Occurrence and Genotyping of Coxiella burnetii in Ixodid Ticks in Oromia, Ethiopia

Bersissa Kumsa, Cristina Socolovschi, Lionel Almeras, Didier Raoult, and Philippe Parola

Unité de Recherche en Maladies Infectieuses et Tropicales Emergentes (URMITE; UM63, CNRS 7278, IRD 198, Inserm 1095), Aix Marseille Université, Faculté de Médecine, Marseille, France

Abstract. This study was conducted from September 2011 to March 2014 to address the occurrence and genotypes of Coxiella burnetii using molecular methods in ticks collected from domestic animals in Ethiopia. Ticks were tested for C. burnetii by quantitative real-time polymerase chain reaction (qPCR) targeting two different genes followed by multispacer sequence typing (MST). An overall prevalence of 6.4% (54/842) of C. burnetii was recorded. C. burnetii was detected in 28.6% (14/49) of Amblyomma gemma, 25% (31/124) of Rhipicephalus pulchellus, 7.1% (1/14) of Hyalomma marginatum rufipes, 3.2% (2/62) of Am. variegatum, 3.1% (4/128) of Am. cohaerens, 1.6% (1/63) of Rh. praetextatus, and 0.6% (1/153) of Rhipicephalus (Boophilus) decoloratus. Significantly higher overall frequencies of C. burnetii DNA were observed in Am. gemma and Rh. pulchellus than in other tick species (Mantel–Haenszel [MH], P < 0.0001). The overall frequency of C. burnetii was significantly higher (MH, P < 0.0001) in ticks from southeastern districts (Arero, Moyale, and Yabelo) than that from other districts. This study demonstrated the presence of C. burnetii genotype MST 18 in ticks in southeastern districts and genotype MST 20 in ticks in central districts. This study highlights the importance of ticks in the epidemiology of C. burnetii in Ethiopia.

INTRODUCTION

Ticks are one of the major causes of health problems in both humans and animals.1–3 Ticks are vectors and reservoirs for several emerging and reemerging infectious pathogens of medical and veterinary importance:4–6 In Ethiopia, ticks are widely distributed in all agro-ecological zones.4 Most previously published data on ticks in Ethiopia focus on species distribution, and thus only a very few molecular studies have been conducted on the occurrence of pathogens in ticks in the country.5

Coxiella burnetii, the causative agent of Q fever, is a small (3–5 μm) polymorphic obligate intracellular gram-negative bacteria belonging to the genus Coxielia, family Coxiellaceae, order Legionellales, class Gammaproteobacteria, and phylum Proteobacteria.6,7 C. burnetii mainly affects macrophages.7 It is able to build highly infective spore-like forms that are resistant to environmental influences and therefore can stay infective for several months.6 Thus, the bacterium is highly resistant to environmental stresses, such as high temperature, osmotic pressure, and ultraviolet light. It can also survive standard disinfectants and is resistant to many other environmental factors.6 Because of its cosmopolitan distribution, high resistance to environmental stresses, aerosol route of infection, nonspecific nature of its symptoms and clinical signs, and low infectious dose (1–10 bacteria), C. burnetii is considered a potential bioterrorist agent.7

In humans, C. burnetii causes nonpathognomonic clinical signs ranging from asymptomatic or mildly symptomatic to fatal disease.5,6,7 Acute Q fever can be asymptomatic or it can manifest as atypical pneumonia, granulomatous hepatitis, or appear as a self-limited flu-like syndrome.8 Syndromes, such as endocarditis, osteomyelitis, septic arthritis, infected aortic aneurysms, chronic hepatitis, or chronic pneumonia, have been described as the chronic type of Q fever.6 In pregnant woman, infection may lead to premature delivery or abortion.8 Farmers, veterinarians, abattoir and dairy workers, and laboratory technicians are populations that are at high risk of contracting infection as an occupational zoonosis.10 Although inhalation of C. burnetii is considered the main means of infection in humans, consumption of raw milk and milk products, transplacental infection, intradermal inoculation, blood transfusion, and contact with urine, feces, and semen of infected animals are also regarded as possible routes of infection.6,8 Excretion of C. burnetii by domestic ruminants is implicated as a major cause of environmental contamination and the source for human infection.6,8

