Ticks and Tick-borne Diseases 10 (2019) 924–928

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

journal homepage: www.elsevier.com/locate/ttbdis

Original article

Presence of *Rickettsia aeschlimannii*, '*Candidatus* Rickettsia barbariae' and *Coxiella burnetii* in ticks from livestock in Northwestern Algeria

Karim Abdelkadir[a], Ana M. Palomar[b], Aránzazu Portillo[b], José A. Oteo[b,*], Khatima Ait-Oudhia[a], Djamel Khelef[a]

[a] High National Veterinary School, Issad Abbes Avenue, Oued Smar, Algiers, Algeria
[b] Center of Rickettsiosis and Arthropod-Borne Diseases, Hospital Universitario San Pedro-CIBIR, Logroño, Spain

ARTICLE INFO

Keywords:
Spotted fever group rickettsiae
Anaplasmataceae
Coxiella burnetii
Livestock
Ticks
Algeria

ABSTRACT

Livestock and their ectoparasites are involved in the epidemiology of several zoonotic diseases. Studies regarding the molecular detection of infectious agents in ticks from Northwestern Algeria are scarce. Thus, the presence of spotted fever group *Rickettsia* spp., Anaplasmataceae microorganisms and *Coxiella burnetii* was investigated in ticks collected from ruminants in Sidi Bel Abbes and Saida provinces. *Rickettsia aeschlimannii* was detected in one *Hyalomma excavatum* pool and one *H. marginatum* pool. Moreover, '*Candidatus* Rickettsia barbariae' was found in one *H. excavatum* and six *Rhipicephalus bursa* pools. Lastly, *Coxiella burnetii* was amplified in two *H. excavatum* and two *R. bursa* pools. No Anaplasmataceae bacterium was detected. This study demonstrates the presence of the tick-associated microorganism '*Candidatus* R. barbariae' in the North of Africa, and corroborates the presence of the zoonotic pathogens *R. aeschlimannii* and *C. burnetii* in Algeria.

1. Introduction

Ticks are considered, after mosquitoes, to be the most important vectors of a large diversity of human and animal pathogens all over the world (Jongejan and Uilenberg, 2004; Parola et al., 2013; García-Álvarez et al., 2013). Livestock such as cattle, sheep and goats are hosts and possible reservoirs of a variety of pathogens carried and transmitted by ticks (Wardeh et al., 2015).

Arthropod-borne rickettsioses, caused by several *Rickettsia* species, are among the oldest known vector-borne diseases (Parola et al., 2013). To date, nine pathogenic spotted fever group (SFG) rickettsiae have been detected in Algeria. These include *Rickettsia conorii* subsp. *conorii*, *Rickettsia aeschlimannii*, *Rickettsia massiliae*, *Rickettsia monacensis*, *Rickettsia africae*, *Rickettsia sibirica* subsp. *mongolitimonae*, *Rickettsia slovaca*, *Rickettsia helvetica* and *Rickettsia felis* (Bitam et al., 2006; Dib et al., 2009; Mouffok et al., 2009; Kernif et al., 2012; Mokrani et al., 2012).

Anaplasmosis and ehrlichiosis are tick-borne diseases that occur in mammals, including cattle, sheep, dogs and humans. They are caused by obligate intracellular bacteria belonging to the family Anaplasmataceae (order Rickettsiales), within the genera *Anaplasma*, *Ehrlichia* and '*Candidatus* Neoehrlichia' (Rar and Golovljova, 2011). Among them, bovine anaplasmosis is prevalent in Algeria and *Ehrlichia canis* infection from domestic animals has been also reported (Bessas et al., 2016; Dahmani et al., 2015; Rjeibi et al., 2018).

Q fever is a zoonosis with a worldwide distribution that is caused by the obligate intracellular bacterium *Coxiella burnetii* (Angelakis and Raoult, 2010). Domestic ruminants (cattle, sheep and goats) are the main reservoirs of this microorganism (Arricau-Bouvery and Rodolakis, 2005). Ticks are involved in the epidemiology of *C. burnetii*, although not as recognized vectors. In Algeria, Q fever was reported for the first time in French soldiers in 1948 and serological studies have confirmed a high seroprevalence in Algerian inhabitants (Pierrou et al., 1956; Lacheheb and Raoult, 2009).

There are few studies on ticks and tick-borne diseases from Algeria and most of them are reported from the northeastern area (Bitam et al., 2006; Leulmi et al., 2016; Aouadi et al., 2017). Therefore, the purpose of the present study was to investigate the presence of bacteria (SFG *Rickettsia* spp., *Anaplasma/Ehrlichia/'Candidatus* Neoehrlichia spp.' and *C. burnetii*) in ticks removed from livestock in Northwestern Algeria.

