Activity dynamics and phylogenetic analyses of *Ixodes ricinus* population in North West Tunisia

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Abstract

The present study aimed to investigate the phenology of *Ixodes ricinus* in Aïn Draham forest (Northwest Tunisia) and evaluate the correlation between abiotic factors (temperature, relative humidity and rainfall). Ticks were collected monthly from September 2016 to August 2017 using dragging techniques. A total of 116 specimens were collected from 5 sites, consisting of 47 adults (19 females and 28 males, sex ratio M: F= 1.47), 45 nymphs and 24 larvae presenting a prevalence of 40.5±8.9%, 38.8±8.9% and 20.7±7.4%, respectively (p=0.001). Adult *Ixodes ricinus* were questing hosts from October to May, while the nymphs were collected between May (n=23) and August (n=1). Larvae were present in the ground between July and September. The number of ticks varied significantly according to sites (p<0.01). The highest number of ticks was recorded in site 2 (37%; n=43) and the lowest (2.58%; n=3) in site 4. This difference was probably associated to the host availability and the characteristic of the sites. Statistical analyses showed a significant correlation between adult's tick number and meteorological parameters such as mean humidity (Pearson r= -0.77; *P* = 0.003), mean temperature (Pearson r= -0.74; *P* = 0.006), mean rainfall (Pearson r= 0.64; *P* = 0.025).

The comparison between twenty 16S rDNA sequences with approximately 444 bp length showed a variability among 11 sequences which were deposited in GenBank. There was a low genetic divergence (1-2%) among the *I. ricinus* isolates collected from the five sites. The amplicons showed 95-100% homology with *I. ricinus* sequences available in GenBank from different countries. These results should be supported by other surveys in other regions from Tunisia to better understand the biology of *I. ricinus* in its wild biotope and the influence of environmental factors on its distribution.

Keys words: *Ixodes ricinus*, abundance, activity dynamics, abiotic factors, Tunisia

Introduction

The *Ixodes ricinus* tick complex which consists of 14 species, is widely distributed in Europe, USA and North Africa (Keirans and Needham, 1999; Xu et al., 2003). From this complex, only *I. ricinus* and *I. inopinatus* have been reported in Tunisia (Estrada-Peña et al., 2014). Because of their high morphological similarities, these two species were previously confounded (Noureddine et al., 2011). In Africa, *I ricinus* has been reported only in some humid and mountainous regions in Tunisia, Algeria and Morocco (Bouattour et al., 1999; Walker et al., 2003). Hoogstraal et al. (1964) recorded few numbers of *I. ricinus* in Egypt that were most likely accidentally introduced from Southern Europe by migratory birds.

The huge interest in *Ixodes ricinus* is due to its vector role of several pathogens including zoonotic: bacteria (*Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum*, *Rickettsia helvetica*, *Rickettsia monacensis*), parasites (*Babesia divergens)* and viruses (tick-borne encephalitis) (Bouattour and Darghouth, 1996; Younsi et al., 2001, 2005; Benredjem et al., 2014).

Ixodes ricinus is a three-host tick species. The immature stages attach to small mammals and reptiles, while adults feed mainly on cattle and sheep but also were found in birds and in vegetation such as ferns (*Pteridium aquilininum*). In Tunisia, the larvae and nymphs were collected from lizards between April and August, whilst, adults are active between September and May and infest almost exclusively cattle (Bouattour, 2001). *I. ricinus* populations are showing a certain level of differences in activity dynamics between North African countries probably related to the presence of specific microclimates (temperature, humidity and rainfall) and differences in host availability (Estrada-Peña et al., 2013).

Indeed, *I. ricinus* biology is widely influenced by environmental and topographical components, such as temperature, humidity, rainfall, slope and altitude (Keirans and Needham, 1999; Estrada-Peña, 2001; Jore et al., 2014). The tick survives at very low temperatures and its distribution is expanding to high elevation that can reach 1250 meter above sea level (a.s.l)

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(Materna et al., 2005; Qviller et al., 2014). For its development, *I. ricinus* requires at least 80% of humidity, which is mostly induced by high rainfall rate and undergrowth thickness (Jore et al., 2014). The nature of vegetation cover is one of the factors regulating the abundance of *I. ricinus* (Alonso-Carné et al., 2016; Estrada-Peña, 2001; Jore et al., 2014). The Normalized Difference Vegetation Index (NDVI) has been the most widely used remotely sensed metric for ecosystem monitoring and land surface process assessment in ticks and tick-borne pathogens studies including *I. ricinus* (Alonso-Carné et al., 2016; Rosà et al., 2018).

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Variability among hosts and microhabitats make their interaction with ticks more complex and can influence the genetic structure of the tick populations through differences in response to environmental changes (Noureddine et al., 2011).

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The work reported herein aimed to follow the activity dynamics of *I.* ricinus under natural conditions in forested areas in Northwest of Tunisia and to investigate for the first time its correlation with abiotic factors. The phylogenetic relationships of the Tunisian *Ixodes* ticks with other tick populations were also studied.

