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 Ticks are vectors and reservoirs for several emerging and reemerging infectious pathogens of medical and veterinary importance.^{2,3} 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 *Coxiella*, 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, non-specific 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.^{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.⁹ 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.^{6,9} 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.¹² 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

*Address correspondence to 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

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 Senegal²¹. 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 ticks 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, Kimbabit 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 south-east 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

FIGURE 1. Map of the Oromia State showing study districts in Ethiopia with the overall prevalence of *C. burnetii*, positive tick spp., and multispacer sequence typing (MST) recorded in each district.

described.⁵ 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.^{28–30} 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.³¹

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.⁵ Each specimen was longitudinally cut into two equal halves.^{32,33} 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.⁵ 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.⁵ 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).⁵ 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.⁵

Molecular detection of *C. burnetii* by quantitative PCR. DNA samples were individually tested using *C. burnetii*-specific quantitative real-timePCR (qPCR) with primers and probes designed for the amplification of the COX spacer sequence as previously described.¹² DNA samples that were positive for the COX spacer sequence were further confirmed by the highly *C. burnetii*-species specific IS30A spacer as has been performed previously using the same primers and probes.^{12,34} 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, 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 Arero 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, Cox5, 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.^{12,24,34} 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 (http://ifr48.timone.univ-mrs.fr/MST_Coxiella/mst/). Detection of amplified products, cleaning of excess primers and nucleotides from DNA, sequencing, assembling, and edition of sequences were all performed as described previously.^{32,33}

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™ 7. 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™ 7. 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), Kimbibit (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* (2 females), *Am. variegatum* (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. praetextatus* (63: 34 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 leachii* (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. praetextatus*, 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. praetextatus* ticks

TABLE 1
Prevalence of *C. burnetii* in ixodid ticks using species-specific gene qPCR in nine districts in Oromia, Ethiopia

