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Improving specific detection and updating phylogenetic data related to *Anaplasma platys*-like strains infecting camels (*Camelus dromedarius*) and their ticks

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ABSTRACT

In camels and their infesting ectoparasites, specific detection of pathogenic *Anaplasma platys* and genetically related strains (*A. platys*-like strains) remains problematic. This requires sequencing of the hemi-nested PCR products specific to *A. platys* and related strains. In this study, a PCR/RFLP method, earlier developed for specific detection of *A. platys*-like strains in animal species other than camels, was adapted in order to subtype *A. platys*-like strains isolated from camels and their ticks and to differentiate them from pathogenic *A. platys* without going through a sequencing step. This approach was used for investigating the infections with *A. platys* and related strains in 412 *Camelus dromedarius* camels and 334 feeding ticks from five Tunisian governorates. Microscopic examination using Giemsa-stained blood smears was performed in order to specify which types of cells were infected. Ticks were identified as *Hyalomma dromedarii* (n = 164, 49%), *H. impeltatum* (n = 161, 48.3%) and *H. excavatum* (n = 9, 2.7%). *A. platys* was not detected in any of the tested camels or ticks. The overall prevalence of *A. platys*-like strains was 5.6% (23/412) in camels and microscopic examination of infected cells showed a tropism for neutrophil granulocytes. One tick identified as *H. dromedarii* out of 327 analyzed ticks was found to be infected with *A. platys*-like strains (0.3%). Alignment, identity comparison and phylogenetic analysis of the 16S rRNA partial sequences obtained in this study suggest that Tunisian dromedaries and feeding ticks are infected with different *Anaplasma* strains genetically related to *A. platys*. Sequence analysis and phylogenetic study based on the *groEL* gene confirm the RFLP results and show that camel strains formed a separate sub-cluster relatively close to *A. platys*-like strains infecting Tunisian cattle. This adapted RFLP assay allows fast and specific detection of pathogenic *A. platys* and *A. platys*-like strains in camels and infesting ticks and has the intrinsic potential of revealing co-infections with these two types of bacteria in the same sample, reducing the time and costs associated with cloning and sequencing during molecular diagnosis.

1. Introduction

Nowadays, emerging and re-emerging diseases occur increasingly in humans and animals and lead to large economic losses especially in developing countries. Due to the climatic changes, numerous infectious agents, showing a potential public health interest, have been detected all over the world. Most of these diseases are caused by various vector-borne bacteria (Ehounoud et al., 2017). Among these emerging bacteria, *Anaplasma* spp. occupy an important place. They are Gram-negative, obligate intracellular bacteria belonging to the order Rickettsiales, family *Anaplasmataceae*. Until now, there are six recognized species belonging to this genus, namely *A. ovis*, *A. bovis*, *A. marginale*, *A. centrale*, *A. phagocytophilum* and *A. platys* (Dumler et al., 2001).

Anaplasma platys, the causative agent of infectious canine cyclic thrombocytopenia, is the only classified rickettsial species known to infect platelets (Ben Said et al., 2018; Dumler et al., 2001). It has been, for a long time, considered as a specific pathogen of dogs but several reports described the infection in humans (Arraga-Alvarado et al., 2014; Breitschwerdt et al., 2014; Maggi et al., 2013), cats (Lima et al., 2010; Salakij et al., 2012) and some ruminants such as camels, buffalo, sheep, goats and red deer (Allsopp et al., 1997; Chochlakis et al., 2009; Djiba et al., 2013; Li et al., 2015, 2016; Lorusso et al., 2016a, b;

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Machado et al., 2016). In Tunisia, *A. platys* infection was detected in dogs (M'ghirbi et al., 2009) and in *Rhipicephalus sanguineus* sensu lato ticks (Sarih et al., 2005).

Additionally to the canine pathogen, *A. platys*-like strains have been found in neutrophils of cattle, sheep and goats (Zobba et al., 2014), and in cat platelets in Italy (Zobba et al., 2015). This bacterium was also detected by PCR and sequencing in Algerian cattle (Dahmani et al., 2015) and in Moroccan camels recently reported by Ait Lbacha et al. (2017) as "*Candidatus* Anaplasma camelii". In ticks, these strains were also identified in *R. sanguineus* s.l. infesting dogs from Costa Rica (Campos-Calderón et al., 2016) and in *R. bursa* collected from Italian ruminants (Chisu et al., 2018). Since the specific detection of these strains genetically related to *A. platys* requires a sequencing step, Ben Said et al. (2017) developed a RFLP assay coupled with hemi-nested *groEL* PCR published earlier by Alberti et al. (2005) in order to discriminate between *A. platys* and genetically related strains. Proved by *in silico* analysis, this method has made it possible to specifically detect *A. platys*-like strains in several wild and domestic animals including goats, sheep, cattle, red deer and cats from Tunisia and/or Italy (Ben Said et al., 2017).

