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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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Abstract

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 populations, who have high contact rates with ticks and their animal hosts.

Keywords: Spotted Fever Group rickettsiae, Zoonoses, Burkina Faso, Ethiopia, real-time PCR

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 contribute 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 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'Élevage 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 animals 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) geigy* (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 tick specimens of the same species and sex belonging to 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 3", 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 10µl 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)

2.5 Sequence analysis and phylogenetic analysis

Chromas 2.0 software (Technelysium, Helensvale, Australia) and BLAST (<http://blast.ncbi.nlm.nih.gov/>) were used to edit the chromatograms and to identify similarities to known sequences. Sequences were aligned with reference sequences available in GenBank using ClustalW (Thompson et al., 1997). ModelTest ver. 3.7 (Posada and Crandall, 2001) and PAUP*(ver. 4.0b10; Swofford, 2003) were used to estimate the model of molecular evolution by a hierarchical likelihood ratio test approach and Akaike information criterion (Akaike, 1973). MrBayes (ver. 3.1.1; Ronquist and Huelsenbeck, 2003) was used to create the phylogenetic trees; a Markov chain Monte Carlo was run for a total of 1,000,000 generations using 2 runs with 4 chains, and results were represented as a 50% majority rule consensus

tree. The pairwise genetic distance comparisons were calculated by the Nei–Gojobori method (Nei and Gojobori, 1986).

3. Results

The newly developed RT-PCR assays showed a high sensitivity and specificity. The detection limit of both assays, based on the standard curve dilutions, resulted less than 10 DNA copies. Positive controls were correctly identified. No cross-reactions with other *Rickettsia* spp. were observed, except for the *R. africae* probe that hybridized to *R. slovaca* as expected. RT-PCR showed a good reproducibility when standards were tested in duplicate.

By testing Burkina Faso ticks, we detected *R. africae* in 75.0% *A. variegatum*, collected from 20 infested animals from the abattoir (number of positive ticks=24). *R. aeschlimannii* infected 7 *H. rufipes* from the abattoir and 6 from the CIRDES collection (from the localities of Fada, Gaoua, Manga, Séba, Tiébora), with an overall prevalence of 50.0%. Moreover, we detected *R. aeschlimannii* in 24% *H. truncatum*, namely 2 specimens from the abattoir and 4 from CIRDES tick collection (from Banfora, Gaoua, Manga, Orodara) (Table 1). As regards Ethiopian samples, *R. africae* infected 28.5% *A. gemma* collected from dromedaries and cattle in Filtu and Dollo. *R. aeschlimannii* was found in 27.0% *H. truncatum* (from dromedaries and sheep, from Dollo), 88.3% *H. rufipes* (from cattle, dromedaries, and goats, from Filtu and Dollo) and in the 4 tested *H. impeltatum* from Dollo (from dromedaries and cattle) (Table 1).

The end-point *OmpA* PCR succeed to identify *R. africae* only in 21.9% of RT-PCR positive samples (n=7) and in 22.9% *R. aeschlimannii* RT-PCR positives (n=8). The sequences obtained from the end-point *OmpA* PCR confirmed the RT-PCR identification results. A 100% identity was observed among our *R. africae* sequences, and among *R. aeschlimannii* ones,

independently from the tick species and geographic area. Phylogenetic analysis indicates that *R. africae* sequences group with the reference sequences from Liberia (JN043509), and that *R. aeschlimannii* samples are 100% similar to strains detected in Egypt (HQ335158) and Senegal (HM050284) (Figure 1). Our sequences were submitted to GenBank with the accession no. KX063614-21.

All *Amblyomma* spp. ticks were negative to *R. aeschlimannii* PCR, and all *Hyalomma* spp. were negative to *R. africae*. No infection by other rickettsial species was detected in the RT-PCR negative samples by testing them with the classical PCR.

Tick infection prevalence was not significantly associated with tick sex, engorgement index (for individually extracted ticks), and with the fact that DNA was extracted from individual or pooled samples. Accordingly, no differences in infection prevalence were found among ticks collected from different vertebrate hosts. There was a significantly higher *R. aeschlimannii* load in *Hyalomma* ticks from Ethiopia compared to Burkina Faso ($p < 0.01$); this difference was not associated with the vertebrate host species, neither with the tick species, and the number of ticks in the extraction pools. We did not record differences in the copy number of the two rickettsiae among the different tick species within the *Hyalomma* and *Amblyomma* genera.

