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Detection of *Rickettsia aeschlimannii* and *Rickettsia africae* in ixodid ticks from Burkina Faso and Somali Region of Ethiopia by new real-time PCR assays

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A B S T R A C T

In the framework of cooperation for development projects in Burkina Faso and Ethiopia, we collected ixodid ticks from cattle, small ruminants and camels. We optimized new TaqMan Probe real-time PCR assays to detect *Rickettsia aeschlimannii* and *Rickettsia africae OmpA* gene in the collected samples.

Rickettsia africae was identified in 75.0% *Amblyomma variegatum* (95%CI: 56.6–88.5), while *R. aeschlimannii* in 24.0% *Hyalomma truncatum* (95%CI: 9.4-45.1) and 50.0% *H. rufipes* (95%CI: 29.9–70.0) collected from cattle in different provinces throughout Burkina Faso. Ticks from the Libaan zone, Somali Region of Ethiopia, were also infected by *R. africae* (28.5% prevalence in *Amblyomma gemma*, 95%CI: 14.7–46.0) and *R. aeschlimannii* (27.0% *H. truncatum*, 95%CI: 5.0–62.9; 88.3% *H. rufipes*, 95%CI: 60.5–99.3). All tested ticks were adults.

The developed diagnostic tools were highly sensitive and enabled us to rapidly classify *R. aeschlimannii* and *R. africae*, which were identified in Burkina Faso and in the Somali Region of Ethiopia for the first time. Further studies are needed to assess the zoonotic risk and prevalence of infection in local human populations, who have high contact rates with ticks and their animal hosts.

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1. Introduction

Tick-borne rickettsiae are pathogens belonging to the Spotted Fever Group (SFG). In the past few years, an increasing number of studies have been performed providing new understanding on the zoonotic role and diversity of these agents. Such researches also contributed to clarify their geographical distribution; indeed, some rickettsiae which were previously considered to be restricted to a specific geographical area have then been detected in different continents (Parola et al., 2013).

Scarce information is available from Africa, as rickettsial agents often cause mild disease and do not usually get diagnosed. *Rickettsia africae* is the most widespread SFG rickettsia in sub-Saharan Africa, where it causes the African tick-bite fever (ATBF; Kelly et al., 1996). The disease was firstly described in the 1930s in South Africa as a rural disease occurring in people having contact with cattle ticks (Pijper, 1934). ATBF is rather common in travellers to rural sub-Saharan Africa and is transmitted by *Amblyomma* ticks, mainly *A. hebraeum* and *A. variegatum* (Jensenius et al., 2003).

Rickettsia aeschlimannii is characterized by a more heterogeneous geographical distribution and was detected in *Hyalomma*, *Rhipicephalus* and *Haemaphysalis* spp. ticks from several continents. *R. aeschlimannii* causes symptoms similar to Mediterranean Spotted Fever, which have been reported so far in patients travelling from Africa or, at a lesser extent, in African patients (Parola et al., 2013).

In the light of the increasing need for sensitive diagnostic tools for identifying emerging and re-emerging rickettsial infections, we set up new quantitative real-time PCR protocols for the detection of *R. africae* and *R. aeschlimannii*, and we applied them to screen ticks from livestock in the framework of two cooperation and research-development projects in the Somali Region of Ethiopia

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Table 1
Prevalence of infection by *R. africae* and *R. aeschlimannii*, obtained by *OmpA* gene real-time PCR, in adult ticks collected on livestock from the Somali Region of Ethiopia and Burkina Faso. CI = confidence intervals.

Study area	Tick species	N tested	% prevalence of *Rickettsia* spp. (95%CI)	
			R. africae	*R. aeschlimannii*
Somali Region of Ethiopia	*A. gemma*	37	28.5 (14.7–46.0)	0
	H. impeltatum	4	0	100 (0.0–100)
	H.rufipes	21	0	88.3 (60.5–99.3)
	H. truncatum	8	0	27.0 (5.0–62.9)
Burkina Faso	*A.variegatum*	32	75.0 (56.6–88.5)	0
	H.rufipes	26	0	50.0 (29.9–70.0)
	H. truncatum	25	0	24.0 (9.4–45.1)

and in Burkina Faso. Our results contribute to the knowledge on the distribution of SFG rickettsiae and their vectors in African Countries.