Camelus dromedarius is a ruminant species of great economic importance in several African countries including Tunisia (Ben Dhia et al., 1999). It is an essential source of revenue exploited for meat and milk production (Ben Dhia et al., 1999; Moslam and Megdiche, 1989). Camels can be infested by various tick species such as *Hyalomma dromedarii*, *H. impeltatum, H. excavatum*, *H. marginatum*, *H. lusitanicum*, *R. sanguineus* s.l., *R. bursa*, *R. pulchellus*, *R. decoloratus*, *Amblyomma variegatum* and *A. gemma* (Elghali and Hassan, 2009; Fard et al., 2012; Gharbi et al., 2013; Loftis et al., 2006; Zeleke and Bekele, 2004). Until now, only one report described the infection with *A. platys*-like strains in Tunisian dromedaries (Belkahia et al., 2015). However, no data is available concerning *Anaplasma* infections in ticks infesting camelids in Tunisia.

In this study, we adapted a PCR/RFLP method, earlier developed by Ben Said et al. (2017) in order to subtype *A. platys*-like strains isolated from camels and infesting ticks and to differentiate them from pathogenic *A. platys* without going through a sequencing step. We used this approach to search the occurrence and the prevalence of *A. platys*-like strains in camels and ticks from five Tunisian governorates. Infected cells were identified by Giemsa-stained blood smears of positive camel samples. Genotyping and phylogenetic analysis based on 16S rRNA and *groEL* genes were also performed on revealed *A. platys*-like strains infecting camels and/or feeding ticks.

2. Materials and methods

2.1. Sampling regions

The present study was carried out from October 2015 to August 2017 in seven farms located in five governorates from central and southern Tunisia (Fig. 1). These governorates are Kebili and Tataouine (Saharan bioclimatic area; temperature average in summer (from June to August): 39 °C and 35 °C, respectively; annual rainfall averages: 94 mm and 134 mm, respectively), Gabes and Kairouan (arid bioclimatic area; temperature average in summer: 33 °C and 35 °C, respectively; annual rainfall averages: 177 mm and 293 mm, respectively) and Sousse (semi-arid bioclimatic area; temperature average in summer: 31 °C; annual rainfall average: 361 mm) (INM, 2018). Studied regions are known for camel breeding and harbored nearly 70% of the national camel population estimated at 56,021 heads (OIE, 2016). Camels of the military mehary unit based in the region of Douz (governorate of Kebili) were visited and examined four times in order to survey the seasonal dynamics of *Anaplasma* infection.

2.2. Samples size determination

The minimal required number of samples may be estimated and determined using the following formula: $N = 1.96^2 * Pexp (1 - Pexp) / d^2$ (Thrusfield, 2005) where required sample size (N) depends on the expected anaplasmosis prevalence (Pexp = 50%) with a confidential interval of 95% and the accepted absolute error (d) of 5%. According to this formula, a total of 384 samples were required in this study (76 samples at the governorate level (n = 5) and 54 samples from each herd (n = 7)). Since it was not possible to examine all camels, sampling was randomly done taking into account a representative number of samples from each farm and governorate.

2.3. Camel blood sampling and tick collection

A total of 412 apparently healthy camels were examined. Whole-blood samples were collected aseptically from the jugular vein of each dromedary using vials containing EDTA. Information concerning animal age, gender, breed and tick infestation were recorded. In addition, 334 ticks were removed, by hand or steel forceps, from different thin parts of dromedary skin as ears, eyelids, interdigital space, udder, inner side of the thighs, perineum and tail. Referring to the taxonomic key of tick identification (Walker et al., 2013), specimens were identified based on various morphologic criteria. Ticks collected from each camel were stored separately, in labeled tubes containing 70% ethanol.

2.4. DNA genomic extraction

The DNA was extracted from 300 µl of each blood sample with the Wizard® Genomic DNA purification kit (Promega, Madison, USA) according to the manufacturer's instructions. Ticks were immersed in distilled water for 10 min, dried on sterile filter paper, crushed individually by shaking with the Tissue Lyser LT (Qiagen, Hilden, Germany), and homogenized. A total of 100 µl of homogenized ticks was used for DNA extraction with the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. DNA was eluted in a final volume of 100 µl. DNA extracts were then stored at -20 °C until use. The search of DNA extraction efficiency was performed in all tick samples with polymerase chain reaction (PCR) amplification of the 16S rRNA mitochondrial gene using tick-specific primers TQ16S + 1 F and TQ16S-2R, as described by Black and Piesman (1994) (Supplementary file 1). Additionally, for blood and tick samples, DNA quantity and quality were estimated by using the Qubit 3.0 Fluorimeter (Thermo Fisher).