Zone	IluAba Bora		East Showa	West Showa	North Showa	Arsi	Borana			Total
District	Abdela	Gachi	Ada'a	Wolmara	Kimbibit	Arsi	Arero	Moyale	Yabelo	
<i>Am. cohaerens</i>	0/52	0/57	—	—	—	1/2 (50%)	1/12 (8.3%)	2/3 (66.7%)	0/2	4/128 (3.1%)
Cattle (N = 58)	0/41	0/55	—	—	—	1/2 (50%)	1/12 (8.3%)	2/3 (66.7%)	0/2	4/115 (3.5%)
Sheep (N = 9)	0/11	0/2	—	—	—	—	—	—	—	0/13
<i>Am. gemma</i>	—	—	0/1	—	—	—	5/15 (33.3%)	7/23 (30.4%)	2/10 (20%)	14/49 (28.6%)
Cattle (N = 25)	—	—	0/1	—	—	—	5/15 (33.3%)	7/23 (30.4%)	2/10 (20%)	14/49 (28.6%)
<i>Am. lepidum</i>	—	—	—	—	—	—	0/1	0/1	—	0/2
Cattle (N = 2)	—	—	—	—	—	—	0/1	0/1	—	0/2
<i>Am. variegatum</i>	—	0/5	0/14	0/9	—	2/31 (6.4%)	0/2	—	0/1	2/62 (3.2%)
Cattle (N = 40)	—	0/3	0/14	0/7	—	2/31 (6.4%)	0/2	—	0/1	2/60 (3.3%)
Sheep (N = 2)	—	0/2	—	—	—	—	—	—	—	0/2
<i>Amblyomma</i> larva	0/10	0/5	0/4	0/2	—	—	—	—	0/1	0/22
Cattle (N = 20)	0/8	0/5	0/4	0/2	—	—	—	—	0/1	0/20
Sheep (N = 2)	0/2	—	—	—	—	—	—	—	—	0/2
<i>Amblyomma</i> nymph	0/26	0/13	0/7	0/1	—	0/10	0/1	0/1	0/1	0/60
Cattle (N = 44)	0/13	0/11	0/6	0/1	—	0/10	0/1	0/1	0/1	0/44
Sheep (N = 14)	0/13	0/2	—	—	—	—	—	—	—	0/15
Cat (N = 1)	—	—	0/1	—	—	—	—	—	—	0/1
Overall <i>Amblyomma</i> spp.	0/88	0/80	0/26	0/12	—	3/43 (7%)	6/31 (19.3%)	9/28 (32.1%)	2/15 (13.3%)	20/323 (6.2%)
<i>Rh. (Bo.) decoloratus</i>	0/14	0/17	0/11	0/37	0/23	1/37 (2.7%)	0/10	0/3	0/1	1/153 (0.6%)
Cattle (N = 104)	0/14	0/16	0/11	0/37	0/20	1/37 (2.7%)	0/10	0/3	0/1	1/149 (0.7%)
Sheep (N = 2)	—	0/1	—	—	0/3	—	—	—	—	0/4
<i>Rh. (Bo.) decoloratus</i> larva	—	—	0/2	—	0/2	—	—	—	—	0/4
Cattle (N = 2)	—	—	0/2	—	0/2	—	—	—	—	0/4
<i>Rh. (Bo.) decoloratus</i> nymph	0/3	0/6	0/4	0/11	0/6	—	0/1	—	—	0/31
Cattle (N = 31)	0/3	0/6	0/4	0/11	0/6	—	0/1	—	—	0/31
<i>Rh. praetextatus</i>	—	—	0/12	—	1/38 (2.6%)	0/13	—	—	—	1/63 (1.6%)
Cattle (N = 30)	—	—	0/12	—	1/26 (3.8%)	0/13	—	—	—	1/51 (2%)
Sheep (N = 9)	—	—	—	—	0/12	—	—	—	—	0/12
<i>Rh. e. evertsi</i>	0/2	0/4	0/11	0/6	0/7	0/12	0/1	0/1	—	0/44
Cattle (N = 23)	0/2	0/3	0/11	0/4	0/7	0/12	0/1	0/1	—	0/41
Sheep (N = 2)	—	—	—	—	—	—	—	—	—	0/3
<i>Rh. pulchellus</i>	—	—	0/4	—	—	—	15/58 (25.9%)	12/40 (30%)	4/24 (16.7%)	31/126 (24.6%)
Cattle (N = 50)	—	—	0/2	—	—	—	15/58 (25.9%)	12/40 (30%)	4/24 (16.7%)	31/124 (25%)
Dog (N = 1)	—	—	0/2	—	—	—	—	—	—	0/2
<i>Rh. sanguineus</i>	—	—	0/14	—	—	—	—	—	—	0/14
Dog (7)	—	—	0/14	—	—	—	—	—	—	0/14
<i>Rhipicephalus</i> spp. nymph	—	—	0/5	—	—	—	0/2	0/2	—	0/9
Cattle (N = 5)	—	—	0/1	—	—	—	0/2	0/2	—	0/5
Dog (3)	—	—	0/4	—	—	—	—	—	—	0/4
Overall <i>Rhipicephalus</i> spp.	0/19	0/27	0/63	0/54	1/76 (1.3%)	1/62 (1.6%)	15/72 (20.8%)	12/46 (26.1%)	4/25 (16%)	33/444 (7.4%)
<i>Hy. m. rufipes</i>	—	—	0/2	0/3	0/2	—	0/2	1/5 (20%)	—	1/14 (7.1%)
Cattle (N = 5)	—	—	0/2	0/3	0/2	—	0/2	1/5 (20%)	—	1/14 (7.1%)
<i>Hy. truncatum</i>	—	—	0/29	0/6	0/2	0/16	—	—	—	0/53
Cattle (N = 27)	—	—	0/29	0/6	0/2	0/16	—	—	—	0/53
Overall <i>Hyalomma</i> spp.	—	—	0/31	0/9	0/4	0/16	0/2	1/5 (20%)	—	1/67 (1.5%)
<i>Hae. leachii</i>	—	—	0/6	—	—	—	—	—	—	0/6
Dog (1)	—	—	0/1	—	—	—	—	—	—	0/1
Cat (2)	—	—	0/5	—	—	—	—	—	—	0/5
<i>Hae. spinulosa</i>	—	—	0/2	—	—	—	—	—	—	0/2
Dog (1)	—	—	0/2	—	—	—	—	—	—	0/2
Overall <i>Haemaphysalis</i> spp.	—	—	0/8	—	—	—	—	—	—	0/8
Overall prevalence in ticks	0/107	0/107	0/128	0/75	1/80 (1.2%)	4/121 (3.3%)	21/105 (20%)	22/79 (27.8%)	6/40 (15%)	54/842 (6.4%)

qPCR = quantitative real-time polymerase chain reaction.