In animals, C. burnetii is known to cause infection in a wide range of species, including ruminants, companion animals, birds, and reptiles.10 However, domestic ruminants are implicated as the common source of infection for humans. As is the case of humans, infections in animals are generally asymptomatic.10 In small ruminants, C. burnetii causes reproductive disorders, such as abortions, premature delivery, delivery of weak offspring, stillbirth in the final stage of gestation, postpartum metritis, and infertility.10,11 In cattle, C. burnetii generally causes less obvious clinical signs, but recent studies have shown that abortion and irregular repeat breeding, metritis, and infertility are important risk indicators.11 In ruminants, C. burnetii is shed via birth fluids and the placenta during normal parturition or abortion and in vaginal mucus, milk, feces, urine, and semen.11 Inhalation or ingestion of C. burnetii with contaminated feed and water from the environment is the major source of infection in animals.10,11

Hard and soft ticks are one of the most important arthropods known to be naturally infected with C. burnetii.6,7,9 So far, C. burnetii has been reported in more than 40 different tick species in several countries.9 Ticks acquire C. burnetii during a blood feeding on infected animals and can transmit the bacterium to other mammals during the next blood meal or by aerogenic spread of dried tick fecal excretions, thus playing a role in the maintenance of C. burnetii in the environment.11 In infected ticks, C. burnetii multiplies in the middle gut cells resulting in high titers of viable organisms that are expelled with feces.7 Ticks are considered as important reservoirs and potential vectors due to both transstadial and transovarial transmission of C. burnetii to their offspring in some tick species.6,7 Furthermore, it has been postulated that there is an increase in the virulence of C. burnetii after passage through ticks.9 Ticks play an important role in the
epidemiology of Q fever by contaminating the environment by excreting *C. burnetii* via their feces, saliva, and coxal fluids.6,7 However, no evidence is available reporting the transmission of *C. burnetii* to humans by blood-feeding ticks.7 Recently, *C. burnetii* DNA has been detected in 8.3% of *Ornithodoros sonrai* in Senegal,12 in fleas in Cyprus13 and Egypt,14 and in biting flies in the United States.15

In sub-Saharan African countries, including Ethiopia, the importance of *C. burnetii* as a cause of febrile illness in humans is poorly understood due to the lack of capacity of developed laboratories.16 Included in the few studies from African countries are reports of antibodies against *C. burnetii* in the sera of 1–24% of humans in 7 west African countries:17 30.9% of humans, 28.3% of cattle, 18.2% of sheep and 32% of goats in Kenya,18 24.2% of goats and 18.5% of sheep in Gambia,19 *C. burnetii* DNA in the blood of 7.8% of cattle and 7.5% of goats in Tanzania,20 and 0.3% of febrile patients in Algeria and 0.4% in Senegal.21 A previous study performed nearly half a century ago in Ethiopia reported the detection of *C. burnetii* in 5.3% of *Amblyomma variegatum* and 10.8% of *Hyalomma truncatum* ticks.22 More recently, an epidemiological study highlighted the seroprevalence of *C. burnetii* in 31.6% of cattle, 90% of camels, and 54.2% of goats in eastern Ethiopia.23

Genotypic characterization of *C. burnetii* is a very important information for epidemiological investigation of Q fever outbreaks and is necessary to determine the epidemiological link between the source of the outbreak, human cases, and hosts involved in the life cycle.24 Multispacer sequence typing (MST) is one of the more recently developed techniques to genotype and characterize *C. burnetii* strains and is known to be reliable, reproducible, and possesses high discriminatory power.24–26

To obtain better information about the involvement of tick species as reservoirs or vectors of Q fever, in this study, we aimed to determine the occurrence and genotype of *C. burnetii* in different tick species collected from domestic animals in different agro-ecological zones and animal management systems in nine districts in the Oromia Regional State of Ethiopia.

**MATERIALS AND METHODS**

**Study areas and animals.** Ixodid ticks were collected from cattle, sheep, dogs, and cats in the Arsi, Wolmara, Kimbibit Ada’a, Abdela, Gachi, Arero, Moyale, and Yabelo districts in Oromia as described in a previous publication.5 These districts are located in six zones of the central, southwestern, and southeast parts of Ethiopia with various climates and agro-ecological and animal management systems (Figure 1). Livestock comprising cattle, goats, sheep, and camels in Arero, Moyale, and Yabelo districts (Borana zone) are kept under a pastoral type of animal management.23,27 This system is characterized by very large animal populations, and livestock are the main source of human food from milk and milk byproducts and the main source of income for farmers, whereas in all other study districts, animals, including cattle, sheep, goats, and equines, are kept under a crop–animal mixed type of management with extensive production systems.23,27