2.5. Molecular detection of A. platys-like strains

Primer pair EHR16SD/ EHR16SR was used in a catch-all PCR for amplifying 345 bp of 16S rRNA gene of the *Anaplasmataceae* bacteria (Parola et al., 2003, Supplementary file 1). PCR reaction was performed in a final volume of 50 µl containing 0.125 U/µl Taq DNA polymerase (Biobasic Inc, Canada), 1x PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 2 µl (50 to 150 ng) genomic DNA, 0.5 µM of primers. Thermal cycling profile was as described by Parola et al. (2003). Positive 16S rRNA samples were used for the identification of *A. platys* and related strains infections using hemi-nested PCR with outer primers EphplgroEL-F and EphplgroEL-R, and inner primer EplgroEL-R, amplifying 515 bp of the *groEL* gene (Alberti et al., 2005; Alberti and Sparagano, 2006; Ben Said et al., 2017, Supplementary file 1). Each reaction was performed in a final volume of 50 µl containing 0.125 U/µl Taq DNA polymerase (Biobasic Inc, Canada), 1x PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 2 µl genomic DNA (50 to 150 ng) and 0.5 µM of primers. Thermal cycling reactions were performed by using an automated DNA thermal cycler (Applied Biosystems 2720 Thermal Cycler, Foster City, California, USA) as described by Alberti et al. (2005). One microliter of each amplicon was used for PCR reaction with specific primers under

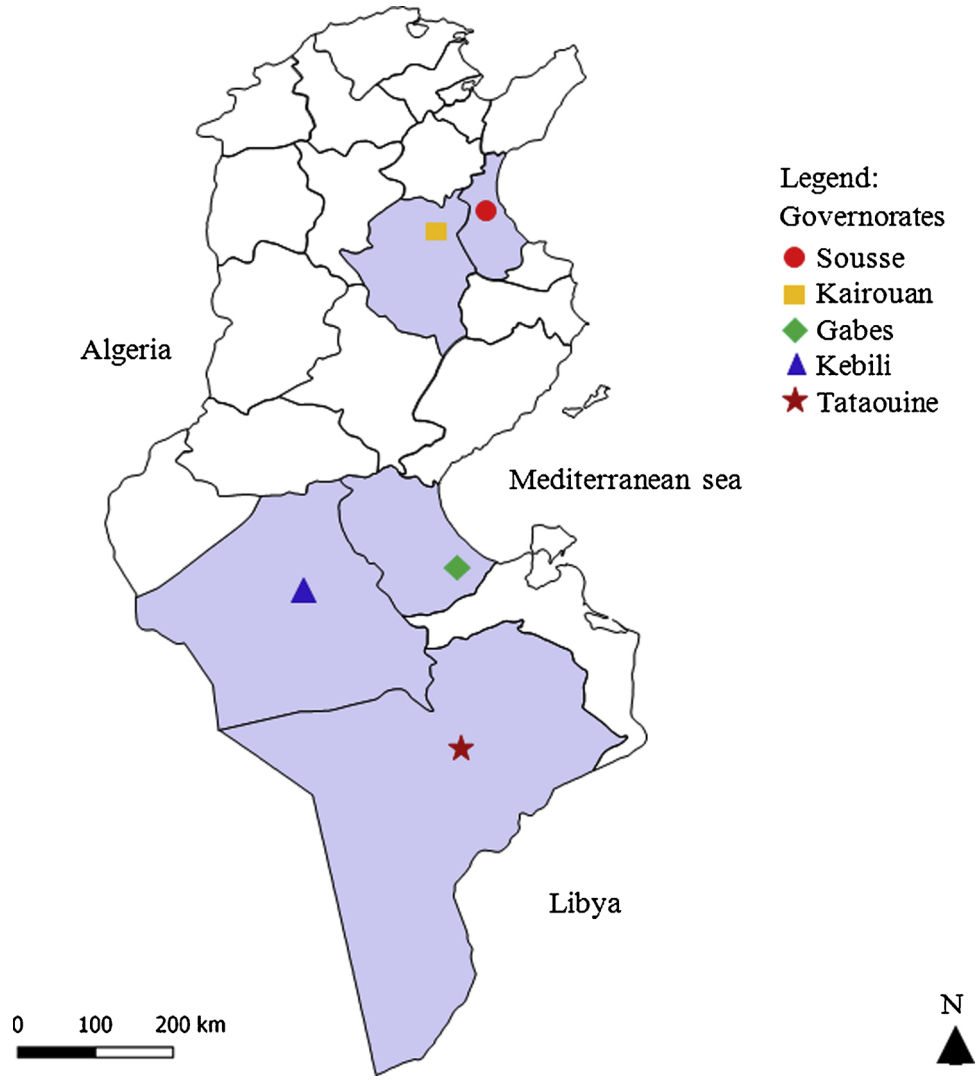

Fig. 1. Map of Tunisia showing investigated governorates.

the same conditions as for the first PCR. The PCR products were electrophoresed in 1% agarose gel containing 0.5 µg/ml of ethidium bromide. Distilled water and DNA samples positive to *A. platys* and/or to *A. platys*-like strains from sheep, goats and cattle (Ben Said et al., 2017) were used as negative and positive controls, respectively, in each PCR run.