* Corresponding autor at: Departamento de Enfermedades Infecciosas, Hospital Universitario San Pedro-CIBIR, C/ Piqueras, 98-7ª N.E., 26006, Logroño, La Rioja, Spain.
E-mail addresses: karimeabdelkadir@gmail.com (K. Abdelkadir), ampalomar@riojasalud.es (A.M. Palomar), aportillo@riojasalud.es (A. Portillo), jaoteo@riojasalud.es (J.A. Oteo), aitoudhia.ensv@gmail.com (K. Ait-Oudhia), djamelkhelef@yahoo.fr (D. Khelef).

https://doi.org/10.1016/j.ttbdis.2019.04.018
Received 16 October 2018; Received in revised form 9 April 2019; Accepted 22 April 2019
Available online 27 April 2019
1877-959X/

2. Methods

2.1. Tick collection

Tick surveys were conducted from April to September 2017 in 31 ruminant farms from two districts of Northwestern Algeria (Sidi Bel Abbes and Saida). The farms included in the present study consisted of 17 cattle and 14 sheep farms; seven of them were pure-bred (three Holstein for cattle, and three Ouled djellal and one Hamra for sheep). The collection of ticks was performed during the ordinary visit of private veterinarians to the ruminant farms with owners' permission. Ticks were collected from animal hosts using fine forceps, and immediately stored in 70% alcohol. Specimens were transported to the National Veterinary Higher School (Algiers), and subsequently to the Center of Rickettsiosis and Arthropod-Borne Diseases, Hospital Universitario San Pedro-CIBIR (La Rioja, Spain). Ticks were identified by morphological approaches through taxonomic keys (Walker et al., 2003; Estrada-Peña et al., 2004).

2.2. DNA extraction

Ticks were pooled (2–5 ticks per pool) taking into account the tick species and the origin and, whenever possible, the gender. Adult ticks were rinsed in sterile water and laterally bisected using a sterile scalpel, and a half of each adult tick was stored at -80 °C for future analysis, if needed. The second half of ticks was chopped with sterile scalpels, grouped into pools (as described above) and washed three times with phosphate buffered saline for blood removal, prior to DNA extraction. The DNA from each pool was extracted using the Qiagen DNA Mini kit (Qiagen, Hilden, Germany), following the manufacturer's recommendations.

2.3. Validation of the tick classification

At least one specimen of each tick species and doubtful specimens were also classified using molecular detection as previously described by Black and Piesman (1994) (Supplementary Table).

2.4. Bacterial screening

The adequate DNA extraction and the control of inhibitors of the PCR technique in the pool samples were checked using PCR assays that amplify fragment genes of the mitochondrial ribosomal DNA of ticks. All the samples were analysed using the 16S rRNA PCR assay (Black and Piesman, 1994), and those that gave doubtful or negative results were also analyzed using the PCR assay that amplifies the 12S rRNA gene (Beati and Keirans, 2001) (Supplementary Table). Pools that gave positive results for these PCR assays were analysed for presence of SFG *Rickettsia* spp., *Anaplasma* spp., *Ehrlichia* spp., '*Candidatus* Neoehrlichia spp.' and *C. burnetii* using specific PCR assays. These assays were carried out as described by the respective authors using KAPA Taq PCR Kit (Kapa Biosystem, Wilmington, MA, USA) and Premix Ex Taq (Probe qPCR) (Takara Bio Inc., Shiga, Japan) for conventional and real time PCR assays respectively, following the manufacturer's recommendations. PCR primer pairs, size of the amplicons (bp) and annealing temperatures of the assays are shown in Supplementary Table.

A positive control (*Ixodes ricinus* tick DNA extract, *Rickettsia slovaca*, *Anaplasma phagocytophilum* or *C. burnetii* DNA) and two negative controls, one of them containing water instead of template DNA and the other with template DNA but without primers, were included in the PCR assays.

2.5. Identification of bacterial species

All PCR products were sequenced in both senses using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Forest City, CA, USA) with sequence product resolution (Prism 3130; ABI) at the Sequencing Unit, CIBIR, Spain. The nucleotide sequences were analysed and compared to those available in NCBI database using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The obtained sequences that differed from any other sequence available from GenBank were submitted using Sequin software (https://www.ncbi.nlm.nih.gov/Sequin/).

A phylogenetic tree was constructed by the neighbour-joining method using MEGA version 7 (www.megasoftware.net). Confidence values for individual branches of the resulting tree were determined by bootstrap analysis with 1000 replicates. The evolutionary distances were computed using the maximum composite likelihood (MCL) approach.

3. Results

3.1. Tick collection

A total of 149 engorged or partially engorged ticks were collected. All the ticks were adult specimens morphologically classified within four different species: *Rhipicephalus bursa* (n = 74; 49.7%), *Hyalomma excavatum* (n = 68; 45.6%), *Hyalomma scupense* (n = 3; 2%) and *Hyalomma marginatum* (n = 4; 2.7%). The gender and origin of the tick specimens are specified in the Table 1. The molecular classification of six *R. bursa*, four *H. excavatum*, one *H. scupense* and one *H. marginatum* specimens confirmed morphological data. Specifically, three *R. bursa* specimens showed 100% identity with the sequence KR870983 deposited in GenBank, and the three remaining ones, 99.8% of identity with this sequence. The nucleotide sequences obtained from *H. excavatum* specimens, which were identical to each other, and the one from *H. scupense* showed 99.8% identity with the sequences under GenBank accession numbers KU130429 and KC203349, respectively. The remaining 16S rRNA sequence corresponded to a *H. marginatum* specimen and it was 100% identical to the sequence deposited under GenBank accession number L34307.