**Collection and identification of ticks.** Ticks were collected from September through November 2011. All the body surfaces of each study animal were thoroughly examined visually to establish the presence or absence of ticks.5 Ticks attached to the skin of each animal were carefully removed manually using forceps or by hand to avoid any damage to the body and placed into separate, prelabeled, small, plastic tubes containing 70% ethanol for subsequent identification as previously...
described. Ticks from the same animal were put in the same vial and transported to the laboratory (URMITE, Marseille, France). Ticks were morphologically identified under a microscope using identification keys described previously. Species determination was possible for adult specimens, whereas identification to the genus level was performed for larvae and nymphs. For each tick specimen, the sex and developmental stage was determined and a photograph of the dorsal and ventral body parts was captured. Tick genera are abbreviated as has been described previously.

DNA extraction from ticks. Before DNA extraction, each tick specimen was rinsed twice in sterile water for 15 minutes and then dried on sterile filter paper. Each specimen was longitudinally cut into two equal halves. One half of each specimen was kept as reserve sample to avoid the risk of losing samples for any reason during or after DNA extraction. Genomic DNA was individually extracted from a total of 842 tick specimens using the QIAamp DNA tissue extraction kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. The DNA from each tick specimen was eluted in 100 µL of Tris EDTA (TE) buffer and stored at −20°C under sterile conditions to preclude contamination until the sample was used for polymerase chain reaction (PCR). To avoid cross-contamination among samples during DNA extraction, all parts of the DNA extracting EZI Advanced XL Robot (Qiagen, Hilden, Germany) were disinfected after each batch of extraction as per recommendations of the manufacturers. The second half of each tick specimen was stored at −80°C as a backup sample.

Molecular detection of C. burnetii by quantitative PCR. DNA samples were individually tested using C. burnetii-specific quantitative real-time PCR (qPCR) with primers and probes designed for the amplification of the COX spacer sequence as previously described. DNA samples that were positive for the COX spacer sequence were further confirmed by the highly C. burnetii-specific species-specific IS30A spacer as has been performed previously using the same primers and probes. Sterile water was used as a negative control whereas DNA from C. burnetii was used as a positive control. A sample was considered positive when the PCRs were positive for the two different abovementioned C. burnetii specific genes.

Genotyping of C. burnetii detected in ticks. For genotyping from the total of 54 positive samples, 19 ticks including Amblyomma gemma (N = 6; three from Arero, two from Moyale, one from and one from Yabelo districts), Rhipicephalus pulchellus (N = 9; three from Arero, four from Moyale, and two from Yabelo districts), one Hyalomma marginatum rufipes from Moyale, one Am. variegatum and one Rhipicephalus (Boophilus) decoloratus from Arsi district, and one Am. cohaerens from Arsi district that harboured sufficient amount of C. burnetii DNA (with Ct < 35) were selected.

MST was used to determine the genotypes of C. burnetii in randomly selected representative tick DNA samples that were positive for C. burnetii by qPCR. The spacer regions in the C. burnetii genome that exhibited higher variation for differentiating the genotypes (Cox2, Cox3, Cox18, Cox37, Cox56, Cox57, and Cox61 manufactured by Eurogentec, Seraing, Belgium) were selected for standard PCR using similar PCR conditions and sequences of primers as described previously. The genotypes identified by MST were compared with genotypes included in the MST database containing C. burnetii genotypes from countries in Europe and other parts of the world. Detection of amplified products, cleaning of excess primers and nucleotides from DNA, sequencing, assembling, and edition of sequences were all performed as described previously.

Ethical statement. Ethical approval for the collection of ticks from domestic animals was obtained from the animal research ethics board (Agreement number 14/160/550/2011) of the College of Veterinary Medicine and Agriculture of Addis Ababa University. All necessary permits were obtained from the administration and agricultural office of each district and from each animal owner.

Data analysis. Microsoft Excel was used for data management. Descriptive statistics, such as percentages and means, were used to summarize the proportions of ticks positive for C. burnetii DNA. Statistical analyses were performed with EpiInfo™. Associations between the prevalence of C. burnetii DNA among different tick species and genera and districts of collection were determined using the Mantel–Haenszel (MH) test with the statistical software EpiInfo™. All differences were considered significant at P values < 0.05.