In order to sub-type revealed strains and to found any eventual co-infection between *A. platys* and *A. platys*-like strains infecting camels and/or infesting ticks, the *groEL* partial sequences obtained in this study were used for the RFLP assay recently developed and applied by Ben Said et al. (2017). This method based on *Stu*I restriction enzyme (New England Biolabs, Hitchin, UK) was able to discriminate between canine *A. platys* and *A. platys*-like strains present in sheep, goats and cattle (Ben Said et al., 2017). Thus, this same RFLP assay was used in a first step, to differentiate between canine *A. platys* and camel *A. platys*-like strains.

2.6. Sequencing and phylogenetic analysis

A selection of PCR products obtained with primers EHR16SD/EHR16SR and EphplgroEL-F/EplgroEL-R, respectively, representative of the *Anaplasmataceae* (18 amplicons of 16S rRNA partial sequence) and *A. platys* and related strains complex (8 amplicons of *groEL* partial sequence), were purified with the GF-1 Ambi Clean kit (Vivantis, USA) according to manufacturer's instructions. Purified DNA fragments were sequenced in both directions, using the same primers as for the PCR amplifications step (Supplementary file 1). The reaction was performed using a conventional Big Dye Terminator cycle sequencing ready reaction kit (Perkin Elmer, Applied Biosystems, Foster City, USA) and an ABI3730XL automated DNA sequencer (Macrogen Europe, Amsterdam, The Netherlands). Chromatograms were edited with Chromas Lite v 2.01. The DNAMAN program (Version 5.2.2; Lynnon Biosoft, Que., Canada) was used to perform multiple sequence alignments of 16S rRNA and *groEL* sequences and to translate nucleotide to amino-acid GroEL sequences. Identity searches were conducted by using BLAST (http://blast.ncbi.nlm.nih.gov, Altschul et al., 1997). DNAMAN program was also used to calculate genetic distances computed by the maximum composite likelihood method (Tamura and Nei, 1993). Neighbor-joining trees were built by using the same software (Saitou and Nei, 1987). Statistical support for internal branches was established by bootstrap analysis with 1000 reiterations. The 16S rRNA and *groEL* nucleotide partial sequences of *Anaplasma* sp. isolates genetically related to *A. platys* were deposited in the GenBank under accession numbers from MH510072 to MH510097 (Tables 2 and 3).