4. Discussion

As far as we know, no data on SFG rickettsiae in Burkina Faso are available in the literature, so we report for the first time *R. aeschlimannii* and *R. africae* in this Country. We also describe the two rickettsial species in the Somali Region of Ethiopia, integrating previous reports from the Didessa valley (South-Western Ethiopia; Hornok et al., 2014) and Oromia region (Kumsa et al., 2015).

The *OmpA* gene amplification is widely used for the molecular diagnosis of rickettsiosis and species characterization (Fournier et al., 2003). Our *OmpA* gene RT-PCR assays are rapid to perform and very sensitive. They could be employed also in leading research labs in developing countries, where local researchers have nowadays good experience in molecular techniques; besides, these assays are run in a total of 10ul MasterMix, and being this relatively cheap, it makes their use sustainable also in limited-resource laboratories in African Countries.

As regards the *R. africae* probe, it can be also usefully employed for the detection of *R. slovaca* in endemic areas, due to its ability to identify the two pathogens; further discrimination is needed when testing samples in areas where both *R. africae* and *R. slovaca* are, or might be present (Mediterranean countries).

The limitations of this study are the few sequences obtained, from the *OmpA* classic PCR, to compare with the real time PCR results. However, the correct identification of the negative and positive controls used throughout the experiment confirmed the specificity of the assay.

Our finding of a 75% *R. africae* infection in *A. variegatum* from Burkina Faso is in accordance with other studies in Africa, where remarkably high *R. africae* infection rates -up to 100%- are reported in *Amblyomma* spp. (Parola et al., 2005). Transstadial and transovarial transmission were demonstrated in *A. variegatum* (Socolovschi et al., 2009), which is recognized as primary vector of *R. africae* together with *A. hebraeum*. Studies in other Western Africa sub-Saharan countries (Table 2) reported *A. variegatum* infection prevalence varying from 62% to 100%. *R. africae* has been detected in other *Amblyomma* species, including *A. gemma*, as we also found in the present study: 28.5% *A. gemma* from the Somali Region were infected, showing a prevalence almost identical to that reported in the Oromia Region of Ethiopia (28.6%) by

Kumsa et al. (2015). A higher infection rate (over 60%) had been observed in *A. gemma* from Kenya (Mutai et al., 2013).

Contrary to our findings, *R. africae* is reported in literature from *Hyalomma* spp. ticks. Indeed, it was detected in *H. truncatum* and *H. rufipes* in several North African and sub-Saharan Countries (Table 2). Other findings refer to Israel, including infection in *H. impeltatum* (Kleineremann et al., 2013), Turkey (Orkun et al., 2014) and to *Hyalomma* spp. collected from migratory birds in southern Europe (Wallmenius et al., 2014; Toma et al., 2014).

We detected *R. aeschlimannii* in *H. rufipes* and *H. truncatum* from both Burkina Faso and Ethiopia. This is in accordance with previous studies in West and East Africa (Table 3). *R. aeschlimannii* was shown to be transmitted transstadially and transovarially in *Hyalomma* spp. (Matsumoto et al., 2004; Socolovschi et al. 2009). The few *H. impeltatum* collected in Ethiopia were also positive, as previously reported in Senegal and Egypt (Table 3).

R. africae and *R. aeschlimannii* infection rates in endemic areas can thus be high in the vector ticks (Parola et al., 2001; Nakao et al., 2013), which can act as reservoir when the transovarial and transstadial transmission are efficient (Abdel Shafy et al., 2012). Little is known about the reservoir role of vertebrate hosts for several *Rickettsia* species (Parola et al., 2013). Reye et al. (2012) suggested the possible implication of cattle as reservoir hosts for *R. africae* and *R. aeschlimannii*, but this hypothesis needs further support. We did not find any differences in the infection prevalence and rickettsial load among ticks collected on different hosts and in ticks with different engorgement index and sex. This supports the hypothesis of a negligible contribution of vertebrate hosts to the infection of feeding ticks, which is maintained in the tick host.

