holartica*, and *F. novicia*), *Rickettsia bellii*, and *R. felis* (0.8%; 95% CI: 0.02%–2.4%), and one case of *Bartonella rochalimae* (0.3%; 95% CI: 0.01%–1.6%). The sequence of the positive *Bartonella* was 100% identical to *B. rochalimae* (GenBank accession no. MK693114). The sequence of *R. bellii* was 98% identical for two patients and 97% identical for one patient (GenBank accession nos. MK693110 and MK693111, respectively). The sequence of *R. felis* was 100% identical for two patients (GenBank accession nos. MK693112 and MK693113).

Table 1 gives the epidemiological, clinical, and analytical characteristics of patients with and without positive amplification of DNA in blood. In patients with amplification of DNA, the following symptoms are significantly more common: generalized pain (100% vs. 18.6%; $p < 0.001$), arthralgia–myalgia (69.2% vs. 18.4%; $p < 0.001$), skin rash (38.5% vs. 3.9%; $p < 0.001$), headache (92.3% vs. 51.4%; $p = 0.004$),

Table 1. Epidemiological, Clinical, and Analytical Characteristics of Patients

	DNA amplification		
	Negative (n = *381**)	*Positive* (n = *13*[†])	p
Age			
Median (IQR)	21 (10–35)	35 (23.5–39)	0.083
Range	2–70	2–50	
<15 years old	194 (66.2)	9 (81.8)	0.348
Gender, female	208 (54.6)	9 (69.2)	0.297
Symptoms			
Headache	196 (51.4)	12 (92.3)	0.004
Nausea	113 (29.7)	3 (23.1)	0.763
Shivers	35 (9.2)	3 (32.1)	0.120
Generalized pain	71 (18.6)	13 (100)	<0.001
Chest pain	48 (12.6)	2 (15.4)	0.674
Abdominal pain	149 (39.1)	5 (38.5)	0.963
Stomach burning	70 (18.4)	4 (30.8)	0.260
Vomiting	27 (7.1)	4 (30.8)	0.014
Difficulty of moving	21 (5.5)	2 (15.4)	0.172
Dyspnea	6 (1.6)	1 (7.7)	0.211
Sweating	52 (13.6)	5 (38.5)	0.027
Coughing	123 (32.3)	7 (53.8)	0.133
Arthralgia–myalgia	70 (18.4)	9 (69.2)	<0.001
Diarrhea	7 (12.3)	2 (15.49	0.669
Astenia	70 (18.4)	1 (7.7)	0.478
Skin rash	15 (3.9)	5 (38.5)	<0.001
Signs			
Hepatomegaly	5 (1.3)	0 (0.0)	1.0
Splenomegaly	2 (0.5)	0 (0.0)	1.0
Laboratory			
Hb, median (IQR)	13 (11.4–14.3)	12.4 (11.5–13.7)	0.615
WBC, median (IQR)	7.4 (5.7–10.4)	7.9 (6.1–9.0)	0.675
Outcome			
Hospitalization	51 (13.4)	1 (7.7)	1.0

All values are presented as *n* (%) unless stated otherwise.
*Missing values for participants testing negative: age, $n = 88$; hemoglobin, $n = 44$; white blood count, $n = 110$.
[†]Missing values for participants testing positive: age, $n = 2$; hemoglobin, $n = 1$; white blood count, $n = 7$.
Hb, hemoglobin; IQR, interquartile range; WBC, white blood cells.

vomiting (30.8% vs. 7.1%; $p=0.014$), and sweating (38.5% vs. 13.6%; $p=0.027$).

