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Original article

# Spotted fever group rickettsiae in ixodid ticks in Oromia, Ethiopia

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ABSTRACT

In Ethiopia, information on the transmission of human zoonotic pathogens through ixodid ticks remains scarce. To address the occurrence and molecular identity of spotted fever group rickettsiae using molecular tools, a total of 767 ixodid ticks belonging to thirteen different species were collected from domestic animals from September 2011 to March 2014. *Rickettsia africae* DNA was detected in 30.2% (16/53) *Amblyomma variegatum*, 28.6% (12/42) *Am. gemma*, 0.8% (1/119) *Am. cohaerens*, 18.2% (4/22) *Amblyomma* larvae, 6.7% (2/60) *Amblyomma* nymphs, 0.7% (1/139) *Rhipicephalus* (*Boophilus*) *decoloratus* and 25% (1/4) nymphs of *Rh.* (*Bo.*) *decoloratus*. A markedly low prevalence of *R. africae* was recorded in both *Am. cohaerens* and *Rh. (Bo.) decoloratus* ($p < 0.0001$) compared with that in *Am. variegatum* and *Am. gemma*. The prevalence of *R. africae* was markedly low in the western districts (Gachi and Abdela) ($p < 0.0001$); however, the prevalence of *R. africae* was relatively high in the central (Ada'a, Wolmara and Arsi) and eastern (Arero, Moyale and Yabelo) districts, where *Am. variegatum* and *Am. gemma* were predominantly associated with *R. africae*, respectively. *R. aeschlimannii* DNA was detected in 45.4% (5/11) *Hyalomma marginatum rufipes* and 2.2% (1/46) *Hy. truncatum*. Moreover, the first report of *R. massiliae* DNA in 1.9% (1/52) *Rhipicephalus praetextatus* ticks in Ethiopia is presented herein. Altogether, these results suggest that the transmission of spotted fever group rickettsiae through ixodid ticks is a potential risk for human health in different parts of Ethiopia. Clinicians in this country should consider these pathogens as a potential cause of febrile illness in patients.



## Introduction

Ticks are obligate hematophagous arthropods that act as reservoirs and vectors for a wide range of human and animal pathogens worldwide. Approximately 10% of the currently recognized tick species carry human and animal pathogens. Currently, ticks and mosquitoes are the major vectors for human and animal disease agents (Jongejan and Uilenberg, 2004; Parola and Raoult, 2001). Recent studies have indicated an increase in the spectrum of tick-borne pathogens affecting humans and animals (Dantas-Torres et al., 2012; de la Fuente and Estrada-Pena, 2012; Nicholson et al., 2010).

Ticks are common and widely distributed throughout Ethiopia (Mekonnen et al., 2001). Several species of ixodid ticks have been identified in Ethiopia and are considered to have more veterinary significance than medical importance (Kumsa et al., 2012). Most of the studies on the role of ixodid ticks as vectors of human pathogens in Ethiopia were conducted before the discovery of well-advanced molecular tools during the 1950s and 60s (Burgdorfer et al., 1973; Philip et al., 1966). Indeed, the isolation of *Rickettsia prowazekii*, the causative agent of epidemic typhus, from *Amblyomma* ticks feeding on Ethiopian cattle remains a mystery and had never been confirmed in any other studies conducted in Ethiopia (Burgdorfer et al., 1973).

Spotted fever group (SFG) rickettsiae are small, obligate intracellular, short rod, Gram-negative bacteria belonging to the genus *Rickettsia*, the family Rickettsiaceae and the order Rickettsiales (Parola et al., 2013). The genus *Rickettsia* comprises 3 main biogroups: the 'spotted fever group' (SFG), primarily transmitted through ixodid ticks, except *R. felis* and *R. akari*, which are vectored through fleas and mites, respectively; the 'typhus group' (TG), transmitted through fleas and lice; and the 'scrub typhus group' (STG), primarily vectored through chiggers (Parola, 2011; Parola et al., 2013). Currently, the genus *Rickettsia* comprises 31 species that cause diseases in vertebrate hosts, including humans, domestic animals, birds, and wildlife. Some ticks have also been implicated as SFG rickettsiae reservoirs, as these insects maintain rickettsiae both transstadially and transovarially.

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In humans, spotted fever rickettsia induces symptoms that generally include fever, headache, myalgia, rash, local lymphadenopathy, and an eschar at the site of the tick bite, which might be useful in diagnosis (Parola et al., 2013). *Rickettsia africae*, the causative agent of ATBF, typically causes symptoms, including inoculation eschars, fever, regional lymphadenopathies and the frequent lack of cutaneous rash or pale vesicular eruptions, and the distribution of this pathogen is consistent with the geographical distribution of *Amblyomma* ticks (Parola et al., 2013). *Rickettsia aeschlimannii* is an emerging pathogen recently detected in human patients in Morocco, South Africa, Algeria, Tunisia and Greece, causing clinical symptoms resembling Mediterranean spotted fever caused by *R. conorii* (fever, generalized maculopapular rashes and an escar at the tick-bite site) (Parola et al., 2013). *Rickettsia massiliae* is a pathogenic SFG rickettsia associated with clinical symptoms, such as fever, eschar, night sweats, headache, maculopapular rash and necrotic skin lesions, in human patients in America, Europe and Africa (Parola et al., 2013).