Materials and methods

Study area and characterization of study sites

The study was conducted in “Col des Ruines” locality (Aïn Draham region) in Northwest forests of Tunisia (36°46'N, 8°41'E) representing an extension of the forest mountain of North Africa, namely Kroumirie. The region is situated in the humid bio-climatic zone and is characterized by Mediterranean climate with distinct seasonal variations. Winter is specified by heavy rainfall and frequent snow spells (During the coldest months of December, January and February). Mean annual temperature and rainfall are 15.0°C and 1389 mm, respectively (Climate-Data.org). The forest occupies 20% of the total surface area of the region (Madyouni, 2015) and the rich hydrographical network is characterized by low pH (Ghrabi-Gammar et al., 2009). The vegetation cover is dominated by different oak species: cork (*Quercus suber*), zeen (*Quercus canariensis)* and kermes (*Quercus coccifera)*. The forest undergrowth is dominated by high density of ferns (*Pteridium aquilininum*) reflecting the high humidity of this region and tree heath (*Erica arborea*).

Five sampling sites with different characteristics were selected to provide enough area for *I. ricinus* collection (Table 1 and Figure 1).

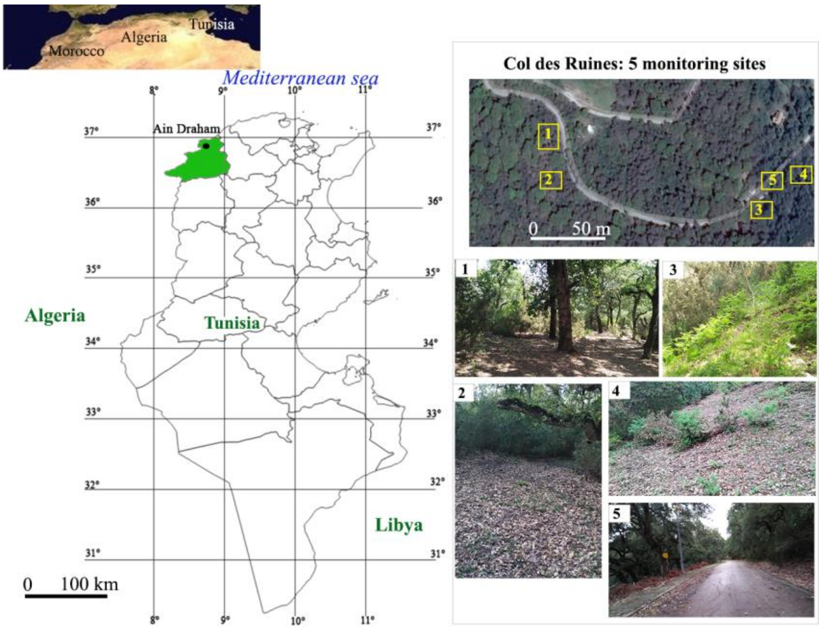

Figure 1: Location of Aïn Draham forest (District of Jendouba, Northwest Tunisia) and habitat of the 5 study sites

All sites are poorly exposed to sunlight and they are mountainous. The slopes range between 1° at the road borders and 79° at site 3, respectively. This mountainous area is suitable for the passage and grazing of domestic animals such as cattle (*Bos taurus*), sheep (*Ovis aries*) and goats (*Capra aegagrus hircus*). Footprints of wild boar (*Sus scrofa*) were frequently encountered in multiple sites and when temperatures rise (early spring), large lizards

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(*Psammodromus algirus*) were also observed. The other animals that are present in the region are hares (*Lepus timidus*), foxes (*Vulpes vulpes*) and birds (Stambouli-Essassi et al., 2007).

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Table 1: Abiotic characteristics of the 5 sampling sites in Aïn Draham region (Jendouba district)

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Site number	**Latitude**	**Longitude**	**Average slope [Range] (%)**	**Average altitude (m)**	**Characteristics**	**Approximate surface (m^2)**
1	36,7912	8,6811	16 [3-29]	745	Partially covered by few trees Presence of dry undergrowth Low density of ferns	558
2	36,7903	8,6817	17 [4-30]	751	High trees density Presence of dry undergrowth Low density of ferns Presence of trace of wild boars	352
3	36,7903	8,6844	39 [3-75]	775	Low density trees Presence of water source High humidity High density of ferns	199
4	36,7907	8,685	40.5 [2-79]	774	Low density of trees Absence of ferns and undergrowth Low density of vegetation	295
5	36,791	8,6849	13.5 [1-26]	750	Presence of trees by the side of the roads Passage way of domestic animals Presence of faeces of small ruminants	307

Tick sampling

Ticks were collected monthly between September 2016 and August 2017 in the five sites using the dragging technique (Tack et al., 2011). Dragging was done between 10 a.m. and 1 p.m. A one-meter square white towel was dragged over the ground for a distance of 10 m, or over ferns, then checked for the presence of ticks. Each tick was individually placed in labelled Eppendorf tube. The specimens were identified under stereomicroscope according to the key of Walker et al. (2003) based on morphological criteria. Ticks were stored at -20°C until analysed.

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Meteorological data collection and remotely sensed NDVI values

Relative humidity (RH, %) in the studied region and during the study period were obtained from the Weather Underground report (http://www.weatherunderground.com). The slope and GPS coordinates were recorded with GPS calculator.