RESULTS

Ticks in the present study were collected from a total of 245 animals (207 cattle, 29 sheep, 7 dogs, and 2 cats) in 9 districts: Abdela (37 cattle and 15 sheep), Gachi (29 cattle and 3 sheep), Ada’a (20 cattle, 7 dogs, and 2 cats), Wolmara (17 cattle and 1 sheep), Kimbibi (23 cattle and 10 sheep), Arsi (31 cattle), Arero (23 cattle), Moyale (18 cattle), and Yabelo (9 cattle). The 842 ticks collected consisted of 373 males, 343 females, 100 nymphs, and 26 larvae. Overall, tick species collected comprised Am. cohaerens (128: 76 males, 52 females), Am. gemma (49: 27 males, 22 females), Am. lepidum (62: 51 males, 11 females), Amblyomma larvae (22), Amblyomma nymphs (60), Rh. (Bo.) decoloratus (153: 37 males, 116 females), Rh. (Bo.) decoloratus larvae (4), Rh. (Bo.) decoloratus nymphs (31), Rh. praevertatus (63: 29 males, 29 females), Rh. pulchellus (126: 69 males, 57 females), Hy. m. rufipes (14: 10 males, 4 females), Rhipicephalus spp. nymphs (9), Hy. truncatum (53: 33 males, 20 females), Rh. sanguineus (14: 9 males, 5 females), Haemaphysalis leachi (6: 2 males, 4 females) and Hae. spinulosa (2 females). The majority of the ticks were collected from cattle 90.5% (762/842) and a small proportion was collected from sheep 6% (51/842), dogs 2.7% (23/842), and cats 0.7% (6/842).

This study recorded an overall frequency of 6.4% (54/842) of C. burnetii DNA in seven species of ixodid ticks out of the total of 13 different species tested using molecular tools (Table 1). C. burnetii DNA was detected in 28.6% (14/49) of Am. gemma, 25% (31/124) of Rh. pulchellus, 7.1% (1/14) of Hy. m. rufipes, 3.2% (2/62) of Am. variegatum, 3.1% (4/128) of Am. cohaerens, 1.6% (1/63) of Rh. praevertatus, and 0.6% (1/153) of Rh. (Bo.) decoloratus (Table 1). Photographs of ticks positive for C. burnetii are depicted on Figure 2. The overall prevalence of C. burnetii DNA was significantly higher in both Am. gemma (28.6%; 14/49) and Rh. pulchellus (25%; 31/124) than the sum of all the other positive tick species (2.1%; 9/420) (MH, P < 0.0001). This study is the first to report finding of C. burnetii DNA in Am. gemma, Rh. pulchellus Rh. (Bo.), decoloratus, Hy. m. rufipes, and Rh. praevertatus ticks.
from Ethiopia. The overall frequency of *C. burnetii* DNA in the *Rhipicephalus* and *Amblyomma* (6.9%; 53/767) genera was higher than in the *Hyalomma* and *Haemaphysalis* (1.3%; 1/75) genera of ticks (Table 1). However, this variation was not statistically significant (MH, *P* = 0.06).

The overall proportion of *C. burnetii* DNA in ticks was 1.2% (1/80) in Kimubit, 3.3% (4/121) in Arsi, 20% (21/105) in Arero, 27.8% (22/79) in Moyale, and 15% (6/40) in Yabelo districts (Table 1). Conversely, *C. burnetii* DNA was not detected in any ticks from the Abdela (0/107), Gachi (0/107), Ada’a (0/128), and Wolmara (0/75) districts in western and central Oromia (Table 1 and Figure 1). The overall frequency of *C. burnetii* DNA was significantly higher in ticks collected from southeastern districts in Borana zone (Arero, Moyale, and Yabelo) (21.9%; 49/224) than in ticks collected from central districts (Kimubit and Arsi) (2.5%; 5/201) of Oromia regional state (MH, *P* < 0.0001) (Figure 1). In this study, 7.1% (54/762) of the ticks from cattle were positive for *C. burnetii* DNA; however, *C. burnetii* DNA was not detected in ticks taken from sheep (0/51), dogs (0/23), and (0/6) cats (Table 1).

**MST** of *C. burnetii* from positive tick samples showed the presence of two different genotypes: the first genotype, MST

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**Table 1**

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<th>Region</th>
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<th>Overall <em>Hyalomma</em> spp.</th>
<th>Overall <em>Haemaphysalis</em> spp.</th>
<th>Overall <em>Hae. leachi</em></th>
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qPCR = quantitative real-time polymerase chain reaction.

