ORIGINAL ARTICLE

Seasonal distribution of *Rickettsia* spp. in ticks in northeast Algeria

L. Dib[1], I. Lafri[2,3], M. Boucheikhchoukh[1], Z. Dendani[1], I. Bitam[2,4] and A. Benakhla[1]

1) *Université Chadli Bendjdid, Département des Sciences Vétérinaires, El Tarf, Algeria,* 2) *Aix-Marseille Université, IRD, VITROME, Service de Santé des Armées, Assistance Publique-Hôpitaux de Marseille, IHU Méditerranée-Infection, Marseille, France,* 3) *Institut des Sciences Vétérinaires, Université Blida, Blida and* 4) *Ecole Supérieure en Sciences de l'Aliment et des Industries Agroalimentaires (ESSAIA), Algiers, Algeria*

Abstract

This article discusses research conducted on the sampling of two tick species: *Ixodes ricinus* and *Rhipicephalus bursa*. Ticks were collected in northern Algeria (El Tarf) in 2014 and studied for differences in abundance and seasonal distribution of population dynamics, as well as tested by PCR for the presence of *Rickettsia* spp. By molecular tools, four *Rickettsia* pathogens agents were detected: *R. helvetica, R. monacensis, R. raoultii* and *R. massiliae.*

Keywords: Algeria, *Ixodes*, PCR, *Rhipicephalus*, *Rickettsia*, ticks
Original Submission: 17 July 2018; **Revised Submission:** 17 October 2018; **Accepted:** 25 October 2018
Article published online: 2 November 2018

Corresponding author: L. Dib, Université Chadli Bendjdid, Département des Sciences Vétérinaires, El Tarf, 36000, Algeria.
E-mail: db.loubna@yahoo.fr

Introduction

Ticks are obligatory haematophagous arthropods that parasitize all classes of vertebrates in almost all regions of the world. They are currently considered the second most common vector of human infectious diseases in the world after mosquitoes. Each tick species has privileged environmental conditions and biotopes that determine the geographical distribution of ticks and consequently the areas at risk of tick-borne diseases [1].

Ticks are limited to geographic locations where climatic conditions are conducive to the completion of their life cycle, and changing climatic conditions (temperature and precipitation) can alter the geographical extent and seasonal period of disease risk and subsequent transmission dynamics in endemic areas, leading to changes in spatial and temporal patterns of human disease [2]. The impact of ticks on human public health was recognized with the emergence of Lyme disease 25 years ago [3].

Since then, about 15 tick rickettsioses have appeared [4]. Tick rickettsiosis is an infection caused by a mandatory Gram-negative intracellular bacterium of the spotted fever group with the genus *Rickettsia* within the *Rickettsiaceae* family of the order *Rickettsiales* [5]. These zoonoses are now recognized as important and emerging vector-borne infections throughout the world. Rickettsial disease is widespread throughout the world, with a seasonal epidemic appearance. In a study of tick-borne encephalitis virus, Korenberg [6] reported that seasonal changes in the prevalence of infection in active, unfed adult ticks in a natural population are determined by the virus content of individual ticks at the time of their activation and also by the duration of subsequent virus persistence in ticks.

Knowledge of the seasonal abundance of ticks and the prevalence of tick-borne pathogens is therefore essential to describe in order to better understand the risk of tick-borne diseases. Many studies are therefore being conducted on the detection of *Rickettsia* in different tick species, but few are studying the dynamics of transmission of this bacterium. The Mediterranean region is a complex biogeographical area thanks to its the diversity of habitats and sudden landscape changes, which are the result of variations in altitude. This region is mainly characterized by a warm climatic season (summer), which coincides with the driest period of the year; the rainy season coincides with the coldest period of the year. The original forests of the

https://doi.org/10.1016/j.nmni.2018.10.008

Mediterranean region comprise *Quercus* spp. and *Olea europaea,* which define the region's main regions [7].

The variability of climatic characteristics provides a tick fauna rich in species, some of which are specific to the Mediterranean region. Because of these main climatic characteristics, the composition of tick fauna in the Mediterranean region is highly variable, and the distribution of the most important tick species can change significantly depending on the specific characteristics of the region concerned.