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Land surface temperature (LST, °C) and rainfall (mm) that were recorded by MODIS satellite at a resolution of 1 km were obtained from the Google Earth Engine (GEE) catalogue (https://code.earthengine.google.com/dataset/MODIS/006/MOD11A2).

The Normalized Difference Vegetation Index (NDVI) was computed using the Landsat 8 Surface Reflectance dataset distributed by the USGS (MASEK et al., 2013) and available through the Google Earth Engine (GEE) catalogue with a resolution of 500 meters.

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NDVI time-series buffered by 30 m spanning the entire tick samples collecting period from September 2016 to August 2017 for each sampling site has been extracted (Figure 2).

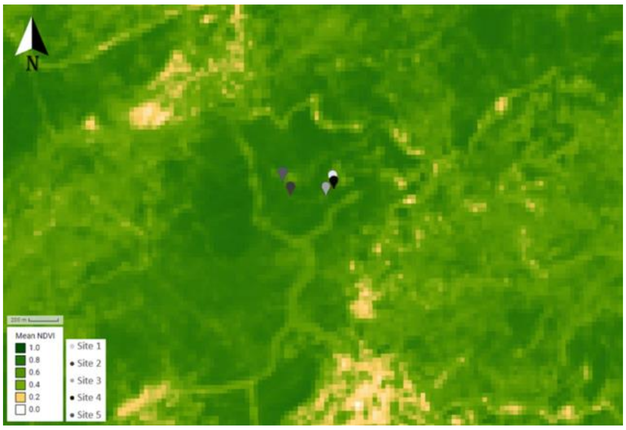

Figure 2: Average NDVI map during the study period in the 5 sites of Aïn Draham (Jendouba district, Northwest Tunisia)

DNA extraction and PCR

Genomic DNA was extracted from all the tick samples using the Wizard® Genomic DNA Extraction Kit (Promega, Madison, USA) according to the manufacturer's instructions. PCR reactions were performed in 30 µl reaction volume made up of 1x PCR buffer, 2.25 mM $MgCl_2$, 0.8 mM dNTP, 0.5 µM of each primer, and 1U Taq DNA polymerase (Bio Basic, Canada). The target was to amplify the 16S rDNA gene using the following primer pairs 16S-F 5’TGCTGTGGTATTTTGACTATAC3’ and 16S-R 5’CCGGTCTGAACTCAGATCAAGT3 which amplified a fragment of 444 base pairs (Noureddine et al., 2011). PCR reactions involved the following cycling profiles; an initial denaturation at 95°C for five minutes, followed by 35 amplification cycles made up of first denaturation step at 95°C for 30 sec, annealing at 59.4°C for 30 sec and elongation at 72°C for 1 minute. A final elongation step at 72°C for 15 minutes completed the PCR reactions. PCR products were analysed by electrophoresis in a 1% agarose gel.

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Sequencing and data analysis

A total of 20 PCR products (eight of them were isolated from *I. ricinus* collected from cows grazing in the study region, these samples were included only in the study of *Ixodes* populations genetic) were purified and sequenced by a private service provider in both directions with the same primers used in PCR. The evaluation of the chromatograms and pairwise sequence alignments were performed with MEGA 7 software (Tamura et al., 2011). The sequences were compared with those available at the GenBank database by BLAST search. A phylogenetic tree was constructed using the neighbour-joining method implemented in MEGA 7 to evaluate genetic relationships and clustering patterns between *Ixodes* isolates from Aïn Draham region and the global ones that were downloaded from the NCBI Genbank database. A total of 11 haplotypes generated in this study have been deposited with the NCBI GenBank database under the following accession numbers: MH375816, MH375817, MH375818, MH375819, MH375820, MH375821, MH375822, MH375823, MH375824, MH375825 and MH375826.

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Statistical analyses

The abundance of *I. ricinus* was estimated as the number of ticks collected in each site during each visit. A Chi-square test was performed using SPSS software (version 21, IBM, USA) (Schwartz, 1993) to estimate the significance (*P*-value < 0.05) in variation in tick number according to months and sites. The Pearson correlation coefficient was estimated to determine the magnitude of relationship between tick number and studied environmental factors i.e. rainfall, humidity, Land Surface Temperature, altitude, slope and NDVI values.

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Results

Seasonal variation of tick number according to the studied factors

During the study period (1 year), a total of 116 ticks were collected from the 5 sites. All specimens were morphologically identified as *Ixodes ricinus* consisting of 47 adults (28 males and 19 females, sex ratio M:F= 1.47), 45 nymphs and 24 larvae, presenting 40.5±8.9%, 38.8±8.9% and 20.7±7.4% of the tick population, respectively (*P* = 0.001). Adult *Ixodes ricinus* were collected between October and May; the highest number (n=11 ticks) was recorded in April (*P*=0.1). The nymphs were active between May and August with a peak number (23 nymphs) in May (P=0.3). Larvae were collected between July and October with peak in July (n=19 larvae) (P=0.02) (Figure 3).