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 Kimbibit, 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 (Kimbibit 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

FIGURE 2. Picture of ixodid ticks positive for *C. burnetii* collected from domestic animals in nine districts in Oromia, Ethiopia.

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

districts (89.5%; 17/19) than the overall prevalence of genotype MST 20 in central districts (10.5%; 2/19) of Oromia regional state in Ethiopia.

DISCUSSION

The overall frequency of *C. burnetii* (6.4%; 54/842) in seven species of ixodid ticks collected from cattle may highlight the possible importance of ticks in the epidemiology of *C. burnetii* in Ethiopia. A reliable molecular strategy was used that has been validated in recent studies, and our findings were confirmed by positive and negative controls and by amplification of several target genes as previously described.¹² The overall frequency of *C. burnetii* (6.4%; 54/842) in our study is lower than the overall prevalence of 10.8% (32/295)³⁵ in ticks along Didessa valley recently reported from Ethiopia. This difference is most probably attributed to factors like in our study a sample was considered positive when it was positive for recently developed two different types of genes (COX and IS30A spacers); however, the previous study used only one gene (IS1111 spacer). The other possible factors are differences in the number of species and quantity of ticks tested, geographical locations and seasons of tick collections by the two studies might affect the prevalence. We have tested a total of 842 ticks consisting 13 different species from 9 districts in Oromia; however, the previous study³⁵ tested only 295 ticks comprising 7 species from one region along Didessa valley in 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,³⁶ 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. rufipes*, *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. rufipes* 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,^{34,37} 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 south-eastern Oromia in the Borana zone,⁴ 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 significantly 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 (Kimbibit 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 Wolmara (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,²⁶ 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,²⁴ 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.^{25,26} *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.¹²

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 2009.²⁴

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.

Received November 27, 2014. Accepted for publication March 11, 2015.

Published online September 21, 2015.

Acknowledgments: We greatly acknowledge domestic animal owners in the different districts of Oromia for their kindness in allowing us to collect ticks from their animals.

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.