Algeria is one of the Mediterranean regions known for its great bioclimatic variety. Our objectives were to clarify the differences in abundance and dynamics of tick populations, and to determine the seasonal distribution of *Rickettsia* in the far east of Algeria by real-time PCR.

Materials and methods

Study area

Ticks were collected monthly between January and December 2014 from cattle in the El Tarf region of the northeastern border of Algeria (near the Tunisian border) (GPS coordinates 36°45′7.0 N; 8°10′0 E). The climate is Mediterranean, with a rainy season from autumn to spring, and a long dry and hot season in summer [8]. All ticks were adult. We identified them morphologically using the usual taxonomic keys of the species or genus. Ticks were stored in ethanol at 90°C at room temperature.

DNA extraction

The ticks were rinsed with distilled water for 10 minutes, dried on sterile filter paper in a laminar flow hood and individually ground in Eppendorf sterile tubes (Hamburg, Germany). Total genomic DNA of bacterial strains was extracted with the Qiagen QIAamp Blood Kit (Qiagen, Hilden, Germany).

Real-time PCR

In order to increase the size of the treated sample and to see if there was a seasonal effect on the prevalence of *Rickettsia*, a probe was specifically designed (Table 1).

PCR was performed using a Smart Cycler instrument (Cepheid, Sunnyvale, CA, USA). The PCR mixture included a final volume of 20 µL with 10 µL from the Probe Master kit (Qiagen), 0.5 µL (10 pmol/µL) from each primer, 2 µL (2 µmol/µL) probe, 2 µL (2 µmol/µL) probe, 2 µL distilled water and 5 µL extracted DNA. The real-time quantitative PCR reactions were incubated in the Smart Cycler at 94°C for 2 minutes, followed by 50 cycles of a two-step amplification protocol of 94°C for 5 seconds and 60°C for 30 seconds. Fluorescence was monitored during the annealing phase of each cycle, and the results were analysed with Smart Cycler 2.0c (Cepheid). A negative control consisting of DNA extracted from uninfected laboratory ticks and a positive *Rickettsia* control (one positive control every 20 samples) was included in each test.

Results

In this part of our survey, hard ticks were collected monthly between January and December 2014 from cattle in the El Tarf region (Fig. 1).

Several cattle farms were part of our survey. A total of 656 ticks were collected, 475 *Ixodes ricinus* and 181 *Rhipicephalus bursa* (Table 2).

All ticks collected were identified by entomologic keys [7].

A total of 120 *I. ricinus* individuals and 60 of *Rh. bursa* were analysed by molecular tools for the detection of *Rickettsia* pathogens (Table 3). Four species of *Rickettsia* were detected: *R. helvetica, R. monacensis* and *R. raoultii* from *I. ricinus,* and *R. massiliae* from *Rh. bursa,* with different prevalence during the fourth season of the year (Tables 3 and 4).

Discussion

During the research phase, a total of 656 ticks were collected in the study area. Relative abundance analysis revealed that *I. ricinus* species ticks were dominant compared to *Rh. bursa* (475/181). The seasonal dynamics of ticks were assessed from January to December. *I. ricinus* had seasonal activity; its activity

TABLE 1. List of primers and probes used for PCR

Rickettsia sp.	Probe	Primers
R. massiliae	6-FAM-GTGCAAGCAGCGGCACAACC-TAMARA	R: TTGGATGAGTGTGACGGACT F: TACCGGAACCGTTGCTTTAC
R. helvetica	6-FAM-CCTGTGTAGACGATTCAAGAGGGATGA-TAMRA	R: GTTTAATGGGCATTCGGCTA F: ACTTGGATGAGGCAAACACC
R. raoultii	6-FAM-TGGGGCTTTTTCATGTCCTAAGCACA-TAMRA	R: AAATTGATGGTGCAGGAGTGG F: CCAATACCTTGCCCAAAACA
R. monacensis	6-FAM-AACTGTTGAGGTAGAAGCATTCTGCTCATGGTCTG-TAMRA	R: GTTCTCTTTCGGCATTTTAC F: GCAAAAGGGTTAGCTCCRA