2.7. Adapting RFLP method for specific detection of camel A. platys-like strains

Since the available RFLP assay did not discriminate between *A.*

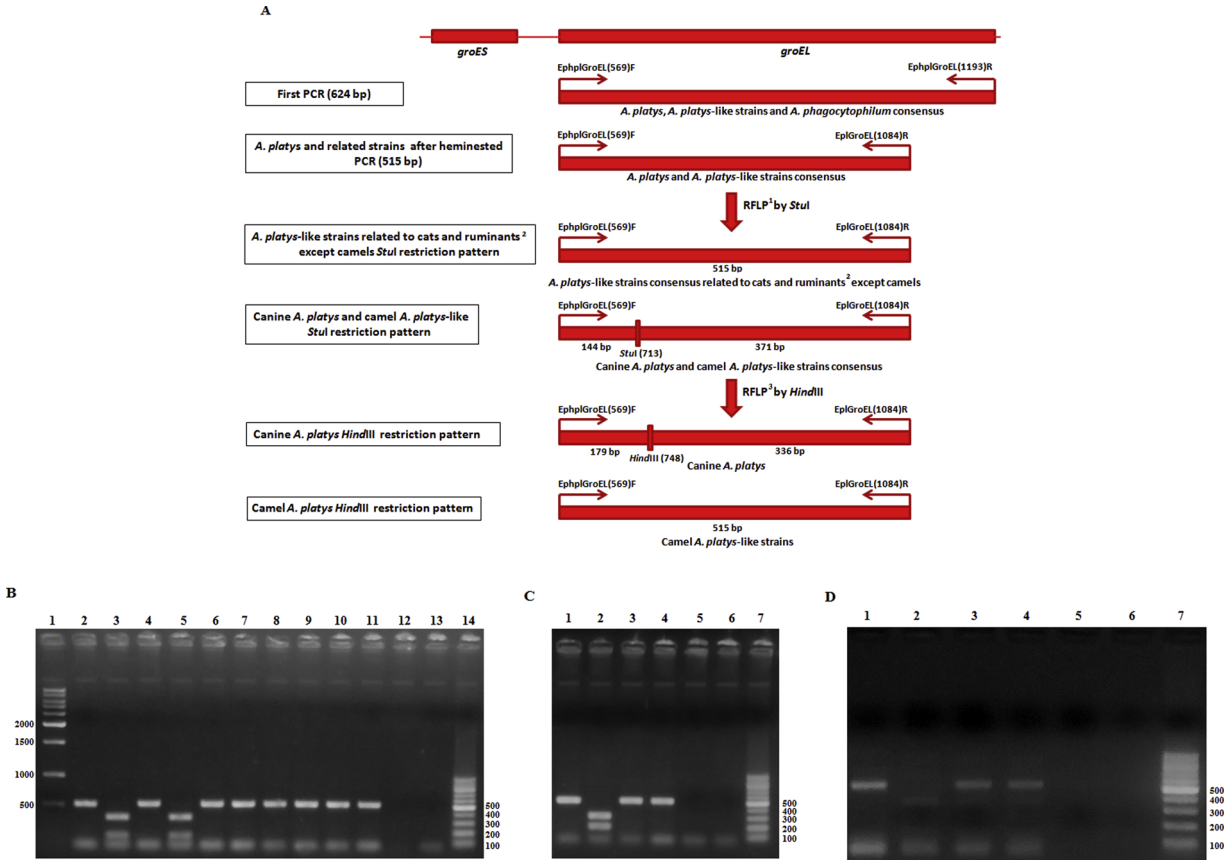

Fig. 2. Adapted RFLP strategy and results of restriction analysis. (A) RFLP strategy adapted and validated during this study for the specific detection and differentiation between canine *A. platys* and camel *A. platys*-like strains. Numbers in parentheses indicate positions of primers and restriction sites of *Stu*I and *Hind*III enzymes calculated using the sequence of NCH-1 strain of *A. phagocytophilum* as a reference; [1]: A *Stu*I site (AGG/CCT), at the position 713 referring to the full *groEL* sequence, was identified in all analyzed canine *A. platys* and camel *A. platys*-like sequences and was absent in all other analyzed *A. platys*-like sequences caused by the substitution of "G" by "A" and of "T" by "G" in positions 713 and 716, respectively. [2]: Ruminants other than camels are cattle, sheep, goats and deer. [3]: A *Hind*III site (A/AGCTT), at the position 748 referring to the full *groEL* sequence, was identified in all analyzed canine *A. platys* sequences and was absent in all camel *A. platys*-like sequences caused by the substitution of "T" by "C" in position 753. (B) Results of restriction analysis using *Stu*I enzyme on DNA analyzed samples. 1 Kb ladders (line 1) and 100 bp ladders (line 14), canine *A. platys* (515 bp) products before RFLP assay isolated from dogs (line 2) and *A. platys*-like PCR (515 bp) isolated from camels (line 4), cattle (line 6), sheep (line 8) and goats (line 10); PCR products after RFLP assay of canine *A. platys* (line 3) and camel *A. platys*-like strains (line 5) (144 and 371 bp) isolated from dogs and camels, respectively, and *A. platys*-like strains (515 bp) isolated from cattle (line 7), sheep (line 9) and goats (line 11). PCR results of negative control before (line 12) and after (line 13) RFLP assay. (C) Results of restriction analysis using *Hind*III enzyme on DNA analyzed samples. Lines 1 and 3: *A. platys* and *A. platys*-like PCR products (515 bp) before RFLP assay isolated from dogs and camels, respectively; lines 2 and 4: PCR products after RFLP assay of *A. platys* and *A. platys*-like strains isolated, respectively, from dogs (199 bp and 316 bp) and camels (515 bp); lines 5 and 6: PCR results of negative control before and after RFLP assay, respectively; and line 7: 100 bp ladder. (D) Results of restriction analysis using *Stu*I and *Hind*III enzymes on DNA analyzed tick sample positive to camel *A. platys*-like strains. Line 1 and 3: PCR products from the tick sample (515 bp) before RFLP assay. Line 2 and 4: PCR products from the tick sample, respectively, after *Stu*I (144 bp and 371 bp) and *Hind*III digestion (515 bp). Lines 5 and 6: PCR results of negative control before and after *Stu*I RFLP assay, respectively and line 7: 100 bp ladder. Since PCR product (515 bp) isolated from the tick sample has a weak band, only one band at 371 bp can be visualized after the digestion by *Stu*I restriction enzyme.