3.2. Bacterial screening

Ticks were grouped in 38 pools. From them, 11 pools corresponding

Table 1
Ticks collected in two districts (Sidi Bel Abbes and Saida) of Algeria from May to September 2017.

		No. of ticks (no. of pools) collected/ No. of ticks (no. of pools) for bacteria screening							
		R. bursa		*H. excavatum*		*H. scupense*		*H. marginatum*	
Origin	District	M	F	M	F	M	F	M	F
Cattle	Sidi Bel Abbes	9(2)/9(2)	3(1)/3(1)	25(5)/25(5)	0	0	0	0	2(1)/0
	Saida	0	3(1)/3(1)	25(5)/20(4)	9(3)/9(3)	0	3(1)/3(1)	0	0
Sheep	Sidi Bel Abbes	17(3)/8(1)	23(8)/18(6)	2(1)/2(1)*	3(1)/3(1)*	0	0	1(1)/1(1)**	1(1)/1(1)**
	Saida	5(1)/0	14(4)/0	0	4(1)/4(1)	0	0	0	0

R.: *Rhipicephalus*; *H.*: *Hyalomma*; M: male; F: female; *; **: Corresponding to the same pool that included both genders.

Table 2
Microorganisms amplified in this study.

Bacterium (No. of positive pools)	Tick species (no. of pools)	Origin/District (no. of pools and gender)	Gene	% dentity (bp)-GenBank no.	Gene	% dentity (bp)-GenBank no.
Rickettsia aeschlimannii (2)	*H. excavatum* (1)	Cattle/Saida (1 M)	*ompA*	100 (590/590)-U43800	*ompB*	99.6 (483/485) AF123705 100 (471/471) KJ663755
	H. marginatum (1)	Sheep/ Sidi Bel Abbis (1 M/F)	*ompA*	100 (491/491)-HQ335157	*ompB*	100 (382/382)-KJ663755
Candidatus R. barbariae (7)	*H. excavatum* (1)	Cattle/ Sidi Bel Abbis (1 M)	*ompA*	100 (487/487)-JF700253	*ompB*	100 (367/367)-KY233287
	R. bursa (6)	Cattle/ Sidi Bel Abbis (3 M/F)	*ompA*	100 (484/484)-JF700253	*ompB*	100 (367/367)-KY233287[b]
		Sheep/ Sidi Bel Abbis (3 M/F)	*ompA*	100 (484/484)-JF700253[a]	*ompB*	100 (367/367)-KY233287[b]
Coxiella burnetii (4)	*H. excavatum* (2)	Cattle/Sidi Bel Abbis (1 M)	*IS30*	100 (122/122)-CP014563	*IS1111*	99.6 (550/552)-CP018150
		Sheep/Saida (1 F)	*IS30*	100 (122/122)-CP014563	*IS1111*	100 (604/604)-CP018150
	R. bursa (2)	Sheep/ Sidi Bel Abbis (2 F)	*IS30*	100 (122/122)-CP014563	*IS1111*	NA

NA: Not amplified; M: Male; F: Female; *H.*: *Hyalomma*; *R. barbariae: Rickettsia barbariae; R.*: *Rhipicephalus*.

[a] A longer fragment gene (587 bp) was obtained from a pool that showed homology (100% identity) with the sequence from GenBank with accession number KU645284.

[b] A longer fragment gene (471 bp) was obtained from two out of the three pools that showed a maximum identity of 99.8% (419/420 bp) with the sequence from GenBank with accession number KY233287.

to 40 ticks (33 *R. bursa*, 5 *H. excavatum* and 2 *H. marginatum* specimens) gave negative results for the PCR assays that amplified fragment genes of the ticks (16S rRNA and 12S rRNA) and were not included in the bacterial screening (Table 1). Therefore, 27 pools (109 specimens) were processed for the bacterium detection (Table 1).