REFERENCES

1. Jongejan F, Uilenberg G, 2004. The global importance of ticks. *Parasitology* 129 (Suppl): S3–S14.
2. Dantas-Torres F, Chomel BB, Otranto D, 2012. Ticks and tick-borne diseases: a One Health perspective. *Trends Parasitol* 28: 437–446.
3. De la Fuente J, Estrada-Pena A, 2012. Ticks and tick-borne pathogens on the rise. *Ticks Tick Borne Dis* 3: 115–116.
4. Regassa A, 2001. Tick infestation of Borana cattle in the Borana Province of Ethiopia. *Onderstepoort J Vet Res* 68: 41–45.
5. Kumsa B, Socolovschi C, Raoult D, Parola P, 2015. Spotted fever group rickettsiae in ixodid ticks in Oromia, Ethiopia. *Ticks Tick Borne Dis* 6: 8–15.
6. Angelakis E, Raoult D, 2010. Q fever. *Vet Microbiol* 140: 297–309.
7. Maurin M, Raoult D, 1999. Q fever. *Clin Microbiol Rev* 12: 518–553.
8. Georgiev M, Afonso A, Neubauer H, Needham H, Thiery R, Rodolakis A, Roest H, Stark K, Stegeman J, Vellema P, van der Hoek W, More S, 2013. Q fever in humans and farm animals in four European countries, 1982 to 2010. *Euro Surveill* 18: pii: 20407.
9. Cutler SJ, Bouzid M, Cutler RR, 2007. Q fever. *J Infect* 54: 313–318.
10. Guatteo R, Seegers H, Taurel AF, Joly A, Beaudeau F, 2011. Prevalence of *Coxiella burnetii* infection in domestic ruminants: a critical review. *Vet Microbiol* 149: 1–16.
11. Agerholm JS, 2013. *Coxiella burnetii* associated reproductive disorders in domestic animals—a critical review. *Acta Vet Scand* 55: 13.
12. Mediannikov O, Fenollar F, Socolovschi C, Diatta G, Bassene H, Moles JF, Sokhna C, Trape JF, Raoult D, 2010. *Coxiella burnetii* in humans and ticks in rural Senegal. *PLoS Negl Trop Dis* 4: e654.
13. Psaroulaki A, Chochlakis D, Ioannou I, Angelakis E, Tselentis Y, 2014. Presence of *Coxiella burnetii* in Fleas in Cyprus. *Vector Borne Zoonotic Dis* 14: 685–687.
14. Loftis AD, Reeves WK, Szumlas DE, Abbassy MM, Helmy HM, Moriarity JR, Dasch GA, 2006. Surveillance of Egyptian fleas for agents of public health significance: *Anaplasma*, *Bartonella*, *Coxiella*, *Ehrlichia*, *Rickettsia*, and *Yersinia pestis*. *Am J Trop Med Hyg* 75: 41–48.
15. Nelder MP, Lloyd JE, Loftis AD, Reeves WK, 2008. *Coxiella burnetii* in wild-caught filth flies. *Emerg Infect Dis* 14: 1002–1004.
16. Vanderburg S, Rubach MP, Halliday JE, Cleaveland S, Reddy EA, Crump JA, 2014. Epidemiology of *Coxiella burnetii* infection in Africa: a One Health systematic review. *PLoS Negl Trop Dis* 8: e2787.
17. Dupont HT, Brouqui P, Faugere B, Raoult D, 1995. Prevalence of antibodies to *Coxiella burnetii*, *Rickettsia conorii*, and *Rickettsia typhi* in seven African countries. *Clin Infect Dis* 21: 1126–1133.
18. Knobel DL, Maina AN, Cutler SJ, Ogola E, Feikin DR, Junghe M, Halliday JE, Richards AL, Breiman RF, Cleaveland S, Njenga MK, 2013. *Coxiella burnetii* in humans, domestic ruminants, and ticks in rural western Kenya. *Am J Trop Med Hyg* 88: 513–518.
19. Klaassen M, Roest HJ, van der Hoek W, Goossens B, Secka A, Stegeman A, 2014. *Coxiella burnetii* seroprevalence in small ruminants in the Gambia. *PLoS ONE* 9: e85424.
20. Qiu Y, Nakao R, Namangala B, Sugimoto C, 2013. First genetic detection of *Coxiella burnetii* in Zambian livestock. *Am J Trop Med Hyg* 89: 518–519.
21. Angelakis E, Mediannikov O, Socolovschi C, Mouffok N, Bassene H, Tall A, Niangaly H, Doumbo O, Znazen A, Sari M, Sokhna C, Raoult D, 2014. *Coxiella burnetii*-positive PCR in febrile patients in rural and urban Africa. *Int J Infect Dis* 28: 107–110.
22. Philip CB, Hoogstraal H, Reiss-Gutfreund R, Clifford CM, 1966. Evidence of rickettsial disease agents in ticks from Ethiopian cattle. *Bull World Health Organ* 35: 127–131.
23. Gumi B, Firdessa R, Yamuah L, Sori T, Tolosa T, Aseffa A, Zinsstag J, Schelling E, 2013. Seroprevalence of brucellosis and Q-fever in southeast Ethiopian pastoral livestock. *J Vet Sci Med Diagn* 2: 1–2.
24. Glazunova O, Roux V, Freylikman O, Sekeyova Z, Fournous G, Tyczka J, Tokarevich N, Kovacava E, Marrie TJ, Raoult D, 2005. *Coxiella burnetii* genotyping. *Emerg Infect Dis* 11: 1211–1217.
25. Astobiza I, Tilburg JJ, Pinero A, Hurtado A, Garcia-Perez AL, Nabuurs-Franssen MH, Klaassen CH, 2012. Genotyping of *Coxiella burnetii* from domestic ruminants in northern Spain. *BMC Vet Res* 8: 241.
26. Sulyok KM, Kreizinger Z, Hornstra HM, Pearson T, Szigeti A, Dan A, Balla E, Keim PS, Gyuranecz M, 2014. Genotyping of *Coxiella burnetii* from domestic ruminants and human in Hungary: indication of various genotypes. *BMC Vet Res* 10: 107.
27. CSA, 2008. *Ethiopian Agricultural Sample Survey Report on Livestock and Livestock Characteristics*, Volume II, 2007/08. Addis Ababa, Ethiopia: CSA.
28. Hoogstraal H, 1956. *African Ixodidae. I. Ticks of the Sudan (with Special Reference to Equatorial Province and with Preliminary Reviews of the Genera Boophilus, Margaropus and Hyalomma)*. 1st edition. Washington DC: Department of the Navy, Bureau of the Medicine and Surgery, U.S. Government Printing Office.