2. Materials and methods

2.1. Tick collection

Ticks from Burkina Faso were collected in 2005–2006, during a nation-wide tick survey conducted by CIRDES (*Centre International de Recherche-Développement sur l'Elevage en zone Subhumide*) (Adakal et al., 2013), and in June-July 2012, from cattle at the Bobo-Dioulasso abattoir ('*abattoir frigorifique*'; 11°21′N, 4°17′W). CIRDES ticks originated from the localities of Banfora (10°38′N, 4°45′W), Fada (12°04′N, 00°21′E), Gaoua (10°20′N, 3°11′W), Manga (11°39′N, 01°3′W), Orodara (10°58′N, 4°54′W), Séba (13°25′N, 0°32′E), Tiébora (10°40′N, 4°15′W). They included 35 *Hyalomma truncatum* and 51 *H. rufipes* adults. As regards the abattoir ticks, they were randomly gathered in different body sites, during a limited time period (around 5 min per animal), soon before slaughter; most of the cattle were from villages within a 25–30 km radius from town. Ticks from each animal were preserved in separate labelled vials containing 70% ethanol, and subsequently identified to species level using the identification keys described in Walker et al. (2000, 2003). Ninety-nine of the examined animals at the Bobo Dioulasso abattoir were infested by ticks; we collected 145 specimens, namely 50 *A. variegatum* (35 males, 10 females, 5 nymphs), 22 *H. truncatum* (13 males, 9 females), 44 *H. rufipes* (31 males, 13 females), 18 *Rhipicephalus (Boophilus) geigyi* (one male, 17 females), 6 *R. (Bo.) decoloratus* females and 5 *R. (Bo.)* spp. females, which could not be identified to species level since they were damaged.

Ticks from Ethiopia were collected in 2006–2007 in Filtu and Dollo districts, Libaan zone (located from 03°58′ to 5°19′N, and from 39°56′ to 42°03′E), Somali Region, from nomadic herds of cattle (Borana zebu), dromedaries, sheep and goats. Numbers, species and stage of collected ticks are described in Tomassone et al. (2012).

2.2. Tick processing and DNA extraction

A sample of adult *Hyalomma* spp. and *Amblyomma* spp. ticks from Burkina Faso was randomly selected (Table 1). As far as ticks collected from the abattoir, we chose two specimens of each species, if present, from each animal. We also tested *Hyalomma* and *Amblyomma* spp. DNA extracts from Ethiopian ticks; these samples comprised individual ticks and pooled ticks in batches of 2–3 specimens of the same species and sex from the same animal (Tomassone et al., 2012). In both cases, ticks were cut with a sterile blade and homogenized with a pestle in microcentrifuge tubes; DNA was extracted by DNeasy Blood and Tissue kit (QIAGEN, Valencia, CA, USA). The tick engorgement index was evaluated as the ratio of body length to *scutum* width (Yeh et al., 1995) for ticks extracted individually.

2.3. Molecular analysis

Real-time quantitative PCR (RT-PCR) targeting *R. africae* and *R. aeschlimannii OmpA* gene were developed *ad hoc* for this study. SFG rickettsiae OmpA gene sequences were aligned to design the primers (RafrS: AATGATATAACGGCTGAAG, RafrA: GTAAGTAAATGCCATACCA; RaeschS: ATGATATAACGGCTGAAG, RaeschA: GTAAGTAAATGCCATACCA) and the specific TaqMan probes (R. africae: CTAATGGTACTCCTGTTGATGGTCC, R. aeschlimannii: CTAATGGTACTCCTGTTGATGGTCC) marked 5′-FAM e 3′-TAMRA. The amplified fragments of *OmpA* gene were 80 bp and 73 bp respectively. The first probe hybridised to the OmpA genes of both *R. africae* and *R. slovaca*, due to their high similarity. The PCR assays were run in an Applied biosystems 7300 thermal cycler using the following protocol: 50 °C for 2′, 95 °C for 3′, [35x] 95 °C for 3änd 60 °C for 30″. Plasmid standard curves for absolute quantification of DNA copy number were used. The two amplicons were cloned into pCR-XL-TOPO cloning vector (Life Technologies). The identity of inserts were confirmed by sequencing. After plasmids preparation, the standard curves with known copy numbers of insert were generated from the plasmids by amplification of 100 fold serial dilutions of 10^7 to 10^1 DNA copies per reaction. The dilutions were also used to test the sensitivity limits of the assays: a log-10 titration series of plasmid indicated that less than 10 copies of *OmpA* gene segment could be detected. All experiments were conducted in duplicate, in a 10 µl volume. To evaluate the RT-PCR specificity, the DNA of *R. conorii, R. massiliae, R. monacensis, R. parkeri, R. raoultii, R. rickettsii* and *R. slovaca* were tested.

All the samples included in this study were subjected to RT-PCR and to end-point classical diagnostic PCR for *gltA* (CS-78 and CS-323 primers; Labruna et al., 2004) and *OmpA* genes (Rr190.70F and Rr190.602R primers; Regnery et al., 1991). Amplicons from classical *OmpA* PCR were purified using ExoSAP-IT PCR Clean-up Kit (GE Healthcare Limited, Chalfont, UK) and sent to an external service (Macrogen Inc., Amsterdam, The Netherlands) for automatic sequencing.

In all PCR reactions, *R. africae* and *R. aeschlimannii* DNA were used as positive controls and distilled water as negative control.

2.4. Statistical analysis

Prevalence of PCR-positive results per geographic area and tick species were calculated, with 95% exact binomial confidence intervals (95%CI). Prevalence of infection in tick pools were calculated by using the Pooled Prevalence Calculator (Sergeant, 2009), with a 95% confidence level. We evaluated the association of possible risk factors (animal hosts, and tick species, sex, engorgement index) with the infection prevalence (using Fisher Exact test) and with rickettsial load (using Wilcoxon signed rank test or Kruskal-Wallis test). Analyses were performed by using R software (R Development Core Team, 2015)

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