Rickettsia-specific *ompA* and *ompB* amplicons were obtained from nine pools. The analysis of the sequences showed the presence of *R. aeschlimannii* from two pools containing *H. excavatum* or *H. marginatum*. In addition, '*Candidatus* Rickettsia barbariae' was found in one and six pools of *H. excavatum* and *R. bursa*, respectively. The sequences obtained from the second round of the *ompA* (484–487 bp) and *ompB* (367 bp) PCR assays corresponding to the seven samples were identical to each other and were 100% identical to sequences under GenBank accession numbers JF700253 and KY233287, respectively. Moreover, a longer fragment of the *ompA* gene (587 bp) and two of the *ompB* gene (420 bp) were obtained from *R. bursa* pools from the first rounds of the respective PCR assays. The longer fragment of the *ompA* sequence was identical to the sequence JF700253 but the two longer *ompB* sequences showed a change with the sequence KY233287 (Table 2). The phylogenetic tree based on the longest *ompA* fragment gene obtained showed homology (100% identity) with a strain of this *Candidatus* species detected in China (GenBank accession number KU645284) (Fig. 1). *Anaplasma* spp., *Ehrlichia* spp. and '*Candidatus* Neoehrlichia spp.' were not identified in any of the samples with the target genes used. *Coxiella burnetii* was amplified from 2 *H. excavatum* pools using two target genes. Moreover, two pools of *R. bursa* tested positive results for this bacterium only with one target gene. These results are summarized in the Table 2.

3.3. Sequences submission to a Public database

The sequences obtained in this study that were different from those previously deposited on a public database were submitted to GenBank under accessions nos.: MK601703- *Rhipicephalus bursa* 16S rRNA gene; MK601704- *Hyalomma excavatum* 16S rRNA gene; MK601704- *Hyalomma scupense* 16S rRNA gene; MK028342- *Rickettsia aeschlimannii ompB* gene; MK028340-'*Candidatus* Rickettsia barbariae' *ompA* gene; and MK028339-'*Candidatus* Rickettsia barbariae' *ompB* gene; and MK028341- *Coxiella burnetii* IS*1111* gene.

4. Discussion

Rickettsia aeschlimannii and *C. burnetii* have been found in ticks from Northwestern Algeria and '*Candidatus* R. barbariae' has been detected for the first time in the North of Africa. The presence of a bacterium in engorged ticks does not demonstrate their role as vectors and/or reservoirs, but proves the circulation of a microorganism in an area and gives information about its ecoepidemiology.

The presence of *R. aeschlimannii* in *H. excavatum* and *H. marginatum* has been previously documented from Algeria (Bitam et al., 2006; Parola et al., 2013; Leulmi et al., 2016), and *H. marginatum* is considered the most important vector of this zoonotic agent. Endemic cases of *R. aeschlimannii* infection occurred in Algeria, although the pathogenicity of this microorganism does not seem to be severe (Oteo et al., 2005; Mokrani et al., 2008; Portillo et al., 2015).

Molecular techniques have allowed the identification of several candidatus to *Rickettsia* species that remain uncultivated, among them '*Candidatus* R. barbariae'. Fragment genes (*gltA*, *ompA* and *ompB* genes) of this '*Candidatus*' were amplified for the first time from *R. bursa* ticks from Portugal, and later a deeper genetic characterization was performed from a strain obtained from *R. turanicus* removed from domestic animals in Italy (de Sousa et al., 2006; Mura et al., 2008). Until now, '*Candidatus* R. barbariae' has been reported from ticks from Europe (Portugal, Italy, Greece, Cyprus and France), from Asia (China, Lebanon, Israel and Palestinian Territories) and recently from Africa (Cameroon and South Africa) (de Sousa et al., 2006; Mura et al., 2008; Chochlakis et al., 2012; Socolovschi et al., 2012; Waner et al., 2014; Papa et al., 2016; Guo et al., 2016; Ereqat et al., 2016 Fernández de Mera et al., 2018; Vanegas et al., 2018; Halajian et al., 2018). In addition, new '*Candidatus* spp.' have been proposed for closely related strains in South Africa and Kenya, named '*Candidatus* Rickettsia rhabdomydis' and '*Candidatus* Rickettsia moyalensis' respectively (Kimita et al., 2016; Essbauer et al., 2018). Nevertheless, up to our knowledge it has not been previously detected in the North of Africa. The bacterium has been mainly amplified from ticks, specifically from *Rhipicephalus* species (*R. turanicus* and *R. sanguineus* in the majority of the cases but also from *R. bursa*, *Rhipicephalus annulatus*, *Rhipicephalus lunulatus* or *Rhipicephalus simus*) (de Sousa et al., 2006; Mura et al., 2008; Halajian et al., 2018). It has also been detected in other tick genera such as *Amblyomma* or *Hyalomma*, but not in *H. excavatum* (Ereqat et al., 2016; Kimita et al., 2016; Fernández de Mera et al., 2018; Vanegas et al., 2018). Moreover, it has been found in other arthropods such as the flea *Vermipsylla alakurt* (Zhao et al., 2016). Nevertheless, all but a few questing *R. turanicus* and *R. sanguineus* ticks were fed arthropods, as well as the ticks analysed in our study, and the infection could be due to the just taken blood meal (Socolovschi et al., 2012; Waner et al., 2014). Thus, the bacterium has been amplified from polecats (*Vormela peregusna*) or rodents (*Rhabdomys pumilio*) tissues (Liu et al., 2018; Essbauer et al., 2018). The pathogenic potential of '*Candidatus* R. barbariae' remains unknown. It has not been associated with human or animal diseases, although it has been identified in a *R. bursa* tick removed from a woman in Greece (Papa et al., 2016). More studies should be performed to elucidate the vectors, reservoirs and the pathogenic potential of this microorgamism.