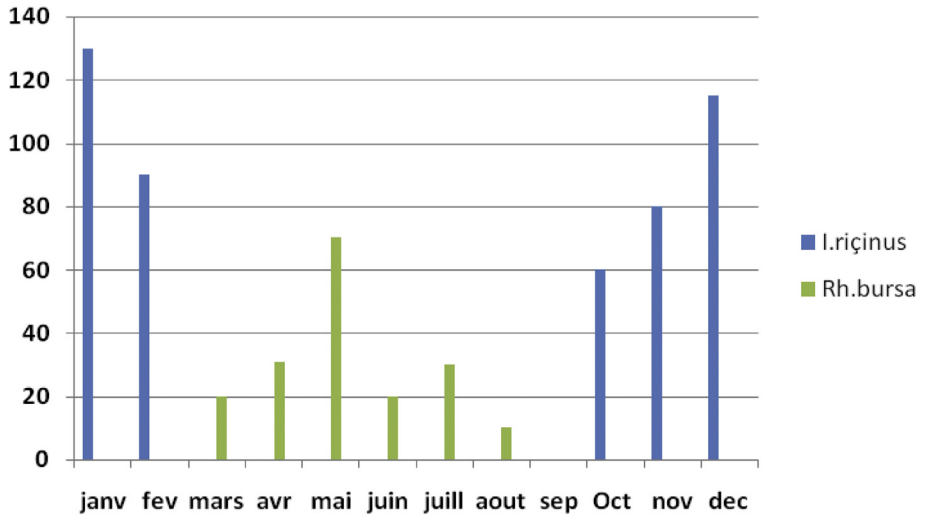

FIG. 1. Seasonal dynamics of tick collection.

TABLE 2. Number of ticks collected by month

Month	*Ixodes ricinus*	*Rhipicephalus bursa*
January	130	
February	90	
March		20
April		31
May		70
June		20
July		30
August		10
September		
October	60	
November	80	
December	115	
Total	475	181

seemed to be limited to cooler months (autumn–winter), although the largest number of ticks was recorded in winter (n = 335) compared to autumn (n = 140). *Rh. bursa* has an activity adapted to the warmer months, and the number of species is higher in spring (n = 121) than in summer (n = 560).

In North Africa, the range of *I. ricinus* is limited to the humid climatic zone; the biotopes of this species essentially correspond to oak formations (*Quercus faginea* and *Q. suber*) [9], which explains the frequency of this species in the study area considered among the most humid in North Africa (1300 mm of rain per year). The maximum abundance of *Rh. bursa* has been reported in spring compared to summer. *Rh. bursa* habitat is formed by grasslands and forest edges, especially in areas of low hills [10].

In Tunisia, Bouattour et al. [9] showed that the adult activity of this tick varies from April to August. In this study, a total of 460 ticks were identified as *I. ricinus* or *Rh. bursa,* and were analysed by specific *Rickettsia* probes. The DNA of *R. helvetica, R. monacensis* and *R. raoultii* were detected in *I. ricinus,* and the DNA of *R. massiliae* was detected in *Rh. bursa.* The seasonal prevalence of *Rickettsia* detected showed 43.33% of *I. ricinus* were infected with *R. helvetica* in winter, but in autumn only 20% of this *Rickettsia* were detected. *R. monacensis* showed a high prevalence in winter (53.33%) compared to autumn (26.66%).

However, *R. raoultii* showed a limited presence in winter. Otherwise, *I. ricinus* is the main vector of the number of human pathogens, which is why *R. monacensis* was described when it was isolated from ticks of *I. ricinus* collected in 1998 from an urban park in Munich, Germany [11]. *R. helvetica* was isolated for the first time in Switzerland [12,13] from *I. ricinus* (the main vector of *Lyme borreliosis*). DNA of *R. raoultii* was found on *Ixodes* spp. ticks in southern Poland and on *Dermacentor* spp. ticks in northeastern Poland.