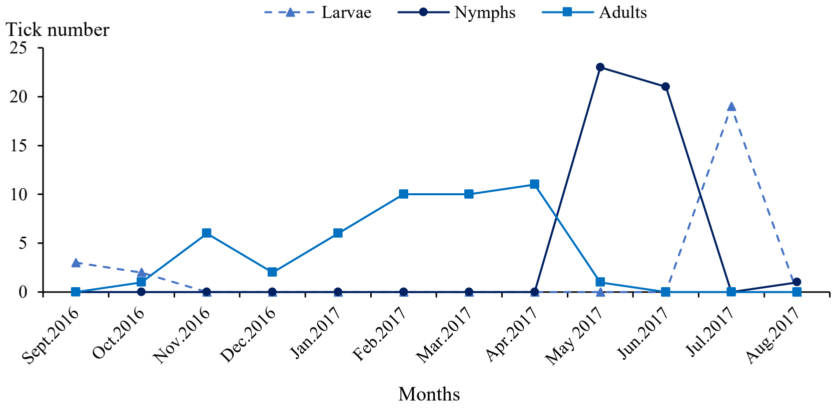

Figure 3: Monthly variation of tick number according to tick stages

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The highest number of ticks was recorded in site 2 (37%; n = 43) and the lowest number in site 4 (2.5%; n = 3) (*P* < 0.01) (Figure 4).

The tick number was negatively correlated with altitude (Pearson r=-0.91; *P*=0.03) and slope (Pearson r=-0.88; *P*=0.04) characterizing each site.

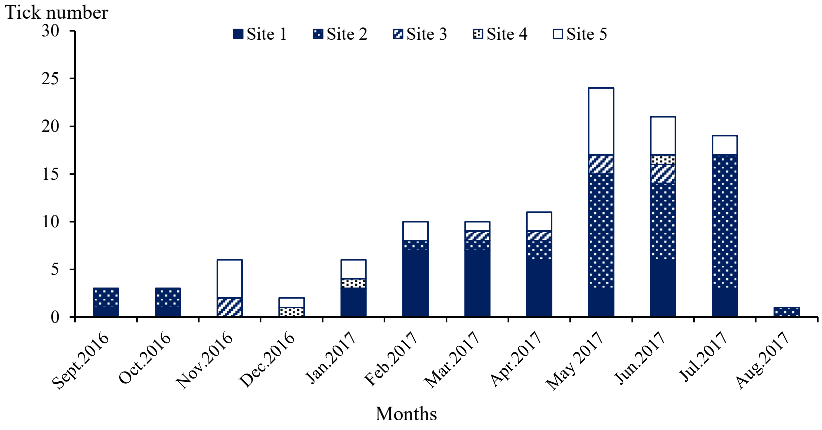

Figure 4: Monthly variation of the number of ticks collected in the 5 surveyed sites in Aïn Draham (Jendouba District, Northwest Tunisia)

The monthly variation of NDVI during the whole study period for the selected area was computed (Figure 5). Despite the significant variation of NDVI according to months (Pearson r=0.652; *P*=0.02), there was no significant correlation between NDVI values and the total tick number collected each visit (Pearson r=0.127; *P*=0.6).

The statistical analyses showed a significant correlation between adult's tick number and the mean humidity (Pearson r= -0.775; *P* = 0.003), Land Surface Temperature (Pearson r= -0.568; *P* =0.05) and the mean rainfall (Pearson r= 0.642; *P* = 0.02). There was no correlation between neither larvae' or nymphs' numbers and the environmental factors.

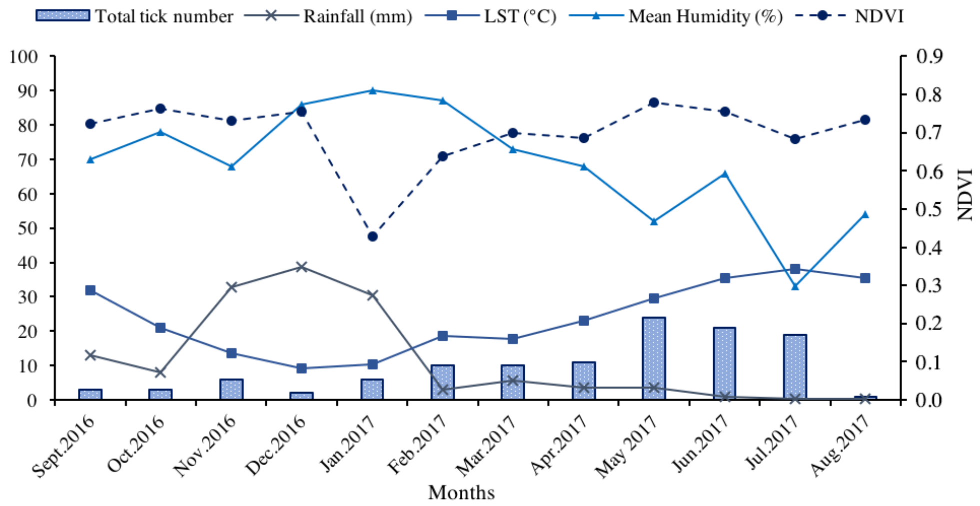

Figure 5: Monthly variation of tick number according to meteorological parameters and NDVI values

Phylogenetic analysis of* Ixodes ricinus *population in Aïn Draham forest

The BLAST search comparison between twenty 16S rDNA sequences showed a difference between 11 haplotypes (98-99% homology) which were submitted to GenBank.