Some *Amblyomma* and *Hyalomma* species commonly feed on humans (Estrada-Peña and Jongejan, 1999). However, in African rural areas, arboviral or rickettsial infections are rarely

taken into consideration when diagnosing acute febrile illnesses in humans (Ndip et al., 2004a). Moreover, some authors suggest a reduced virulence of some *R. africae* variants in the indigenous human populations, owing to hosts adaptation and competitive exclusion (Maina et al., 2014). Cases of acute rickettsiosis (likely ATBF) in natives were demonstrated by serology and PCR in Cameroon (Ndip et al., 2004a, b), where a 26.9% *R. africae* seroprevalence was registered from rural villages (Ndip et al., 2011). Spotted fever group rickettsiae seroprevalence ranged from 21.4 to 51% in rural population in Senegal (Mediannikov et al., 2012), from 0.5% to 3.1% in healthy pregnant women in Madagascar (Keller et al., 2016), while 8.0% of febrile patients were seropositive in Moshi, Kenya (Prabhu et al., 2011). Further investigations are needed to evaluate the extent of the zoonotic risk and prevalence of infection in livestock keepers, local villagers and resident populations in Africa, who have high contact rates with ticks and their vertebrate hosts.

Travel-associated rickettsial infections, on the other side, are rather frequent in tourists which go hunting, trekking and visiting rural areas of sub-Saharan Africa (Jensenius et al., 2002). Although most cases are clinically mild, cardiac and neurological complications are possible in case of ATBF (Delord et al., 2014). Thus, an increase in the awareness and surveillance on these zoonotic agents is needed also in continental Europe, where pathogens and infected ticks can be spread by avian hosts along their migratory routes, as it was highlighted by the recent findings of *Hyalomma* spp. nymphs infected by *R. aeschlimannii* in Germany and Hungary (Rumer et al., 2011; Hornok et al., 2013).

In this framework, there is a need of enhancing the collaboration between veterinarians and medical doctors and raise the awareness of SFG rickettsiae as public health problem, and, at the same time, contributing to spread and divulgate the so-called 'One Health' approach.

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Tables and Figure

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 <i>Rickettsia</i> spp. (95%CI)	
			<i>R. africae</i>	<i>R. aeschlimannii</i>
Somali Region of Ethiopia	<i>A. gemma</i>	37	28.5 (14.7-46.0)	0
	<i>H. impeltatum</i>	4	0	100 (0.0-100)
	<i>H. rufipes</i>	21	0	88.3 (60.5-99.3)
	<i>H. truncatum</i>	8	0	27.0 (5.0-62.9)
Burkina Faso	<i>A. variegatum</i>	32	75.0 (56.6-88.5)	0
	<i>H. rufipes</i>	26	0	50.0 (29.9-70.0)
	<i>H. truncatum</i>	25	0	24.0 (9.4-45.1)

Table 2. Bibliographic reports of *R. africae* in ixodid ticks in Africa, by tick species and Country.