platys and *A. platys*-like strains found in camels and ticks, and the sequencing of *groEL* amplicons confirmed the presence of *A. platys*-like strains in dromedaries (Fig. 2 and Supplementary file 2), we adapted this RFLP method in order to be able to discriminate between canine *A. platys* and camel *A. platys*-like strains. *In silico* analysis of *A. platys* and camel *A. platys*-like *groEL* gene was performed in order to identify restriction site useful to differentiate between these two types of strains. Briefly, 28 *groEL* partial sequences (476 bp, excluding the two primers regions) derived from 19 *A. platys* and 8 camel *A. platys*-like strains, were aligned (Supplementary file 2). A *Hind*III site (A/AGCTT), at position 748 referring to the full *groEL* sequence, was identified in all analyzed *A. platys* sequences and was absent in all camel *A. platys*-like sequences caused by the substitution of "T" by "C" in position 753 (Fig. 2; Supplementary file 2). All hemi-nested PCR products obtained in this study were digested by *Hind*III to discriminate among *A. platys* and camel *A. platys*-like strains. Restriction reaction was performed in a final volume of 20 µl containing 10 µl PCR product, 2 µl buffer (10x), one µl *Stu*I/HindIII enzyme (10,000 U/ml) (Biolabs, New England, UK) and 7 µl distilled water. Digested products were incubated during one hour at 37 °C and run in a 3% high-resolution agarose gel by electrophoresis in TAE buffer (0.04 M Tris, 0.4 mM EDTA, pH 7.7–8.8) at 100 V for 60 min, and subsequently visualized at UV light after ethidium bromide staining.

2.8. Blood smears management

Three randomly selected samples revealed positive to *A. platys*-like and negative to other intracellular bacteria known to infect camels were subjected to blood smears on a microscopic slide for direct search of these *Anaplasma* organisms and identify which types of cells were specifically colonized by these bacteria. Small drop of blood was spotted on a slide and left dry. Slide was then fixed by methanol for three minutes and stained using Giemsa solution during ten minutes. Colored slides were visualized microscopically. Photos were captured

and saved directly using a high resolution microscopic camera related to computer (Leica Microsystems, Wetzlar, Germany).

2.9. Statistical analyses

Exact confidence intervals (CI) for prevalences at the 95% level were calculated. To assess the possible influence of abiotic factors (geographic location, bioclimatic area and season), and factors related to camels (gender, age, breed and presence of ticks) on the molecular prevalence of *A. platys*-like strains, chi square test or Fisher's exact test were performed using Epi Info 6.01 (CDC, Atlanta, USA) with a cut-off value of 0.05.

3. Results

3.1. Parasitological data and efficiency of DNA extraction from ticks

Among 412 dromedaries, 118 (28.6%) were found to be infested by ticks. A total of 334 ixodid ticks (320 adults and 14 nymphs) were collected. The obtained specimens belonged to three *Hyalomma* species. *H. dromedarii* (n = 164, 49%) and *H. impeltatum* (n = 161, 48.3%) were the dominant tick species, followed by *H. excavatum* (n = 9, 2.7%) ($p < 0.001$). Interestingly, *H. dromedarii* was collected from camels mainly located in the governorate of Kebili. In contrast, *H. impeltatum* seems to be preferentially associated with other investigated regions. DNA extraction was applied to all specimens. Partial sequence of 16S rRNA gene was amplified, by using primers specific to all tick genera, in 327/334 samples (97.9%). No amplification products were obtained for seven samples, reflecting a probable failure of the DNA extraction, and were thus excluded from the analysis.

3.2. Specific detection of camel A. platys-like strains

The overall prevalences of the *Anaplasmataceae* bacteria were 35% (144/412) for camels and 8% (26/327) for analyzed ticks. The DNA of *A. platys* and related strains was amplified from 24 positive samples (23 from camels and one from ticks) resulted from analyzing *groEL* positive samples, one canine *A. platys*-positive control isolated from dog and three *A. platys*-like positive controls isolated from sheep, goats and cattle. All these DNA samples produced a clear single band on agarose electrophoresis at 515 bp after the amplification step. Amplicons digestion with *Stu*I allowed differentiation of *A. platys* from *A. platys*-like strains from sheep, goats and cattle, but not from the 23 positive camels (Fig. 2B) and the one positive tick (Fig. 2D). However, digestion of the same amplicons with *Hind*III differentiated camel *A. platys*-like strains from the canine *A. platys* (Fig. 2C and D). More specifically, after restriction reaction, the canine *A. platys* generated two fragments of 336 and 179 bp while *A. platys*-like strains infecting camels was not digested by the same enzyme in the 23 positive camel samples (Fig. 2C) and in the one positive tick (Fig. 2D).