TABLE 3. Seasonal prevalence of *Rickettsia* spp. in *Ixodes ricinus*

	R. helvetica		*R. monacensis*		*R. raoultii*	
I. ricinus	Autumn	Winter	Autumn	Winter	Autumn	Winter
No. of ticks analysed	120	120	120	120	120	120
No. of positive ticks	24	52	32	64	—	36
Prevalence of infection	20%	43.33%	26.66%	53.33%	—	30%

TABLE 4. Seasonal prevalence of *Rickettsia massiliae* in *Rhipicephalus bursa*

	R. massiliae	
Characteristic	**Spring**	**Summer**
No. of *Rh. bursa* ticks analysed	60	60
No. of positive ticks	38	8
Prevalence of infection	63.33%	13.33%

Until recently, *R. raoultii* had only been reported on *Dermacentor nuttalli, Rhipicephalus* sp. and *Dermacentor* spp. in Europe and Asia (i.e. Siberia and Astrakhan area) [14,15]. In our survey, *R. massiliae* was found with a prevalence of 31.66% in spring and 11.66% in summer on *Rh. bursa.*

Elsewhere, *R. massiliae* was isolated from ticks of *Rhipicephalus sanguineus* collected near Marseille in 1992. It was characterized as a distinct species within the spotted fever group of rickettsiae and named *R. massiliae* [16]. In Algeria, *R. conorii, R. aeschlimannii* and *R. massiliae* were detected by PCR on ticks [17]. Therefore, *I. ricinus* showed a high prevalence in 3 *Rickettsia* species in winter, but *Rh. bursa* showed a high prevalence rate in spring.

One of our main objectives was to understand what factors can influence the evolution of the intensity of tick infection by pathogens. This may include the density of the vector or the reactivation phenomenon. In fact, the relationships between ticks and *Rickettsia* are complex, and the mechanism used by *Rickettsia* to survive in unfed wintering ticks or during molting is poorly understood, although experiments have deciphered the phenomenon of reactivation of rickettsian virulence after infected ticks have taken a blood meal. The underlying molecular events after feeding have not yet been elucidated; a tick larva enters a rest period before becoming a searching nymph the following year. Even though the precise mechanism of *Rickettsia* reactivation is not known, it is believed that temperature variations and blood sampling reactivate *Rickettsia.* As in the *Borrelia burgdorferi–Ixodes scapularis* model, *Rickettsia* transmission probably cannot occur until 24 hours after tick fixation, which gives *Rickettsia* time to grow [18].

In another study, Korenberg [6] reported the seasonal populations dynamics of *Ixodes* ticks and tick-borne encephalitis virus, demonstrating that seasonal changes in the prevalence of infection in active nonfed adult ticks in a natural population are generally determined by the virus content of individual ticks at their activation and the duration of tick virus persistence or the rate of virus loss during the subsequent period.

In different regions, these processes may have some specific characteristics. In general, the infection parameters of adult ticks *Ixodes persulcatus* are initially higher and then gradually decrease during their activity period [19]. In addition, the seasonal dynamics of infectivity in adult *I. persulcatus* ticks can change from year to year, even in the same natural outbreak. For example, 2-year studies in European southern taiga forests on the same test plot, using the same tick collection and virologic analysis methods, yielded the following results.

During the first year, the frequency of tick-borne encephalitis virus isolation in May (15% of bioassays were positive) was about twice as high as in June and July. The following year, this parameter remained at about the same level throughout the tick activity period: 7.2% in May, 7.9% in June and 5.5% in July [20]. In *I. ricinus* ticks, the initial prevalence of infection in a new generation in the autumn is apparently more or less unchanged during the cold winter period. Therefore, studies conducted in regions with such a climate do not reveal any seasonal dynamics distinct from infection in nymphs and adult ticks during the period of their activity [21].

Conclusion

On the basis of our results, we conclude that there are few data to explain our results. Hence, studies on *Rickettsia*'s seasonal dynamics remain poor, which requires further research.

Acknowledgements

We acknowledge all our contributors. My research was carried in the laboratory of IHU Méditerranée-Infection, Marseille, France and all products and primers used were financed by the laboratory.

Conflict of interest

None declared.

References

[1] Parola P, Raoult D. Ticks and tickborne bacterial diseases in humans: an emerging infectious threat. Clin Infect Dis 2001;32(6):897–928.
[2] Gage KL, Burkot TR, Eisen RJ, Hayes EB. Climate and vectorborne diseases. Am J Prev Med 2008;35:436–50.
[3] Wormser GP. Hematogenous dissemination in early Lyme disease. Wien Klin Wochenschr 2006;118:634–7.
[4] Parola P, Paddock C, Raoult D. Tick-borne rickettsioses around the world: emerging diseases challenging old concepts. Clin Microbiol Rev 2005;18:719–56.
[5] Bechah Y, Capo C, Mege JL, Raoult D. Epidemic typhus. Lancet Infect Dis 2008;8:417–26.