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18, was found in ticks from the Borana zone (Arero, Moyale, and Yabelo districts), whereas the second genotype, MST 20, was detected in ticks from the central districts (Kimbibit and Arsi) (Figure 1). In southeastern districts, genotype MST 18 was detected in *Am. cohaerens* in the Arero and Moyale districts, in *Hy. m. rufipes* in the Moyale district, and in *Rh. pulchellus* and *Am. gemma* in the Arero, Moyale, and Yabelo districts. In the central districts, genotype MST 20 was detected in *Rh. praetextatus* in the Kimbibit district and in *Am. cohaerens, Am. variegatum*, and *Rh. (Bo.) decoloratus* in the Arsi district. The overall prevalence of MST 18 was significantly higher (MH, *P* < 0.000003) in southeastern
these two spp. in the epidemiology of this zoonosis than the tick spp. most probably indicates the greater importance of

(20.9% of been reported in 30% of this country. Consistent with our observation, knowledge about the species of ticks that host

and Rh. praetextatus decoloratus, Rh. pulchellus

studies are the only reports available in ticks from Africa. Am. variegatum findings, some 48 years ago in central Ethiopia.22 In line with our

southwest Ethiopia.

The detection of C. burnetii in Am. variegatum for the second time in Ethiopia confirms the earlier report made some 48 years ago in central Ethiopia.22 In line with our findings, C. burnetii has been recently detected in 19.6% of Am. variegatum in Nigeria,30 up to 37.6% of A. variegatum in two regions of Senegal,12 2.5% of pools of Am. variegatum from cattle, and 20% of pools from dogs in Kenya.18 These studies are the only reports available in ticks from Africa.

Our observation of C. burnetii in Am. gemma, Rh. (Bo.) decoloratus, Rh. pulchellus, Hy. m. ruifpes, Am. cohaerens, and Rh. praetextatus for the first time in Ethiopia is additional knowledge about the species of ticks that host C. burnetii in this country. Consistent with our observation, C. burnetii has been reported in 30% of Rh. (Bo.) decoloratus and in 1.7% to 20.9% of Hy. m. ruifpes in Senegal12 and in 20% of pools of Rh. (Bo.) decoloratus in Kenya.18 Similarly, C. burnetii has recently been detected in different species of ixodid ticks in France,34,35 Italy,38 Spain,39 Germany,40 Australia,41 Argentina,42 Slovakia and Hungary,43 Japan,44 and in eight species of ticks in China.45

The finding of significantly higher C. burnetii in both Am. gemma and Rh. pulchellus compared with all the other tick spp. most probably indicates the greater importance of these two spp. in the epidemiology of this zoonosis than the other ticks. When considering the established fact that these two tick species are the most predominant species in southeastern Oromia in the Borana zone,9 where animals are kept under a pastoral type of management and there is much closer contact between animals and pastoralists due to common consumption of raw milk and meat, it is implied that there is a high likelihood of transmission of C. burnetii to humans in the study districts of this zone. It is hypothesized that ticks become infected during blood feeding on infected animals and then act as reservoirs of C. burnetii and play an important role in main-

taining the bacteria in the environment, which may cause infection in wild vertebrates, domestic animals, and humans.6,11

However, contrary to our present finding, C. burnetii was not detected in ticks studied in Hungary,46 in Germany,40 or in Sweden.47 This variation is most probably attributed to differences in animal management factors, for example, that domestic animals are housed within farms throughout their lifespan and are not allowed to graze on pastures in some European countries and the systematic use of acaricides, such as deltamethrin, that reduce tick populations and alter the ability of ticks to carry C. burnetii. This is different from Ethiopia where animals graze in communal pastures throughout the year that serve as an infectious source for blood-feeding ticks, and there is no regular use of acaricides as has been shown before.39 In support of this fact, a recent serological study reported antibodies against C. burnetii in 54.2% of goats, 31.6% of cattle, and 90% of camels in eastern parts of Ethiopia.23