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I.ricinus K2(MH375820)    1 tttgactatacgaaggtattgaaataagattttaaatgagtgctaagagt-tgattaaacaattaaaagctttctttaattgaataattgaatttaat-tttttagtgcaaaagcaaaaataaaaattag
I.ricinus K9(MH375816)    1 tttgactatacgaaggtattgaaataagattttaaatgagtgctaagagaatgattaaacaattaaaagctttctttaattgaataattgaatttaatttttt-agtgcaaaagcaaaaataaaaattag
I.ricinus K14(MH375824)   1 tttgactatacgaaggtattgaaataagattttaaatgagtgctaagagaatgattaaacaattaaaagctttctttaattgaataattgaatttaatttttttagtgcaaaagcaaaaataaaaattag
I.ricinus K16(MH375823)   1 tttgactatacgaaggtattgaaataagattttaaatgagtgctaagagaatgattaaacaattaaaagctttctttaattgaataattgaatttaat-tttttagtgcaaaagcaaaaatataaattag
I.ricinus K1(MH375826)    1 tttgactatacgaaggtattgaaataagattttaaatgagtgctaagagaatgattaaacaattaaaagctttctttaattgaataattgaatttaat-tttttagtgcaaaagcaaaaataaaaattag
I.ricinus K8(MH375817)    1 tttgactatacgaaggtattgaaataagattttaaatgagtgctaagagaatgattaaacaattaaaagctttctttaattgaataattgaatttaat-tttttagtgcaaaagcaaaaataaaaattag
I.ricinus K51(MH375818)   1 tttgactatacgaaggtattgaaataagattttaaatgagtgctaagagaatgattaaacaattaaaagctttctttaattgaataattgaatttaat-tttttagtgcaaaagcaaaaataaaaattag
I.ricinus K47(MH375819)   1 tttgactatacgaaggtattgaaataagattttaaatgagtgctaagagaatgattaaacaattaaaagctttctttaattgaataattgaatttaat-tttttagtgcaaaagcaaaaataaaaattag
I.ricinus K18(MH375822)   1 tttgactatacgaaggtattgaaataagattttaaatgagtgctaagagaatgattaaacaattaaaagctttctttaattgaataattgaatttaat-tttttagtgcaaaagcaaaaataaaaattag
I.ricinus K12(MH375825)   1 tttgactatacgaaggtattgaaataagattttaaatgagtgctaagagaatgattaaacaattaaaagctttctttaattgaataattgaatttaat-tttttagtgcaaaagcaaaaataaaaattag
I.ricinus K19(MH375821)   1 tttgactatacgaaggtattgaaataagattttaaatgagtgctaagagaatgattaaacaattaaaagctttctttaattgaataattgaatttaat-tttttagtgcaaaagcaaaaataaaaattag

I.ricinus K2(MH375820)  129 ggacaagaagaccctatgaatttttattaaattttataaaactttttataaaatttaatttaattggggtgatggaaaaagaataaatatc-tttttttaaaagaaaattggttccgtttttagcgatt
I.ricinus K9(MH375816)  130 ggacaagaagaccctatgaatttttattaaattttataaaaagttttataaaatttaatttaattggggtgatggaaaaagaataaatatctttttttt-aaagaaaattggttccgtttttagcgatt
I.ricinus K14(MH375824) 131 ggacaagaagaccctatgaatttttattaaattttataaaaagttttataaaatttaatttaattggggtgatggaaaaagaataaatatctttttttt-aaagaaaattggttccgtttttagcgatt
I.ricinus K16(MH375823) 130 ggacaagaagaccctatgaatttttattaaattttataaaaagttttataaaatttaatttaattggggtgatggaaaaagaataaatatctttttttttaaagaaaattggttccgtttttagcgatt
I.ricinus K1(MH375826)  130 ggacaagaagaccctatgaatttttattaaattttataaaactttatataaaatttaatttaattggggtgatggaaaaagaataaatatc-ttttttt-aaagaaaattggttccgtttttagcgatt
I.ricinus K8(MH375817)  130 ggacaagaagaccctatgaatttttattaaattttataaaactttatataaaatttaatttaattggggtgatggaaaaagaataaatatc-ttttttt-aaagaaaattggttccgtttttagcgatt
I.ricinus K51(MH375818) 130 ggacaagaagacccttgaatttttattaaattttataaaactttatataaaatttaatttaattggggtgatggaaaaagaataaatatc-ttttttt-aaagaaaattggttccgtttttagcgatt
I.ricinus K47(MH375819) 130 ggacaagaagaccctatgaatttttattaaattttataaaactttatataaaatttaatttaattggggtgatggaaaaagaataaatatc-ttttttt-aaagaaaattggttccgtttttagcgatt
I.ricinus K18(MH375822) 130 ggacaagaagaccctatgaatttttattaaattttataaaactttttataaaatttaatttaattggggtgatgaaaaaagaataaatatc-ttttttttaaagaaaattggttccgtttttagcgatt
I.ricinus K12(MH375825) 130 ggacaagaagaccctatgaatttttattaaattttataaaactttttataaaatttaatttaattggggtgatggaaaaagaataaatatc-ttttttttaaagaaaattggttccgtttttagcgatt
I.ricinus K19(MH375821) 130 ggacaagaagaccctatgaatttttattaaattttataaaactttttataaaatttaatttaattggggtgatggaaaaagaataaatatc-ttttttttaaagaaaattggttccgtttttagcgatt