The presence of DNA from *Borrelia* spp. was statistically associated with generalized pain (100%; $p=0.007$) and arthralgia–myalgia (100%; $p=0.007$). Likewise, amplification of *R. bellii* was significantly associated with generalized pain (100%; $p=0.007$), arthralgia–myalgia (100%; $p=0.007$), and skin rash (66.7%; $p=0.05$). Positive PCR of *R. felis* was associated with generalized pain (100%; $p=0.007$) and difficulty moving (66.7% vs. 5.5%; $p=0.010$). And finally, positive PCR results for the *Francisella* group were statistically associated with generalized pain (100%; $p=0.007$), shivers (66.7% vs. 9.2%; $p=0.026$), chest pain (66.7% vs. 12.6%; $p=0.046$), and sweating (66.7% vs. 13.6%; $p=0.05$) (Table 2).

Discussion

In our study, we found DNA from *Bartonella*, *Borrelia*, *Francisella*, and *Rickettsia* in malaria-negative febrile patients attending a rural hospital of Ethiopia. These bacteria are transmitted by arthropod vectors such as lice, fleas, and ticks.

In Ethiopia, *Bartonella* has been found in body and head lice and reported as an infrequent cause of endocarditis in children (Cutler et al. 2012, Tasher et al. 2017). In our series, we found *B. rochalimae* in an outpatient with unknown clinical evolution. To our knowledge, this species has only been described in human clinical infection in one patient returning to the United States from Peru, after receiving multiple insect bites and showing fever, myalgia, nausea, insomnia, anemia, and splenomegaly (Eremeeva et al. 2007). *B. rochalimae* has also been detected in dogs, wild carnivores, rats, and fleas in different parts of the Americas and Europe (Chomel et al. 2009, Diniz et al. 2013).

Louse-borne relapsing fever is responsible for 2.5%–4.5% of febrile episodes in some areas of Ethiopia, and outbreaks incur a severe impact, with mortality rates of 30%–70% (Yimer et al. 2014a, 2014b, Hoch et al. 2015). The organisms belonging to *Borrelia* spp. and identified by 16S rRNA sequencing were associated with febrile episodes, generalized pain, and myalgia. However, there were no hemorrhagic symptoms, contrary to classical clinical manifestations described for this microorganism (Yimer et al. 2014a, 2014b, Hoch et al. 2015).

We found two patients infected by *F. tularensis holarctica* and one patient with a different species of *Francisella*, whose identification was totally compatible with *F. novicida* and *F. hispaniensis*. *F. hispaniensis* was first isolated from a human foot wound in Australia in 2003 (Sjodin et al. 2012). Molecular detection of the *Francisella* group was associated with generalized pain, shivers, chest pain, and sweating. In Kenya, Njeru et al. (2017) found antibodies to *F. tularensis* in 3.7% of their 730 febrile patients. In Sudan, a case report described bacteremia due to *F. turalensis* (Kugeler et al. 2008, Mohamed et al. 2012). In Ethiopia, *Francisella*-like endosymbionts and other atypical *Francisella* have been described in the *Hyalomma rufipes* tick and from human fluids, respectively (Kugeler et al. 2008, Szigeti et al. 2014).

Rickettsia felis has also been reported to be a cause of fever in sub-Saharan Africa, but this association has not been dealt with in depth in Ethiopia (Brown and Macaluso 2016). We found *R. felis* in <1% of the samples, a lower prevalence compared with other studies in Africa, which report a prevalence ranging from 1.5% to 10% of the NMFI (Socolovschi et al. 2010, Mourembou et al. 2015, Sothmann et al. 2017). In our study, *R. felis* was associated with generalized pain and headache. We also found three cases of *Rickettsia bellii*, a microorganism infecting argasid and ixodid tick species, aphids, and whiteflies throughout North and South America, although it has never been identified as a human pathogen (Hecht et al. 2016, Krawczak et al. 2018). There is little research on this microorganism in Africa, although Aarsland *et al*. (2012) did find DNA of *Rickettsia* spp. in 2.9% of 102 children with fever in Ethiopia.