[6] Korenberg E. Seasonal population dynamics of *Ixodes* ticks and tick-borne encephalitis virus. Exp Appl Acarol 2000;24:665–81.
[7] Estrada-Peña A, Bouattour A, Camicas J, Walker AR. Ticks of domestic animals in the Mediterranean region: a guide to identification of species. Utrecht, The Netherlands: International Consortium for Ticks and Tick-Borne Diseases; 2004. p. 52–5.
[8] Emberger L. La végétation de la région méditerranéenne (Essai d'une classification des groupements végétaux). Travaux de botanique et d'écologie. Paris: Edition Masson et Cie; 1971. p. 25–50.
[9] Bouattour A, Darghouth MA, Daoud A. Distribution and ecology of ticks (*Acari: Ixodidae*) infesting livestock in Tunisia: an overview of eighth years field collections. Parasitiologia 1999;41:5–10.
[10] Bourdeau P. Les tiques d'importance Vétérinaire et médicale. 2ère partie: principales espèces de tiques dures (*Ixodidae* et *Amblyommidae*). Point Vet 1993;25:27–41.
[11] Simser JA, Palmer AT, Fingerle V, Wilske B, Kurtti TJ, Munderloh UG. *Rickettsia monacensis* sp. nov., a spotted fever group *Rickettsia,* from ticks (*Ixodes ricinus*) collected in a European city park. Appl Environ Microbiol 2002;68:4559–66.
[12] Beati L, Peter O, Burgdorfer W, Aeschlimann A, Raoult D. Confirmation that *Rickettsia helvetica* sp. nov. is a distinct species of the spotted fever group of rickettsiae. Int J Syst Bacteriol 1993;43:521–6.
[13] Burgdorfer W, Aeschlimann A, Peter O, Hayes SF, Philip RN. *Ixodes ricinus,* vector of a hitherto undescribed spotted fever group agent in Switzerland. Acta Trop 1979;36:357–67.
[14] Mediannikov O, Matsumoto K, Samoylenko I, Drancourt M, Roux V, Rydkina E. *Rickettsia raoultii* sp. nov., a spotted fever group *Rickettsia* associated with *Dermacentor* ticks in Europe and Russia. Int J Syst Evol Microbiol 2008;40:149–56.
[15] Dautel H, Dippel C, Oehme R, Hartelt K, Schettler E. Evidence for an increased geographical distribution of *Dermacentor reticulatus* in Germany and detection of *Rickettsia* sp. RpA4. Int J Med Microbiol 2006;40:149–56.
[16] Beati L, Finidori JP, Gilot B, Raoult D. Comparison of serologic typing, sodium dodecyl sulfate–polyacrylamide gel electrophoresis protein analysis, and genetic restriction fragment length polymorphism analysis for identification of rickettsiae: characterization of two new rickettsial strains. J Clin Microbiol 1992;30:1922–30.
[17] Bitam I, Parola P, Matsumoto K, Rolain JM, Baziz B, Boubidi SC, et al. First molecular detection of *R. conorii, R. aeschlimannii,* and *R. massiliae* in ticks from Algeria. Ann N Y Acad Sci 2006;1078:368–72.
[18] Azad AF, Beard CB. Rickettsial pathogens and their arthropod vectors. Emerg Infect Dis 1998;4:179–86.
[19] Katin AA. Specific spatiotemporal features in the life of natural tick-borne encephalitis foci in relation to the problem of predicting their epidemiological activity. Tick-borne encephalitis and Omsk hemorrhagic fever in the Tyumen region (in Russian). 1993. p. 22–42. Omsk.
[20] Pchelkina A, Korenberg EI, Zemskaya AA, Suvorova LG, Kovalevskii YuV. Studies on virus prevalence in *Ixodes persulcatus* P. Sch. ticks from tick-borne encephalitis foci located in East-European southern taiga forests. In: Markevich A, editor. Abstracts of the second acarological conference. Kyiv, Ukraine: Naukova Dumka; 1970. p. 96–7.
[21] Okulova NM, Bannova GG, Mikhailova IS. Virus prevalence in vectors from a conjugate focus of tick-borne encephalitis and Kemerovo virus located in the southern Kemerovo region. Med Virusol 1973;21:89–93.