I.ricinus K2(MH375820)  258 aaatgaaaaaaatactctagggataacagcgtaataattttggatagttcatatagataaaatagtttgcgacctcgatgttggattaggattctttttttggtgaagaagttaaaaaaa-gaagtttgtt
I.ricinus K9(MH375816)  259 aaatgaaaaaaatactctagggataacagcgtaataattttggatagttcatatagataaaatagtttgcgacctcgatgttggattaggattctttttttggtgaagaagttaaaaaaa-gaagtttgtt
I.ricinus K14(MH375824) 260 aaatgaaaaaaatactctagggataacagcgtaataattttggatagttcatatagataaaatagtttgcgacctcgatgttggattaggattctttttttggtgaagaagttaaaaaaa-gaagtttgtt
I.ricinus K16(MH375823) 260 aaatgaaaaaaatactctagggataacagcgtaataattttggatagttcatatagataaaatagtttgcgacctcgatgttggattaggattctttttttggtgaagaagttaaaaaaa-gaagtttgtt
I.ricinus K1(MH375826)  258 aaatgaaaaaaatactctagggataacagcgtaataattttggatagttcatatagataaaatagtttgcgacctcgatgttggattaggattctttttttggtgaagaagttaaaaaaa-gaagtttgtt
I.ricinus K8(MH375817)  258 aaatgaaaaaaatactctagggataacagcgtaataattttggatagttcatatagataaaatagtttgcgacctcgatgttggattaggattctttttttggtgaagaagttaaaaaaa-gaagtttgtt
I.ricinus K51(MH375818) 258 aaatgaaaaaaatactctagggataacagcgtaataattttggatagttcatatagataaaatagtttgcgacctcgatgttggattaggattctttttttggtgaagaagttaaaaaaa-gaagtttgtt
I.ricinus K47(MH375819) 258 aaatgaaaaaaatactcttgggataacagcgtaataaatttggatagttcatatagataaaatagtttgcgacctcgatgttggattaggattctttttttggtgaagaagttaaaaaaa-gaagtttgtt
I.ricinus K18(MH375822) 259 aaatgaaaaaaatactctagggataacagcgtaataattttggatagttcatatagataaaatagtttgcgacctcgatgttggattaggattctttttttggtgaagaagttaaaaaaa-gaagtttgtt
I.ricinus K12(MH375825) 259 aaatgaaaaaaatactctagggataacagcgtaataattttggatagttcatatagataaaatagtttgcgacctcgatgttggattaggattctttttttggtgaagaagttaaaaaaaagaagtttgtt
I.ricinus K19(MH375821) 259 aaatgaaaaaaatactctagggataacagcgtaataattttggatagttcatatagataaaatagtttgcgacctcgatgttggattaggattctttttttggtgaagaagtt-aaaaaaagaagtttgtt

I.ricinus K2(MH375820)  387 caacttataatttcctacttgatctg
I.ricinus K9(MH375816)  388 caacttataatttcctacttgatctg
I.ricinus K14(MH375824) 389 caacttataatttcctacttgatctg
I.ricinus K16(MH375823) 389 caacttataatttcctacttgatctg
I.ricinus K1(MH375826)  387 caacttataatttcctacttgatctg
I.ricinus K8(MH375817)  387 caacttataatttcctacttgatctg
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I.ricinus K18(MH375822) 388 caacttataatttcctacttgatctg
I.ricinus K12(MH375825) 389 caacttataatttcctacttgatctg
I.ricinus K19(MH375821) 388 caacttataatttcctacttgatctg
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Figure 6: Clustal-W alignment of 16S rDNA amplicons from 11 *Ixodes ricinus* haplotypes in Aïn Draham region (Jendouba district, Northwest Tunisia)

The amplicons showed 95-100% homology with sequences from Algeria, Morocco, France, Germany, Netherlands, Portugal and North America.