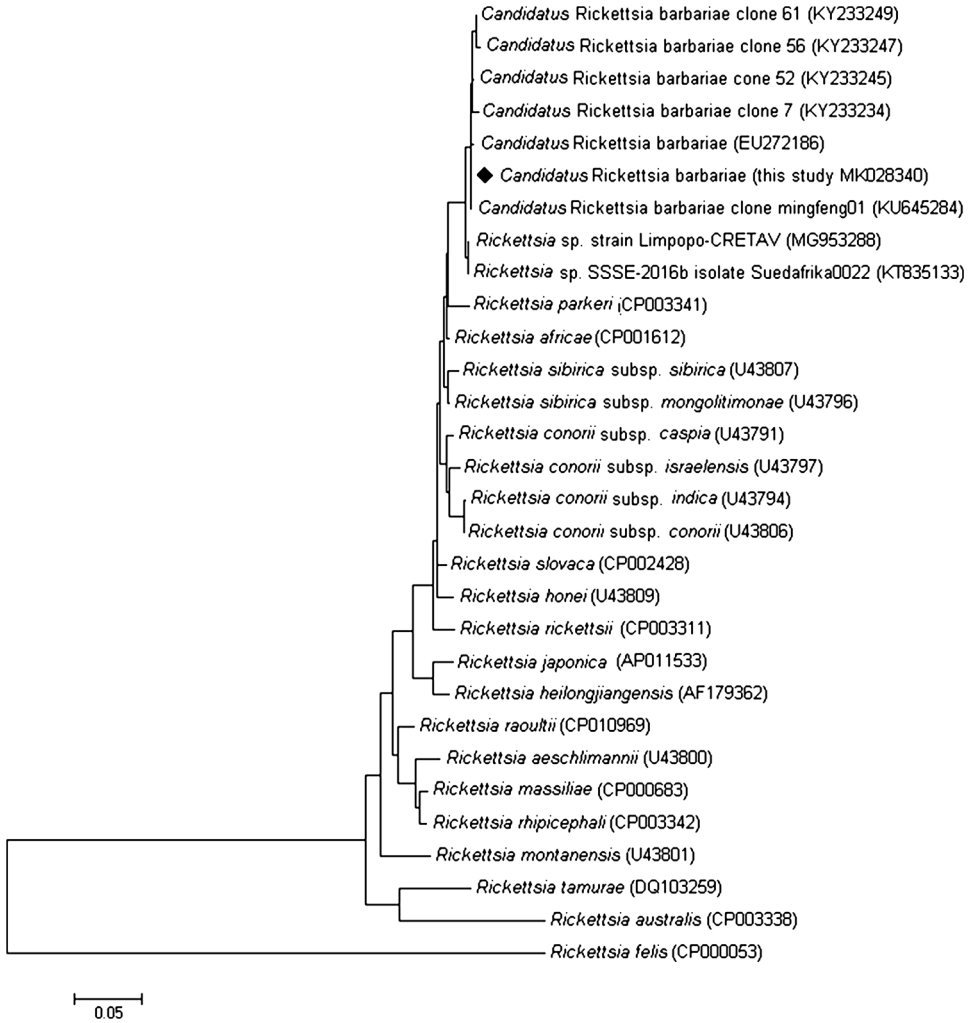

Fig. 1. Unrooted dendrogram showing the phylogenetic position of the strain of '*Candidatus* Rickettsia barbariae' (◆) detected in the present study, among valid *Rickettsia* species and other '*Candidatus*' strains. Phylogeny is inferred from comparison of *ompA* (553 bp) nucleotide sequences by the neighbour-joining method (1000 replicates). GenBank accession numbers of the sequences used in the comparison are shown in brackets.

The infection of livestock with *A. phagocytophilum*, *Anaplasma centrale*, *Anaplasma marginale*, *Anaplasma bovis* and *Anaplasma platys* has been reported from Algeria (Dahmani et al., 2015; Rjeibi et al., 2018). Moreover, *E. canis* and *A. platys* infection in companion animals has been also reported in this country (Bessas et al., 2016). The negative results of the present study were expected due to the absence of the main vectors (*Ixodes ricinus* for *A. phagocytophilum*; *R. microplus* for *A. marginale*; *R. simus* for *A. centrale*; *Amblyomma variegatum* and *Rhipicephalus appendiculatus* for *A. bovis*; and *R. sanguineus* sensu lato for *E. canis* and *A. platys*) (Rar and Golovljova, 2011).