29. Walker AR, Bouattour A, Camicas JL, Estrada-Peña A, Horak IG, Latif AA, Pegram RG, Preston PM, 2003. *Tick of Domestic Animals in Africa: A Guide to Identification of Species*. 1st edition. Edinburgh, Scotland: Bioscience Reports.
30. Walker JB, Keirans JE, Horak IG, 2000. *The Genus Rhipicephalus (Acari, Ixodidae): A Guide to the Brown Cf the World*. 1st edition. Cambridge, United Kingdom: Cambridge University Press.
31. Dantas-Torres F, 2008. Towards the standardization of the abbreviations of genus names of ticks (Acari: Parasitiformes: Ixodidae). *Vet Parasitol* 154: 94–97.
32. Kumsa B, Socolovschi C, Parola P, Rolain JM, Raoult D, 2012. Molecular detection of *Acinetobacter* species in lice and keds of domestic animals in Oromia Regional State, Ethiopia. *PLoS ONE* 7: e52377.
33. Kumsa B, Parola P, Raoult D, Socolovschi C, 2014. *Bartonella melophagi* in *Melophagus ovinus* (sheep ked) collected from sheep in northern Oromia, Ethiopia. *Comp Immunol Microbiol Infect Dis* 37: 69–76.
34. Socolovschi C, Reynaud P, Kernif T, Raoult D, Parola P, 2012. *Rickettsiae* of spotted fever group, *Borrelia valaisiana*, and *Coxiella burnetii* in ticks on passerine birds and mammals from the Camargue in the south of France. *Ticks Tick Borne Dis* 3: 355–360.
35. Hornok S, Abichu G, Meli ML, Tanczos B, Sulyok KM, Gyuranecz M, Gonczi E, Farkas R, Hofmann-Lehman R, 2014. Influence of the biotope on the tick infestation of cattle and on the tick-borne pathogen repertoire of cattle ticks in Ethiopia. *PLoS ONE* 9: e106452.
36. Reye AL, Arinola OG, Hubschen JM, Muller CP, 2012. Pathogen prevalence in ticks collected from the vegetation and livestock in Nigeria. *Appl Environ Microbiol* 78: 2562–2568.
37. Bonnet S, De la Fuente J, Nicolle P, Liu X, Madani N, Blanchard B, Maingourd C, Alongi A, Torina A, Fernandez de Mera IG, Vicente J, George JC, Vayssier-Taussat M, Joncour G, 2013. Prevalence of tick-borne pathogens in adult *Dermacentor* spp. ticks from nine collection sites in France. *Vector Borne Zoonotic Dis* 13: 226–236.
38. Satta G, Chisu V, Cabras P, Fois F, Masala G, 2011. Pathogens and symbionts in ticks: a survey on tick species distribution and presence of tick-transmitted micro-organisms in Sardinia, Italy. *J Med Microbiol* 60: 63–68.
39. Toledo A, Jado I, Olmeda AS, Casado-Nistal MA, Gil H, Escudero R, Anda P, 2009. Detection of *Coxiella burnetii* in ticks collected from Central Spain. *Vector Borne Zoonotic Dis* 9: 465–468.
40. Pluta S, Hartelt K, Oehme R, Mackenstedt U, Kimmig P, 2010. Prevalence of *Coxiella burnetii* and *Rickettsia* spp. in ticks and rodents in southern Germany. *Ticks Tick Borne Dis* 1: 145–147.