In our study, a statistically significant higher (MH, P < 0.0001) prevalence of positive C. burnetii DNA in ticks was observed in southeastern districts from the Borana zone (Arero, Moyale, and Yabelo) than in the central districts (Kimbit and Arsi). This difference is most probably attributable to factors such as the presence of larger population of cattle, goats, sheep, and camel that are usually managed by a pastoral type of management characterized by unrestricted movement of animals in search of water and grazing pasture in districts in the Borana zone. This is in contrast with the central districts that practice a crop–livestock mixed type of farming with restricted movement of ruminants and equines and the absence of camels. In support of the finding of the highest frequency of C. burnetii in camel-rearing districts in the Borana zone of this study, being a camel breeder was reported as a very important risk factor for human Q-fever seropositivity in Chad.48 Despite the greater number of ticks tested in Abdela (0/107), Gachi (0/107), Ada’a (0/128), and Wolmar (0/75) from the western and central districts of Oromia, C. burnetii was not detected (Table 1). In agreement with our report, a significantly higher prevalence of C. burnetii was recorded in ticks from areas where higher populations of ruminants are kept in Senegal12 and Kenya.18 Also in support of our observations, the presence of ticks on animals was reported as the most important risk factor for the occurrence of Q fever and abortion in northern Cyprus.49 Furthermore, several previous studies linked higher populations of ruminants with outbreaks of Q fever in humans in several countries around the world.50,51

For the first time in Ethiopia, this study provides new information on the presence of two different genotypes of C. burnetii in ticks, MST 18 in ticks from animals in the Borana zone, and MST 20 in ticks from animals in the central districts. This finding is in line with previous reports of several genotypes of C. burnetii in ticks.12 Interestingly, a novel MST type (proposed ST52) closely related to MST20 detected in ticks from central Oromia in our study has been recently reported in ticks collected from cattle along Didessa valley in southwest Ethiopia.52 Genotype MST18 was previously identified from human and animal clinical samples in Hungary,53 France, Italy, Romania, Greece, Slovakia, Germany, and Spain.24,25 Similarly, genotype MST 20 has been identified in samples from cattle, goat, and cow’s milk in Hungary,52 from cow milk and milk products from several countries in Europe, including France, the Netherlands, Portugal, Spain, Switzerland, and the
United Kingdom, in human clinical samples from France, in a cow’s placenta from Germany and in rodents and cow’s milk, soil, and goat placental material from the United States, and other parts of the world (Qatar and Saudi Arabia), suggesting the worldwide occurrence of this genotype, which supports our finding. Previous studies have shown that genotype MST 20 is usually associated with cattle and their products. C. burnetii genotyping is important for identifying the source of infection for humans and animals. It is important to note that additional genotypes not detected by our sampling may be circulating in other parts of the country. In the previous report of C. burnetii genotypes from Africa, MST 6, 35, and 36 were detected in ticks in Senegal and genotype MST 19 was recorded in human patients with endocarditis.

Our study has limitation on inference and extrapolation due to very scanty previous information on genotyping of C. burnetii from African countries including Ethiopia and thus our findings were compared with genotypes included in the MST database containing C. burnetii genotypes published in 2009. In conclusion, the findings of this study provide additional information on the geographic distribution and genotypes of C. burnetii in different ticks in Ethiopia. The overall frequency of C. burnetii in ticks from the Borana zone is higher than all other zones. Medical and veterinary professionals in Ethiopia should consider C. burnetii as an emerging pathogen in the country. Ethiopian physicians managing patients with fevers of unknown etiology and travelers with unexplained fevers returning from Ethiopia should consider C. burnetii as one of the potential causative agents. Future studies are necessary to address the isolation, culture, and genotypes of C. burnetii in arthropods, human blood, and domestic and other reservoir host animals and the vector competence of these tick species for C. burnetii and its public health and economic significance in Ethiopia.

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Authors’ addresses: Bersissa Kumsa, Cristina Socolovschi, Lionel Almeras, and Didier Raoult, Unité de Recherche en Maladies Infectieuses et Tropicales Emergentes (URMITE): UM63, CNRS 7278, IRD 198, Inserm 1095, Aix Marseille Université, Faculté de Médecine, Marseille, France, E-mails: bersissak@yahoo.com, cr_socolovschi@yahoo.com, almeras.lionel@gmail.com, and didier.raoult@gmail.com. Philippe Parola, Unité de Recherche en Maladies Infectieuses et Tropicales Emergentes (URMITE): UM63, CNRS 7278, IRD 198, Inserm 1095, Aix Marseille Université, Faculté de Médecine, Marseille, France, E-mail: philippe.parola@univ-amu.fr.

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