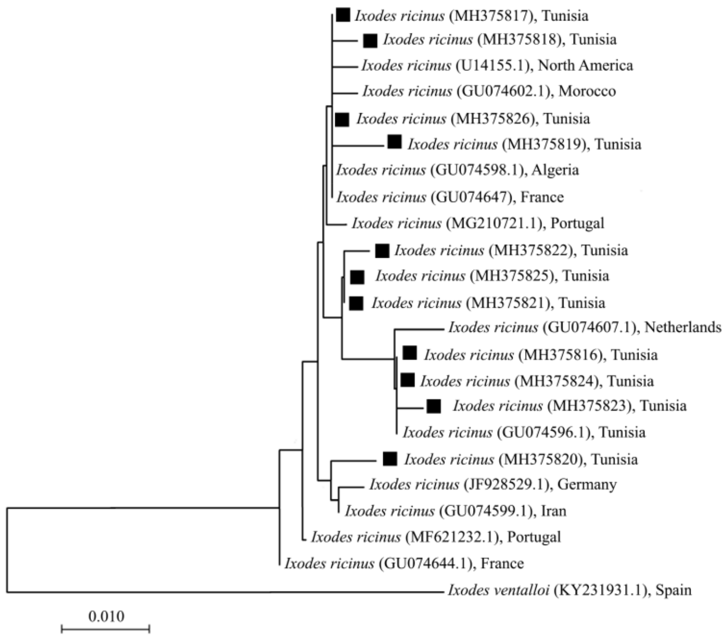

Figure 7: Phylogenetic tree showing the relationships between Tunisian isolates identified in the present study (indicated by black squares) and those deposited in GenBank from different countries based on 16S rDNA gene of *Ixodes ricinus* collected from Northwest Tunisia.

The tree was constructed using the neighbour-joining method of Saitou and Nei (1987) and distance were estimated using the Tamura-Nei model (Tamura and Nei, 1993).

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Discussion

The aim of the present study was to investigate the phenology and phylogeny of *Ixodes ricinus* in 5 contiguous sites in North-western Tunisia and to evaluate the impact of environmental factors i.e. Land Surface Temperature (LST), relative humidity, rainfall, and NDVI on its distribution. The selection of the study sites was based on previous work conducted in the same region and whose ecology was found to be favourable for the presence of *Ixodes ricinus* (Bouattour, 2001; Younsi et al., 2001).

A total of 116 ticks that were morphologically identified as *Ixodes ricinus* were collected during the 12 monthly visits between September 2016 and August 2017 from the five sites. The number of collected *Ixodes ricinus* in the studied sites was lower than that reported previously by Bouattour et al. (2004) in the same region where this species was dominant (n = 421/429 ticks). In another work performed in humid Tunisian regions, *Ixodes ricinus* represented 87% of the total tick population (N = 5146) collected from cattle which represent the preferential host species for *Ixodes* in Tunisia (Bouattour, 2001). Differences could be explained by the duration of the study and the sampling methods since we collected ticks only from vegetation, while other authors collected from domestic animals.

As in Tunisia, in the other North African countries, *Ixodes ricinus* was reported in humid and sub-humid bioclimatic areas. In North west of Morocco, *Ixodes ricinus* represented 26.3% of the tick population collected in Gharb region (N=582/6899) which is situated in 102 m above sea level and belonging to sub-humid bioclimate zone (Laamari et al., 2012). The vegetation is characterized by an abundant forest cover consisting mainly of cork oaks, eucalyptus and ferns. While in Northern Algeria, a small population was collected from cattle in Wilaya of El Tarf which is characterized by a semi-arid climate and has an altitude varied between 800 and 900 m (0.9%; n = 47/5009) (Elfegoun et al., 2013). The differences between ticks abundance in these mountainous regions could be explained by the differences of climate conditions, altitude,

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vegetation type and the cattle breed especially in the presence of local breed which showed in previous studies a resistance against the infestation by some tick species (Ibelli et al., 2012). The prevalence of *Ixodes ricinus* in the European countries is totally different since the number of collected ticks in one year can exceed 3000 ticks as reported in some studies (Remesar et al., 2019). For that reason, in Europe, *Ixodes ricinus* is a serious threat to both humans and animals considering its vector role of zoonotic diseases such as Lyme borreliosis (Benredjem et al., 2014; Estrada-Peña, 2001; Estrada-Peña and de la Fuente, 2017; Hvidsten et al., 2015; Younsi et al., 2005). The variation of population prevalence in these different countries could be associated to the study design and period of the survey, variation in the characteristic of the microenvironment and the presence of different host species.

The present survey showed the occurrence of larvae between July and September while nymphs were active between May and August. Adult *I. ricinus* were present from October to May with peaks during February and April. This is not consistent with the results of Bouattour et al. (1999) who collected immatures instars from lizards between April and June in El Jouza region, a mountainous area belonging to the same bioclimate zone and it is located 52 km far from the study region. The same work revealed that adult *I. ricinus* were observed on cattle between late September and middle March (Bouattour et al., 1999). This few difference could be attributed to the differences of regions, the sampling methods and the variations in climate conditions between the year of the studies.

The activity dynamics of *I. ricinus* in North African regions have the same trend of variation with a little bit of lag, for example in Morocco and Algeria adult *I. ricinus* were observed in cattle between November and April in Gharb region (Laamari et al., 2012) and between December and February in El-Tarf (Elfegoun et al., 2013). This could be explained by the differences in microclimate, the modification of abiotic factors and the vegetation cover in response to changes in environmental factors and climate warming as demonstrated for some

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plant species such as mushroom, golden chanterelle (*Cantharellus cibarius*) and *Anemone nemorosa* (Monteith et al., 1991; De Frenne et al., 2013; Jdaidi and Hasnaoui, 2016). A slightly differences among the NDVI mean values recorded in the study sites might be associated to human-modified landscape or fragmentation of habitat by natural phenomena (Ehrmann et al., 2017).