41. Cooper A, Stephens J, Ketheesam N, Govan B, 2013. Detection of *Coxiella burnetii* DNA in wildlife and ticks in northern Queensland, Australia. *Vector Borne Zoonotic Dis* 13: 12–16.
42. Pacheco RC, Echaide IE, Alves RN, Beletti ME, Nava S, Labruna MB, 2013. *Coxiella burnetii* in ticks, Argentina. *Emerg Infect Dis* 19: 344–346.
43. Spitalska E, Kocianova E, 2003. Detection of *Coxiella burnetii* in ticks collected in Slovakia and Hungary. *Eur J Epidemiol* 18: 263–266.
44. Andoh M, Andoh R, Teramoto K, Komiya T, Kaneshima T, Takano A, Hayashidani H, Ando S, 2013. Survey of *Coxiella burnetii* in ticks collected from dogs in Japan. *J Vet Med Sci* 75: 1115–1117.
45. El-Mahallawy HS, Lu G, Kelly P, Xu D, Li Y, Fan W, Wang C, 2015. Q fever in China: a systematic review, 1989–2013. *Epidemiol Infect* 143: 673–681.
46. Gyuranecz M, Denes B, Hornok S, Kovacs P, Horvath G, Jurkovich V, Varga T, Hajtos I, Szabo R, Magyar T, Vass N, Hofmann-Lehman R, Erdelyi K, Bhidé M, Dan A, 2012. Prevalence of *Coxiella burnetii* in Hungary: screening of dairy cows, sheep, commercial milk samples, and ticks. *Vector Borne Zoonotic Dis* 12: 650–653.
47. Wallmenius K, Pettersson JH, Jaenson TG, Nilsson K, 2012. Prevalence of *Rickettsia* spp., *Anaplasma phagocytophilum*, and *Coxiella burnetii* in adult *Ixodes ricinus* ticks from 29 study areas in central and southern Sweden. *Ticks Tick Borne Dis* 3: 100–106.
48. Schelling E, Diguimbaye C, Daoud S, Nicolet J, Boerlin P, Tanner M, Zinsstag J, 2003. Brucellosis and Q-fever seroprevalences of nomadic pastoralists and their livestock in Chad. *Prev Vet Med* 61: 279–293.
49. Cantas H, Muwonge A, Sareyyupoglu B, Yardimci H, Skjerve E, 2011. Q fever abortions in ruminants and associated on-farm risk factors in northern Cyprus. *BMC Vet Res* 7: 13.
50. De Lange MM, Schimmer B, Vellema P, Hautvast JL, Schneeberger PM, van Duinhoven YT, 2014. *Coxiella burnetii* seroprevalence and risk factors in sheep farmers and farm residents in The Netherlands. *Epidemiol Infect* 142: 1231–1244.
51. Schimmer B, Schotten N, van Engelen E, Hautvast JL, Schneeberger PM, van Duinhoven YT, 2014. *Coxiella burnetii* seroprevalence and risk for humans on dairy cattle farms, the Netherlands, 2010–2011. *Emerg Infect Dis* 20: 417–425.
52. Sulyok KM, Hornok S, Abichu G, Erdelyi K, Gyuranecz M, 2014. Identification of novel *Coxiella burnetii* genotypes from Ethiopian ticks. *PLoS ONE* 9: e113213.
53. Gyuranecz M, Sulyok K, Balla E, Mag T, Balazs A, Simor Z, Denes B, Hornok S, Bajnoczi P, Hornstra H, Pearson T, Keim P, Dan A, 2014. Q fever epidemic in Hungary, April to July 2013. *Euro Surveill* 19: pii20863.