Human and veterinarian cases of infection by *C. burnetii* have been reported from Algeria (Pierrou et al., 1956; Angelakis et al., 2014; Rahal et al., 2018). The infection mainly occurs via inhalation of aerosolized bacteria and livestock is one of the most important reservoirs of this pathogen. The role of ticks as vectors has been suggested but not demonstrated (Eldin et al., 2017). *Coxiella burnetii* has been detected in several tick species, including *R. bursa* in Algeria or *H. excavatum* in Africa (Eldin et al., 2017; Aouadi et al., 2017 Loftis et al., 2006). Thus, the presence of this bacterium in ticks suggests that *C. burnetii* is widely spread although the real role of these arthropods needs further investigations.

5. Conclusion

The present study corroborates the circulation of the zoonotic pathogens *R. aeschlimannnii* and *C. burnetii* in Algeria and demonstrates the presence of '*Candidatus* R. barbariae' in the North of Africa.

COMPETING INTERESTS

The authors declare they have no competing interests.

Acknowledgements

Funding: This study was partially supported by "Fondo Europeo de Desarrollo Regional (FEDER)"

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ttbdis.2019.04.018.

References

Angelakis, E., Raoult, D., 2010. Q fever. Vet. Microbiol. 140, 297–309. https://doi.org/10.1016/j.vetmic.2009.07.016.

Angelakis, E., Mediannikov, O., Socolovschi, C., Mouffok, N., Bassene, H., Tall, A., Niangaly, H., Doumbo, O., Znazen, A., Sarih, 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. https://doi.org/10.1016/j.ijid.2014.05.029.

Aouadi, A., Leulmi, H., Boucheikhchoukh, M., Benakhla, A., Raoult, D., Parola, P., 2017.

Molecular evidence of tick-borne hemoprotozoan-parasites (*Theileria ovis* and *Babesia ovis*) and bacteria in ticks and blood from small ruminants in Northern Algeria. Comp. Immunol. Microbiol. Infect. Dis. 50, 34–39. https://doi.org/10.1016/j.cimid.2016.11.008.

Arricau-Bouvery, N., Rodolakis, A., 2005. Is Q fever an emerging or re-emerging zoonosis? Vet. Res. 36, 327–349.

Beati, L., Keirans, J.E., 2001. Analysis of the systematic relationships among ticks of the genera *Rhipicephalus* and *Boophilus* (Acari: ixodidae) based on mitochondrial 12S ribosomal DNA gene sequences and morphological characters. J. Parasitol. 87, 32–48.

Bessas, A., Leulmi, H., Bitam, I., Zaidi, S., Ait-Oudhia, K., Raoult, D., Parola, P., 2016. Molecular evidence of vector-borne pathogens in dogs and cats and their ectoparasites in Algiers, Algeria. Comp. Immunol. Microbiol. Infect. Dis. 45, 23–28. https://doi.org/10.1016/j.cimid.2016.01.002.

Bitam, I., Parola, P., Matsumoto, K., Rolain, J.M., Baziz, B., Boubidi, S.C., Harrat, Z., Belkaid, M., Raoult, D., 2006. First molecular detection of *R. conorii*, *R. aeschlimannii*, and *R. massiliae* in ticks from Algeria. Ann. N. Y. Acad. Sci. 1078, 368–372.

Black, W.C., Piesman, J., 1994. Phylogeny of hard- and soft-tick taxa (Acari: ixodida) based on mitochondrial 16S rDNA sequences. Proc. Natl. Acad. Sci. U. S. A. 91, 10034–10038.

Chochlakis, D., Ioannou, I., Sandalakis, V., Dimitriou, T., Kassinis, N., Papadopoulos, B., Tselentis, Y., Psaroulaki, A., 2012. Spotted fever group Rickettsiae in ticks in Cyprus. Microb. Ecol. 63, 314–323. https://doi.org/10.1007/s00248-011-9926-4.

Dahmani, M., Davoust, B., Benterki, M.S., Fenollar, F., Raoult, D., Mediannikov, O., 2015. Development of a new PCR-based assay to detect Anaplasmataceae and the first report of *Anaplasma phagocytophilum* and *Anaplasma platys* in cattle from Algeria. Comp. Immunol. Microbiol. Infect. Dis. 39, 39–45. https://doi.org/10.1016/j.cimid.2015.02.002.

de Sousa, R., Barata, C., Vitorino, L., Santos-Silva, M., Carrapato, C., Torgal, J., Walker, D., Bacellar, F., 2006. *Rickettsia sibirica* isolation from a patient and detection in ticks, Portugal. Emerg. Infect. Dis. 12, 1103–1108.

Dib, L., Bitam, I., Bensouilah, M., Parola, P., Raoult, D., 2009. First description of *Rickettsia monacensis* in *Ixodes ricinus* in Algeria. Clin. Microbiol. Infect. 15, 261–262.

Eldin, C., Mélenotte, C., Mediannikov, O., Ghigo, E., Million, M., Edouard, S., Mege, J.L., Maurin, M., Raoult, D., 2017. FromQ FQ fever to *Coxiella burnetii* infection: a paradigm change. Clin. Microbiol. Rev. 30, 115–190.