Previous studies on rickettsiae in Ethiopian ixodid ticks have documented the presence of *Rickettsia* spp. in *Amblyomma variegatum*, *Amblyomma cohaerens* and *Rhipicephalus* spp. in central Ethiopia (Philip et al., 1966) and *Amblyomma* spp. (*Amblyomma gemma, Am. variegatum* and *Am. cohaerens*) in the central and eastern regions of Ethiopia (Burgdorfer et al., 1973). *R. aeschlimannii*, the agent of SFG rickettsiosis, had been detected in *Hyalomma marginatum rufipes* and *R. africae*, the agent of African tick bite fever (ATBF), has been detected in *Amblyomma lepidum* and *Am. variegatum* ticks from eastern Ethiopia (Mura et al., 2008), and also *R. africae* had been detected in pools of *Amblyomma* and *Rhipicephalus* ticks (Pader et al., 2012) (summarized in Fig. 1).

In Ethiopia, where the environment is suitable for ticks, 84% of the population is involved in agriculture and farmers and their family members interact with ectoparasite-infested animals on daily life activities. Moreover, the confirmatory diagnosis of fever and other diseases with unknown etiologies is not commonly practiced, reflecting the lack of health centers in many regions of the country. Therefore, information concerning ectoparasite-borne bacteria is extremely important. To update the knowledge on tick-borne rickettsiae in Ethiopia, the aim of the present study was to address the occurrence and molecular identity of *Rickettsia* species in ixodid ticks collected from domestic animals in nine districts in Oromia Regional State in Ethiopia.

## Materials and methods

### *Study areas and animals*

In this study, tick samples were collected from cattle, sheep, dogs and cats in the following districts in Oromia Regional State in Ethiopia: Arsi (7°56′2.36″ N, 39°39′6.54″ E), Wolmara (9° 6′17.87″ N, 38°28′8.50″ E), Kimbibit (9°24′39.95″ N, 39°21′14.75″ E), Ada'a (9°31′60.00″ N, 38°18′0.00″ E), Bedele (8°27′1.76″ N, 36°21′5.08″ E), Gachi (9°14′2.57″ N, 35°54′48.46″ E), Arero (4°43′33.28″ N, 38°45′46.78″ E), Moyale (3°31′60.00″ N, 39° 3′0.00″ E) and Yabelo (4°53′41.91″ N, 38° 6′0.59″ E). The districts are located in six zones in the central, southwestern and southeast regions of the country, with various climates and agroecology. The livestock in the study areas are traditionally managed under extensive production systems (CSA, 2008).

All the study animals were selected irrespective of sex and age. The animals were categorized into two age groups, young (up to one year) and adult (older than one year), according to a previous publication (Kumsa et al., 2012).

### *Collection and identification of ticks*

Ticks were collected from September through November of 2011. A thorough visual examination of the body surfaces of each study animal was conducted to establish the presence or absence of ticks. All observed ticks attached to the skin of each animal were carefully removed using forceps or by hand to avoid any damage to the body, and the specimen were individually placed into small, pre-labeled plastic tubes containing 70% ethanol for subsequent identification as previously described (Kumsa et al., 2012, 2014a). All ticks from the same animal were placed into the same vial and transported to the Laboratory of the World Health Organization Collaborative Center for Rickettsial Diseases and Arthropod-borne Bacterial Diseases in Marseille, France.

The morphological identification of ticks and molecular studies were performed from January 2012 through March 2014. All adult ticks were identified at the species level, and the larvae and nymphs were microscopically identified at the genus level using previously described morphological identification keys (Hoogstraal, 1956; Walker et al., 2000, 2003). The sex and stage of each tick was determined, and photographs of the dorsal and ventral body parts of each tick specimen were captured. The tick genera are abbreviated as previously described (Dantas-Torres, 2008).

### *DNA extraction from ticks*

Prior to DNA extraction, each tick specimen was rinsed twice in sterile water for 15 min, dried on sterile filter paper, and longitudinally dissected into two equal halves. One half of each specimen was retained as reserve sample to avoid the risk of losing samples for any reason during or after DNA extraction (Kumsa et al., 2014a, 2014b). Genomic DNA was individually extracted from a total of 767 tick specimens using the QIAamp DNA tissue extraction kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. In engorged tick DNA was extracted from small portion of the anterior part of one half of the ticks so as to minimize the PCR inhibitory effect of large amount of blood in the abdomen. 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 PCR. To avoid cross-contamination among the samples during DNA extraction, the DNA extracting EZI Advanced XL Robot (Qiagen, Hilden, Germany) was thoroughly disinfected after each extraction according to the manufacturer's recommendations. The second half of each tick specimen was stored at −80 °C as a backup sample.

### *Molecular detection of Rickettsia species*

As a first step, all DNA samples were individually tested using a genus-specific qPCR system targeting the *glt*A gene (RKND03 system (Rolain et al., 2002): RKND03F-5′-GTG-AAT-GAA-AGA-TTA-CAC-TAT-TTA-T-3′, RKND03R- 5′-GTA-TCT-TAG-CAA-TCA-TTC-TAA-TAG-C-3′ and RKND03P- 6-FAM-CTA-TTA-TGC-TTG-CGG-CTG-TCG-GTT-C-TAMRA) in SFG rickettsiae and the Rpr274P gene in typhus group rickettsiae as previously described (Mediannikov et al., 2010a; Socolovschi et al., 2012). Sterile water was used as a negative control, and DNA from *R. montanensis* and *R. typhi* were used as positive controls for SFG and typhus group *Rickettsia*, respectively. All DNA samples positive for the *glt*A gene (RKND03 system) were further confirmed using species-specific genes for SFG *Rickettsia* mentioned below.

The *Amblyomma* spp. ($n = 37$) and *Rhipicephalus* (*Boophilus*) *decoloratus* ($n = 2$) DNA samples positive for SFG *Rickettsia* were further tested using a previously described *R. africae* species-specific qPCR targeting the *ITS* gene (Mediannikov et al., 2012a). Sterile