3.3. Molecular prevalence of A. platys-like strains in camels and infesting ticks

According to adapted RFLP assay, overall prevalences of *A. platys*-like strains were 5.6% (23/412) and 0.3% (1/327) in camels and ticks, respectively (Table 1). Camels from arid and semi-arid areas were more infected with *A. platys*-like strains (13%) than those from Saharan area (3%) ($p < 0.001$; Table 1). Animals from Kairouan and Gabes showed *A. platys*-like infection rates of 20% (8/40) and 15% (4/26), respectively. Infection rates in these locations were greater than those observed in other governorates ($p < 0.001$; Table 1). In the Kebili governorate, where the seasonality of the infection was monitored, dromedaries sampled during autumn and winter seasons were statistically more infected with *A. platys*-like strains (7% and 4%, respectively) compared to those tested during summer and spring (3% and 1%, respectively) ($p = 0.011$). In addition, no statistically significant differences were found among *A. platys*-like prevalences according to intrinsic factors related to camel gender, age, breed and tick infestation (Table 1).

Table 1
Prevalence of infection with *Anaplasma platys*-like strains in camels according to bioclimatic areas, geographic regions, seasons, gender, age, breed and tick infestation.

Risk factors	Categories	Examined camels	Positive (% ± C.I.[1])	P-value
Bioclimatic area	Saharan	304	9 (3 ± 0.01)	0.000*
	Arid and semi arid	108	14 (13 ± 0.06)	
Region	Gabes	26	4 (15.4 ± 0.13)	0.000*
	Kebili	250	9 (3.6 ± 0.02)	
	Kairouan	40	8 (20 ± 0.02)	
	Sousse	42	2 (4.8 ± 0.06)	
	Tataouine	54	0 (0)	
Season[2]	Autumn	73	5 (6.8 ± 0.05)	0.011*
	Winter	46	2 (4.3 ± 0.05)	
	Spring	91	1 (1.1 ± 0.02)	
	Summer	40	1 (2.5 ± 0.04)	
Gender	Male	176	10 (5.7 ± 0.03)	0.939
	Female	236	13 (5.5 ± 0.02)	
Age	< 5 years	132	5 (3.8 ± 0.03)	0.103
	5 to 10 years	148	6 (4 ± 0.03)	
	> 10 years	132	12 (9.1 ± 0.04)	
Breed[3]	Local	377	23 (6.8 ± 0.02)	0.133
	Other breeds	35	0 (0)	
Tick infestation	Infested	118	9 (7.6 ± 0.04)	0.252
	Not infested	294	14 (4.8 ± 0.02)	
Total		412	23 (5.6 ± 0.02)	

[1] C.I.: 95% confidence interval.
[2] The effect of the season on *A. platys*-like strain has only been investigated on 250 dromedaries located in the governorate of Kebili.
[3] Local breed is Arab breed and the other breeds are Arnouti and Tergui.
* Differences in prevalences of *A. platys*-like strain according to bioclimatic areas, regions and seasons were statistically significant ($p < 0.05$).

3.4. Microscopic findings

The microscopic approach was used in the current study in order to identify the preferential cellular tropism of *A. platys*-like strains in camels. The examination of blood smears derived from *A. platys*-like positive camel samples showed the presence of morulae only in the neutrophil granulocytes. These morulae appeared in the cytoplasm as pleomorphic basophilic inclusions (Fig. 3A to D). Platelets, monocytes and erythrocytes were not found to be infected with this bacterium (Fig. 3E to J).

3.5. Molecular characterization of Anaplasma sp. 16S rRNA genotypes

Eighteen PCR products obtained from 17 randomly selected camels and the only one infected tick with primers EHR16SD/EHR16SR targeting 305 bp of the 16S rRNA gene of the *Anaplasmataceae* were successfully sequenced on both DNA strands. Based on nucleotide alignments, the sequences were grouped in three different genotypes isolated from *C. dromedarius* (Apllike16SCd1 to Apllike16SCd3; GenBank accession numbers MH510072 to MH510088 respectively) and one genotype isolated from *H. dromedarii* tick (Apllike16SHd1; GenBank accession number MH510089). All 16S rRNA sequences obtained in this study shared 99.7% nucleotide identity and differed from each other in three nucleotide positions (Table 2). Based on BLASTN analyses and nucleotide alignments, the four identified genotypes were 99.7–100% identical to those of J3 and E10 *Anaplasma* sp. isolates (GenBank accession numbers JN558826 and JN558821, respectively) found in Chinese goats and considered as *A. platys*-like strains by Liu et al. (2012, Table2). Besides, obtained sequences shared 99.7–100% identity with

Table 2
Designation and information about sequencing 16S rRNA partial sequences of the eighteen *Anaplasma* sp. isolates closely related to *A. platys* infecting camels and ticks in the present study.