Ereqat, S., Nasereddin, A., Al-Jawabreh, A., Azmi, K., Harrus, S., Mumcuoglu, K., Apanaskevich, D., Abdeen, Z., 2016. Molecular detection and identification of spotted fever group rickettsiae in ticks collected from the West Bank, palestinian territories. PLoS Negl. Trop. Dis. 10, e0004348.. https://doi.org/10.1371/journal.pntd.0004348.

Essbauer, S., Hofmann, M., Kleinemeier, C., Wölfel, S., Matthee, S., 2018. *Rickettsia* diversity in southern Africa: a small mammal perspective. Ticks Tick. Dis. 9, 288–301. https://doi.org/10.1016/j.ttbdis.2017.11.002.

Estrada-Peña, A., Bouattour, A., Camicas, J.L., Walker, A.R., 2004. A Guide to Identification of species: Ticks of Domestic Animals in Mediterranean Region. Bioscience Reports, London, UK.

Fernández de Mera, I.G., Blanda, V., Torina, A., Dabaja, M.F., El Romeh, A., Cabezas-Cruz, A., de la Fuente, J., 2018. Identification and molecular characterization of spotted fever group rickettsiae in ticks collected from farm ruminants in Lebanon. Ticks Tick. Dis. 9, 104–108. https://doi.org/10.1016/j.ttbdis.2017.10.001.

García-Álvarez, L., Palomar, A.M., Oteo, J.A., 2013. Prevention and prophylaxis of tick-bites and tick-borne related diseases. Am. J. Infect. Dis. 9, 104–116.. http://thescipub.com/abstract/10.3844/ajidsp.2013.104.116.

Guo, L.P., Jiang, S.H., Liu, D., Wang, S.W., Chen, C.F., Wang, Y.Z., 2016. Emerging spotted fever group rickettsiae in ticks, northwestern China. Ticks Tick. Dis. 7, 1146–1150. https://doi.org/10.1016/j.ttbdis.2016.08.006.

Halajian, A., Palomar, A.M., Portillo, A., Heynec, H., Romero, L., Oteo, J.A., 2018. Detection of zoonotic agents and a new *Rickettsia* strain in ticks from donkeys from South Africa: implications for travel medicine. Travel Med. Infect. Dis. 26, 43–50. https://doi.org/10.1016/j.tmaid.2018.10.007.

Jongejan, F., Uilenberg, G., 2004. The global importance of ticks. Parasitology 129, S3–S14.

Kernif, T., Socolovschi, C., Bitam, I., Raoult, D., Parola, P., 2012. Vector-borne rickettsioses in North Africa. Infect. Dis. Clin. North Am. 26, 455–478. https://doi.org/10.1016/j.idc.2012.03.007.

Kimita, G., Mutai, B., Nyanjom, S.G., Wamunyokoli, F., Waitumbi, J., 2016. Phylogenetic variants of *Rickettsia africae*, and incidental identification of "*Candidatus* Rickettsia moyalensis" in Kenya. PLoS Negl. Trop. Dis. 10, e0004788.. https://doi.org/10.1371/journal.pntd.0004788.

Lacheheb, A., Raoult, D., 2009. Seroprevalence of Q-fever in Algeria. Clin. Microbiol. Infect. 15, 167–168. https://doi.org/10.1111/j.1469-0691.2008.02211.x.

Leulmi, H., Aouadi, A., Bitam, I., Bessas, A., Benakhla, A., Raoult, D., Parola, P., 2016. Detection of *Bartonella tamiae*, *Coxiella burnetii* and rickettsiae in arthropods and tissues from wild and domestic animals in northeastern Algeria. Parasit. Vectors 20 (9), 27. https://doi.org/10.1186/s13071-016-1316-9.

Liu, X., Yang, M., Liu, G., Zhao, S., Yuan, W., Xiao, R., Hazihan, W., Hornok, S., Wang, Y., 2018. Molecular evidence of *Rickettsia raoultii*, "*Candidatus* Rickettsia barbariae" and a novel *Babesia* genotype in marbled polecats (*Vormela peregusna*) at the China-Kazakhstan border. Parasit. Vectors 11, 450. https://doi.org/10.1186/s13071-018-3033-z.

Loftis, A.D., Reeves, W.K., Szumlas, D.E., Abbassy, M.M., Helmy, I.M., Moriarity, J.R., Dasch, G.A., 2006. Rickettsial agents in Egyptian ticks collected from domestic animals. Exp. Appl. Acarol. 40, 67–81.

Mokrani, N., Parola, P., Tebbal, S., Dalichaouche, M., Aouati, A., Raoult, D., 2008. *Rickettsia aeschlimannii* infection. Algeria. Emerg. Infect. Dis. 14, 1814–1815. https://doi.org/10.3201/eid1411.071221.

Mokrani, K., Tebbal, S., Raoult, D., Fournier, P.E., 2012. Human rickettsioses in the Batna area, eastern Algeria. Ticks Tick. Dis. 3, 363–365.