Although we have identified *R. bellii* with 98% of sequence in two patients and 97% in the other, and *B. rochalimae* with sequence of 100% in one case by PCR-reverse line blot assay (RLB), a conventional PCR and sequencing of at least one informative standard amplicon for each genus-specific were performed (Jado et al. 2006, Garcia-Esteban et al. 2008), this finding should be investigated in other studies and in other places in Africa.

Arboviruses are another important source of NMFI. Although there is no literature on coinfection between arbovirus and NMFI, this cannot be excluded. Further studies analyzing arbovirus transmitted by arthropods in NMFI in Africa are warranted.

This study has two main limitations. The first is the absence of a control group of patients without fever, which

Table 2. Symptoms Showing Significant Differences with Group Testing Negative for Bacterial Infection, by Bacterium of Infection

	Bacterium							
Symptom	Borrelia *spp.* (n = *3*)	p*	Rickettsia bellii (n = *3*)	p*	Rickettsia felis (n = *3*)	p*	Francisella *group* (n = *3*)	p*
Shivers	1 (33.3)	0.256	0 (0.0)	1.0	0 (0.0)	1.0	2 (66.7)	*0.026*
Generalized pain	3 (100)	*0.007*	3 (100)	*0.007*	3 (100)	*0.007*	3 (100)	*0.007*
Chest pain	0 (0.0)	1.0	0 (0.0)	1.0	0 (0.0)	1.0	2 (66.7)	*0.046*
Difficulty moving	0 (0.0)	1.0	0 (0.0)	1.0	2 (66.7)	*0.010*	0 (0.0)	1.0
Dyspnea	0 (0.0)	1.0	0 (0.0)	1.0	1 (33.3)	0.054	0 (0.0)	1.0
Sweating	1 (33.3)	0.361	1 (33.3)	0.361	2 (66.7)	*0.053*	1 (33.3)	0.361
Arthralgia–myalgia	3 (100)	*0.007*	3 (100)	*0.007*	1 (33.3)	0.459	2 (66.7)	0.092
Skin rash	1 (33.3)	0.120	2 (66.7)	*0.050*	1 (33.3)	0.120	1 (33.3)	0.120

*Comparison with samples negative for DNA amplification.
Italic values $p<0.05$.

would allow us to know whether the presence of DNA for these pathogens actually represents a symptomless infection. The use of controls could uncover carriers of well-known pathogens, so it is not easy to interpret data about the potential pathogenic role of these microorganisms. Findings of these arthropod-borne bacteria in febrile versus afebrile people are not well characterized. The second important limitation is the poor information about patient outcomes, which is evidence of the difficulty of collecting data in these rural settings.

Our results showed that nucleic acid amplification tests are highly sensitive and can potentially provide more accurate information on the epidemiology of arthropod-borne pathogens in sub-Saharan Africa, where the lack of molecular tools limits the management of all febrile illnesses in these areas. It also suggests that previously ignored organisms, such as *Rickettsia*, *Bartonella*, *Francisella*, and *Borrelia*, should be considered in empiric therapies. These tests could allow a shift in practice from empiric abuse of malarial and antibiotic treatment to more informed decision-making on treatment.

Acknowledgments

We thank all the members of the laboratory and clinical staff at Gambo Rural General Hospital. The study was funded by the Master of Tropical Diseases and International Health of the Department of Medicine, at the Autonomous University of Madrid. Special mention goes to Dr. Francisco Reyes, the managing director of Gambo Hospital, for his support during the study period.

Consent for Publication

The publication of the article was accepted by all authors and by the responsible authorities where the work was carried out, and it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright holder.

Authors' Contributions

J.M.R., R.P.T., M.G., and I.J. were responsible for conception and design of the study. R.P.T., I.M.M., L.P.P., A.T., and G.T. collected the material and data. R.P.T., I.M.M., G.T., R.E., J.G.Z., H.G.G., and I.J. were responsible for the laboratory procedures. J.M.R., R.P.T., L.P.P., M.G., and I.J. were responsible for the logistics and organization of the network. J.M.R., R.P.T., and I.J. interpreted the data. J.M.R. and R.P.T. wrote the main part of the article. All authors read and approved the final version of the article.