Tick species	Country
<i>Amblyomma cohaerens</i>	Ethiopia (Hornok et al., 2014; Kumsa et al., 2015)
<i>Amblyomma compressum</i>	Liberia (Mediannikov et al., 2012)
<i>Amblyomma gemma</i>	Ethiopia (Kumsa et al., 2015; this study), Kenya (Mutai et al., 2013)
<i>Amblyomma hebraeum</i>	Kenya (Mutai et al., 2013)
<i>Amblyomma lepidum</i>	Ethiopia (Hornok et al., 2014), Republic of Djibouti (Socolovski et al., 2007; Mura et al., 2008), Sudan (Parola et al., 2001; Morita et al., 2004)
<i>Amblyomma variegatum</i>	Burkina Faso (this study), Burundi (Parola et al., 2001), Ethiopia (Hornok et al., 2014; Kumsa et al., 2015), Guinea (Mediannikov et al., 2012), Kenya (Macaluso et al., 2003; Mutai et al., 2013; Maina et al., 2014), Liberia (Mediannikov et al., 2012), Madagascar (Keller et al., 2016), Mali (Parola et al., 2001), Niger (Parola et al., 2001), Nigeria (Ogo et al., 2012), Republic of Djibouti (Mura et al., 2008), Senegal (Mediannikov et al., 2010; Sambou et al., 2014), Sudan (Morita et al., 2004; Nakao et al., 2015), Uganda (Lorusso et al., 2013; Nakao et al., 2013), Union of the Comoros (Yssouf et al., 2014)
<i>Haemaphysalis paraleachi</i>	Guinea (Mediannikov et al., 2012)
<i>Hyalomma dromedarii</i>	Algeria (Kernifet et al., 2012), Egypt (Abdel Shafy et al., 2012)
<i>Hyalomma impeltatum</i>	Algeria (Djebou et al., 2012), Egypt (Abdel Shafy et al., 2012)
<i>Hyalomma marginatum</i>	Egypt (Abdel Shafy et al., 2012)
<i>Hyalomma rufipes</i>	Guinea (Mediannikov et al., 2012), Senegal (Sambou et al., 2014)
<i>Hyalomma truncatum</i>	Kenya (Mutai et al., 2013)
<i>Rhipicephalus (Boophilus) annulatus</i>	Kenya (Mutai et al., 2013)
<i>Rhipicephalus (Boophilus) decoloratus</i>	Ethiopia (Hornok et al., 2014; Kumsa et al., 2015), Guinea (Mediannikov et al., 2012), Nigeria (Ogo et al., 2012)
<i>Rhipicephalus (Boophilus) geigy</i>	Liberia (Mediannikov et al., 2012)
<i>Rhipicephalus (Boophilus) microplus</i>	Union of the Comoros (Yssouf et al., 2014)
<i>Rhipicephalus appendiculatus</i>	Kenya (Macaluso et al., 2003; Mutai et al., 2013), Union of the Comoros (Yssouf et al., 2014)
<i>Rhipicephalus evertsi evertsi</i>	Senegal (Mediannikov et al., 2010)
<i>Rhipicephalus pulchellus</i>	Kenya (Mutai et al., 2013)
<i>Rhipicephalus sanguineus sensu lato</i>	Nigeria (Ogo et al., 2012)

Table 3. Bibliographic reports of *R. aeschlimannii* in ixodid ticks in Africa, by tick species and Country.

Tick species	Country
<i>Hyalomma marginatum</i>	Algeria (Bitam et al., 2006), Egypt (Abdel Shafy et al., 2012), Morocco (Beati et al., 1997; Sarih et al., 2008), Sudan (Morita et al., 2004)
<i>Hyalomma rufipes</i>	Algeria (Djebou et al., 2012), Burkina Faso (this study), Chad (Mura et al., 2008), Egypt (Loftis et al., 2006), Ethiopia (Kumsa et al., 2015; this study), Mali (Parola et al., 2001), Niger (Parola et al., 2001), Republic of Djibouti (Mura et al., 2008), Senegal (Mediannikov et al., 2010; Sambou et al., 2014), Sudan (Parola et al., 2001), Zimbabwe (Beati et al., 1995)
<i>Hyalomma truncatum</i>	Burkina Faso (this study), Ethiopia (Kumsa et al., 2015; this study), Kenya (Mutai et al., 2013), Senegal (Mediannikov et al., 2010), Sudan (Morita et al., 2004)
<i>Hyalomma dromedarii</i>	Algeria (Djebou et al., 2012), Egypt (Loftis et al., 2006), Sudan (Morita et al., 2004), Tunisia (Demoncheaux et al., 2012)
<i>Hyalomma impeltatum</i>	Egypt (Abdel Shafy et al., 2012; Loftis et al., 2006), Senegal (Sambou et al., 2014)
<i>Hyalomma aegyptium</i>	Algeria (Bitam et al., 2009)
<i>Hyalomma detritum</i>	Algeria (Bitam et al., 2006)
<i>Rhipicephalus evertsi evertsi</i>	Senegal (Mediannikov et al., 2010; Sambou et al., 2014)
<i>Rhipicephalus (Boophilus) annulatus</i>	Kenya (Mutai et al., 2013)

Figure 1. Phylogenetic tree based on partial *OmpA* gene nucleotide sequences (530bp) of *Rickettsia* spp. Posterior probability values are reported below branches. New Ethiopia sequences are marked with a black point and Burkina Faso ones with a square (this study). Bar: 0.05 substitutions per site.