Mouffok, N., Parola, P., Abdennour, D., Aouati, A., Razik, F., Benabdellah, A., Raoult, D., 2009. Mediterranean spotted fever in Algerian children. Clin. Microbiol. Infect. 15, 290–291.

Mura, A., Masala, G., Tola, S., Satta, G., Fois, F., Piras, P., Rolain, J.M., Raoult, D., Parola, P., 2008. First direct detection of rickettsial pathogens and a new rickettsia, *'Candidatus* Rickettsia barbariae', in ticks from Sardinia, Italy. Clin. Microbiol. Infect. 14, 1028–1033. https://doi.org/10.1111/j.1469-0691.2008.02082.x.

Oteo, J.A., Portillo, A., Blanco, J.R., Ibarra, V., Pérez-Martínez, L., Izco, C., Pérez-Palacios, A., Jiménez, S., 2005. Low risk of developing human *Rickettsia aeschlimannii* infection in the north of Spain. Ann. N. Y. Acad. Sci. 1063, 349–351.

Papa, A., Xanthopoulou, K., Kotriotsiou, T., Papaioakim, M., Sotiraki, S., Chaligiannis, I., Maltezos, E., 2016. *Rickettsia* species in human-parasitizing ticks in Greece. Trans. R. Soc. Trop. Med. Hyg. 110, 299–304. https://doi.org/10.1093/trstmh/trw022.

Parola, P., Paddock, C.D., Socolovschi, C., Labruna, M.B., Mediannikov, O., Kernif, T., Abdad, M.Y., Stenos, J., Bitam, I., Fournier, P.E., Raoult, D., 2013. Update on tick-borne rickettsioses around the world: a geographic approach. Clin. Microbiol. Rev. 26, 657–702. https://doi.org/10.1128/CMR.00032-13.

Pierrou, M., Mimoune, G., Vastel, G., 1956. Une importante épidémie de fièvre Q (175 cas) observée à Batna (Algérie). Presse. Med. 64, 471–473 [Article in French].

Portillo, A., Santibáñez, S., García-Álvarez, L., Palomar, A.M., Oteo, J.A., 2015. Rickettsioses in Europe. Microbes Infect. 17, 834–838.

Rahal, M., Tahir, D., Eldin, C., Bitam, I., Raoult, D., Parola, P., 2018. Genotyping of *Coxiella burnetii* detected in placental tissues from aborted dairy cattle in the north of Algeria. Comp. Immunol. Microbiol. Infect. Dis. 57, 50–54. https://doi.org/10.1016/j.cimid.2018.06.001.

Rar, V., Golovljova, I., 2011. *Anaplasma*, *Ehrlichia*, and "*Candidatus* Neoehrlichia" bacteria: pathogenicity, biodiversity, and molecular genetic characteristics, a review. Infect. Genet. Evol. 11, 1842–1861. https://doi.org/10.1016/j.meegid.2011.09.019.

Rjeibi, M.R., Ayadi, O., Rekik, M., Gharbi, M., 2018. Molecular survey and genetic characterization of *Anaplasma centrale*, *A. marginale and A. bovis* in cattle from Algeria. Transbound. Emerg. Dis. 65, 456–464. https://doi.org/10.1111/tbed.12725.

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. Dis. 3, 355–360. https://doi.org/10.1016/j.ttbdis.2012.10.019.

Vanegas, A., Keller, C., Krüger, A., Manchang, T.K., Hagen, R.M., Frickmann, H., Veit, A., Achukwi, M.D., Krücken, J., Poppert, S., 2018. Molecular detection of spotted fever group rickettsiae in ticks from Cameroon. Ticks Tick. Dis. 9, 1049–1056. https://doi.org/10.1016/j.ttbdis.2018.03.022.

Walker, A.R., Bouattour, A., Camicas, J.L., Estrada-Peña, A., Horak, I.G., Latif, A.A., Pegram, R.G., Preston, P.M., 2003. Ticks of domestic animals in Africa: a guide to identification of species. Bioscience Reports, Edinburgh, UK.

Waner, T., Keysary, A., Eremeeva, M.E., Din, A.B., Mumcuoglu, K.Y., King, R., Atiya-Nasagi, Y., 2014. *Rickettsia africae* and *Candidatus* Rickettsia barbariae in ticks in Israel. Am. J. Trop. Med. Hyg. 90, 920–922. https://doi.org/10.4269/ajtmh.13-0697.

Wardeh, M., Risley, C., McIntyre, M.K., Setzkorn, C., Baylis, M., 2015. Database of host-pathogen and related species interactions, and their global distribution. Sci. Data 15 (2), 150049. https://doi.org/10.1038/sdata.2015.49.

Zhao, S.S., Li, H.Y., Yin, X.P., Liu, Z.Q., Chen, C.F., Wang, Y.Z., 2016. First detection of *Candidatus* Rickettsia barbariae in the flea *Vermipsylla alakurt* from north-western China. Parasit. Vectors 9 (325). https://doi.org/10.1186/s13071-016-1614-2.