Authors' Disclosure Statement

The authors declare that they have no conflict of interests.

References

Aarsland SJ, Castellanos-Gonzalez A, Lockamy KP, Mulu-Droppers R, et al. Treatable bacterial infections are underrecognized causes of fever in Ethiopian children. Am J Trop Med Hyg 2012; 87:128–133.

Brown LD, Macaluso KR. *Rickettsia felis*, an emerging flea-borne rickettsiosis. Curr Trop Med Rep 2016; 3:27–39.

Chomel BB, Henn JB, Kasten RW, Nieto NC, et al. Dogs are more permissive than cats or guinea pigs to experimental infection with a human isolate of *Bartonella rochalimae*. Vet Res 2009; 40:27.

Cutler S, Abdissa A, Adamu H, Tolosa T, et al. *Bartonella quintana* in Ethiopian lice. Comp Immunol Microbiol Infect Dis 2012; 35:17–21.

Diatta G, Mediannikov O, Sokhna C, Bassene H, et al. Prevalence of *Bartonella quintana* in patients with fever and head lice from rural areas of Sine-Saloum, Senegal. Am J Trop Med Hyg 2014; 91:291–293.

Diniz PP, Morton BA, Tngrian M, Kachani M, et al. Infection of domestic dogs in peru by zoonotic *Bartonella* species: A cross-sectional prevalence study of 219 asymptomatic dogs. PLoS Negl Trop Dis 2013; 7:e2393.

Eremeeva ME, Gerns HL, Lydy SL, Goo JS, et al. Bacteremia, fever, and splenomegaly caused by a newly recognized *Bartonella* species. N Engl J Med 2007; 356:2381–2387.

Escudero R, Toledo A, Gil H, Kovacsova K, et al. Molecular method for discrimination between *Francisella tularensis* and Francisella-like endosymbionts. J Clin Microbiol 2008; 46: 3139–3143.

Freedman DO, Weld LH, Kozarsky PE, Fisk T, et al. Spectrum of disease and relation to place of exposure among ill returned travelers. N Engl J Med 2006; 354:119–130.

Garcia-Esteban C, Gil H, Rodriguez-Vargas M, Gerrikagoitia X, et al. Molecular method for *Bartonella* species identification in clinical and environmental samples. J Clin Microbiol 2008; 46:776–779.

Hecht JA, Allerdice ME, Krawczak FS, Labruna MB, et al. Development of a *Rickettsia bellii*-specific TaqMan assay targeting the citrate synthase gene. J Med Entomol 2016; 53: 1492–1495.

Hoch M, Wieser A, Loscher T, Margos G, et al. Louse-borne relapsing fever (*Borrelia recurrentis*) diagnosed in 15 refugees from northeast Africa: Epidemiology and preventive control measures, Bavaria, Germany, July to October 2015. Euro Surveill 2015; 20:1–5.

Jado I, Escudero R, Gil H, Jimenez-Alonso MI, et al. Molecular method for identification of *Rickettsia* species in clinical and environmental samples. J Clin Microbiol 2006; 44:4572–4576.

Jensenius M, Davis X, von Sonnenburg F, Schwartz E, et al. Multicenter GeoSentinel analysis of rickettsial diseases in international travelers, 1996–2008. Emerg Infect Dis 2009; 15:1791–1798.

Krawczak FS, Labruna MB, Hecht JA, Paddock CD, et al. Genotypic characterization of *Rickettsia bellii* reveals distinct lineages in the United States and South America. Biomed Res Int 2018; 2018:8505483.

Kugeler KJ, Mead PS, McGowan KL, Burnham JM, et al. Isolation and characterization of a novel *Francisella* sp. from human cerebrospinal fluid and blood. J Clin Microbiol 2008; 46:2428–2431.