Indeed, each year, during hot and dry weather, there is a dramatic reduction in forest areas due to forest fires caused by hot wind (sirocco) or human activities. Chriha and Sghari (2013) showed that the superficies of destroyed forest as a result of fire during 2012 was estimated to 2000 ha in Tunisia. Nearly, 150 ha are located in the area where this study was carried out. The fire disturbs the ecosystems, kills a part of the fauna and forces another part to migrate. This may explain the lower number of ticks in the present survey compared to previous studies. Eventually, the decrease in rainfall and the increase of temperature during the last decade may be important factors influencing the biotope of ticks then both its distribution and abundance (World Health Organization Regional Office for the Western, 2015), it influences also life cycle pattern of *I. ricinus* and the pathogens it transmits (Bouattour, 2009). C'est de la speculation tu n'as aucun argument objectif ici

By analysing the *I. ricinus* phenology, we observed an overlap of nymphs and adults during May. The presence of nymphs before larvae population is most likely attributed to the diapause period. In fact, it is well established that when the ambient conditions are not favourable, nymph arrest their development by incorporate diapause leading to the occurrence of individuals from different instars at the same time (Gray et al., 2016). Moreover, the seasonal development of nymphs is widely influenced by photoperiod (Belozerov and Naumov, 2002), so the nymphs observed in our study might be from the population of the previous year.

There was a significant difference in tick number between the studied sites which is possibly due to the variability in the microenvironment and the characteristic of each sites in term of

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slope and altitude. The highest tick density was recorded in site 2 which is characterized by high trees density, a thick layer of undergrowth and the presence of wild boars. Whereas, the lowest density was reported in site 4 which is an exposed site and it was characterized by the absence of ferns and undergrowth and it presented a low density of vegetation. The low density of ticks in exposed area was mentioned by Estrada-Peña, (2001). Indeed, *I. ricinus* biology is widely affected by microenvironment factors. In fact more thick is the undergrowth, highest is the humidity which is better suitable for tick development (Hvidsten et al., 2015). This is also consistent with the study of Zingg et al., (2018) that showed a positive correlation between the litter depth and tick abundance. The same authors associated the low abundance of ticks to the influence of formic acid produced by the red wood ants and their predatory behaviour. Similar studies showed that the larval abundance of *Ixodes scapularis* was affected by the soil predator fauna (Burtis et al., 2016) and the humus composition and pH (moder humus with thick layer) was also associated to the nymphs density (Goldstein et al., 2018).

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The number of ticks decreased significantly with slope and altitude. These results are in line with those found by Morán Cadenas et al. (2007) who showed that the tick density was lower in north-facing slope than south-facing slope and it decrease with altitude.

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In spite of different NDVI mean values between the five sites, there was no significant correlation with the tick number. These results are not consistent with previous works carried out in Spain and Italy that showed a significant positive correlation between tick abundance and NDVI values (Estrada-Peña, 2001; Bisanzio et al., 2008).

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This finding could be explained by the fact that the five selected sites were very close to each other and were characterized by the same vegetation cover with lower differences that is not enough to affect the tick distribution.

There was a significant negative correlation between adults *I. ricinus* number and both humidity and LST while the tick number is positively correlated with rainfall. This finding confirm the data provided by previous work (Estrada-Peña, 2001).

The *I. ricinus* amplicons in Aïn Draham forest collected from vegetation and cow are relatively diverse and showed a genetic variability comparing to those from other Mediterranean regions. The Tunisian and the other North African isolates have a close relationship and belong to the same group. This divergence could be explained by the interaction of ticks with their environment and hosts. In fact, the variation among microclimates and the geographical discontinuity could affect the genetic structure and drives the geographic distribution of *I. ricinus* population (Jore et al., 2014). The presence of the Mediterranean Sea could be a natural barrier leading to the isolation of North African tick groups from those present in Europe. Furthermore, the differences in the activity dynamics and the interaction with different host communities could contribute to this divergence. For example, in Europe *I. ricinus* parasitizes mainly sheep but in Tunisia it was found mainly in cattle (Bouattour, 2001; Noureddine et al., 2011).

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To our knowledge, this the first investigation on the activity dynamics of *I. ricinus* regarding temperature, humidity, rainfall and NDVI. Although no significant correlation between the number of ticks collected and the factors cited before, this does not exclude their role as driver of *I. ricinus* phenology. Further studies with extension to other sites where *I. ricinus* was detected before such as El Jouza (Beja district), Oued Abid (Nabeul district) are needed. A study period of at least two years will be suitable for better understand the complete life cycle of *I. ricinus* in Tunisia and the influence of NDVI and meteorological parameters on its distribution.

The sequencing of more samples from other regions in Tunisia would provide more data about the genetic structure and the polymorphism of the species, in particular the possibility of sympatric *Ixodes inopinatus* population.

Acknowledgment

This study was financially supported by the “Laboratory of epidemiology of enzootic infections in herbivores in Tunisia: application to control (Laboratory reference: LR16AGR01)” (Ministry of Higher Education and Scientific Research, Tunisia) and the CGIAR Research program on Livestock. The authors thank Mr. Bechir Guesmi and Mr. Tarek Drissi for their support.

Competing interests

The authors declare that they have no competing interests.

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