Mohamed SE, Mubarak AI, Alfarooq LO. *Francisella tularensis* Bacteremia: A case report from Sudan. Case Rep Infect Dis 2012; 2012:1–2.

Mourembou G, Lekana-Douki JB, Mediannikov O, Nzondo SM, et al. Possible role of *Rickettsia felis* in acute febrile illness among children in Gabon. Emerg Infect Dis 2015; 21: 1808–1815.

Njeru J, Tomaso H, Mertens K, Henning K, et al. Serological evidence of *Francisella tularensis* in febrile patients seeking treatment at remote hospitals, northeastern Kenya, 2014–2015. New Microbes New Infect 2017; 19:62–66.

Okaro U, Addisu A, Casanas B, Anderson B. *Bartonella* species, an emerging cause of blood-culture-negative endocarditis. Clin Microbiol Rev 2017; 30:709–746.

Rijpkema SG, Molkenboer MJ, Schouls LM, Jongejan F, et al. Simultaneous detection and genotyping of three genomic groups of Borrelia burgdorferi sensu lato in Dutch *Ixodes ricinus* ticks by characterization of the amplified intergenic spacer region between 5S and 23S rRNA genes. J Clin Microbiol 1995; 33:3091–3095.

Sjodin A, Svensson K, Ohrman C, Ahlinder J, et al. Genome characterisation of the genus Francisella reveals insight into similar evolutionary paths in pathogens of mammals and fish. BMC Genomics 2012; 13:268.

Socolovschi C, Mediannikov O, Sokhna C, Tall A, et al. *Rickettsia felis*-associated uneruptive fever, Senegal. Emerg Infect Dis 2010; 16:1140–1142.

Sothmann P, Keller C, Krumkamp R, Kreuels B, et al. *Rickettsia felis* Infection in febrile children, Ghana. Am J Trop Med Hyg 2017; 96:783–785.

Szigeti A, Kreizinger Z, Hornok S, Abichu G, et al. Detection of Francisella-like endosymbiont in *Hyalomma rufipes* from Ethiopia. Ticks Tick Borne Dis 2014; 5:818–820.

Tasher D, Raucher-Sternfeld A, Tamir A, Giladi M, et al. *Bartonella quintana*, an unrecognized cause of infective endocarditis in children in Ethiopia. Emerg Infect Dis 2017; 23: 1246–1252.

Toledo A, Jado I, Olmeda AS, Casado-Nistal MA, et al. Detection of *Coxiella burnetii* in ticks collected from Central Spain. Vector Borne Zoonotic Dis 2009; 9:465–468.

Toledo A, Olmeda AS, Escudero R, Jado I, et al. Tick-borne zoonotic bacteria in ticks collected from central Spain. Am J Trop Med Hyg 2009; 81:67–74.

Trataris AN, Rossouw J, Arntzen L, Karstaedt A, et al. *Bartonella* spp. in human and animal populations in Gauteng, South Africa, from 2007 to 2009. Onderstepoort J Vet Res 2012; 79:452.

Yimer M, Abera B, Mulu W, Bezabih B, et al. Prevalence and risk factors of louse-borne relapsing fever in high risk populations in Bahir Dar city Northwest, Ethiopia. BMC Res Notes 2014a; 7:615.

Yimer M, Mulu W, Ayalew W, Abera B. Louse-borne relapsing fever profile at Felegehiwot referral hospital, Bahir Dar city, Ethiopia: A retrospective study. BMC Res Notes 2014b; 7:250.

Zammarchi L, Antonelli A, Bartolini L, Pecile P, et al. Louse-borne relapsing fever with meningeal involvement in an immigrant from Somalia to Italy, October 2015. Vector Borne Zoonotic Dis 2016; 16:352–355.

Address correspondence to:
Ramón Perez-Tanoira
Division of Infectious Diseases
IIS-Fundación Jiménez Díaz
Av. Reyes Católicos, 2
Madrid 28040
Spain

E-mail: ramontanoira@hotmail.com