Anaplasma spp.	Genotype	Isolate[1]	Host or vector	Geographical location	GenBank[2]	16S rRNA variable positions[3]			BLAST analyse
						451	503	703	
A. platys	ZAM dog-72	ZAM dog-72	*Canis lupus familiaris*	Lusaka, Zambia	LC269822	C	C	C	100% *A. platys*
Anaplasma sp.	J3	J3	*Capra hircus*	Zhejiang, China	JN558826	–	–	–	100% *A. platys*
	E10	E10	*Capra hircus*	Zhejiang, China	JN558821	–	–	–	100% *A. platys*
	Apllike16SCd1	GbCd001	*Camelus dromedarius*	Gabes, Tunisia	MH510072	–	–	–	100% *A. platys*
		DzCd060	*Camelus dromedarius*	Douz, Tunisia	MH510073	–	–	–	100% *A. platys*
		DzCd059	*Camelus dromedarius*	Douz, Tunisia	MH510074	–	–	–	100% *A. platys*
		DzCd089	*Camelus dromedarius*	Douz, Tunisia	MH510075	–	–	–	100% *A. platys*
		DzCd090	*Camelus dromedarius*	Douz, Tunisia	MH510076	–	–	–	100% *A. platys*
		DzCd147	*Camelus dromedarius*	Douz, Tunisia	MH510077	–	–	–	100% *A. platys*
		KrCd105	*Camelus dromedarius*	Kairouan, Tunisia	MH510078	–	–	–	100% *A. platys*
		KrCd107	*Camelus dromedarius*	Kairouan, Tunisia	MH510079	–	–	–	100% *A. platys*
		KrCd108	*Camelus dromedarius*	Kairouan, Tunisia	MH510080	–	–	–	100% *A. platys*
		KrCd112	*Camelus dromedarius*	Kairouan, Tunisia	MH510081	–	–	–	100% *A. platys*
		KrCd113	*Camelus dromedarius*	Kairouan, Tunisia	MH510082	–	–	–	100% *A. platys*
		KrCd114	*Camelus dromedarius*	Kairouan, Tunisia	MH510083	–	–	–	100% *A. platys*
		SsCd262	*Camelus dromedarius*	Sousse, Tunisia	MH510084	–	–	–	100% *A. platys*
		TtCd273	*Camelus dromedarius*	Tataouine, Tunisia	MH510085	–	–	–	100% *A. platys*
		TtCd274	*Camelus dromedarius*	Tataouine, Tunisia	MH510086	–	–	–	100% *A. platys*
	Apllike16SCd2	DzCd043	*Camelus dromedarius*	Douz, Tunisia	MH510087	T	–	–	99% *A. platys*
	Apllike16SCd3	DzCd354	*Camelus dromedarius*	Douz, Tunisia	MH510088	–	T	–	99% *A. platys*
	Apllike16SHd1	DzHd030M1	*Hyalomma dromedarii*	Douz, Tunisia	MH510089	–	–	T	99% *A. platys*

[1] Isolate or clone.
[2] GenBank accession number.
[3] Numbers represent the nucleotide position with respect to the HZ strain from USA for *A. phagocytophilum* (GenBank accession number NC_007797) (Lin et al., 2011).

A. platys ZAM dog-72 isolate found in a dog from Zambia (GenBank accession number LC269822) and differed in three nucleotide positions (Table 2). Phylogenetic analysis assembled all obtained sequences in this study in monophyletic clusters including *A. platys* (Fig. 4). In particular, all *Anaplasma* sp. Tunisian strains were closely related to *A. platys*-like strains isolated from Chinese goats and *A. platys* infecting dogs from Zambia and *R. sanguineus* s.l. ticks from India (Fig. 4).

3.6. Molecular characterization of Anaplasma platys-like groEL genotypes

The infection with *Anaplasma platys*-like strains was confirmed by sequencing 476 bp of the *groEL* gene from eight randomly selected positive camel samples. Since PCR product isolated from the only one infected tick has a weak band, we failed to obtain *groEL* partial sequence for this tick sample (Table 3). Sequences alignment revealed six distinct genotypes isolated from *C. dromedarius* camels (ApllikegroelCd1 to ApllikegroelCd6; GenBank accession numbers MH510090 to MH510097) (Table 3). Nucleotide identity among genotypes ranged from 99.2 to 99.8% (Table 4). Revealed genotypes were 87.0 to 91.0% identical on comparison with 27 *A. platys*-like genotypes isolated from Tunisian ruminants and Italian ruminants and cats published in the GenBank. When compared to the *A. platys* reference sequence (GenBank accession number AY848753), nucleotidic identities ranged from 87.0 to 87.8% and differed in 64 nucleotide positions (Tables 4 and 5). Phylogenetic analysis based on the alignment of the six genetic variants obtained in this study was compared with all sequence types of *A. platys*-like strains detected in other Tunisian ruminants (cattle, goats and sheep), in Italian ruminants, cats and ticks, and in one tick from Costa Rica, as well as other *Anaplasma* species found in GenBank (Fig. 5). Identified variants were classified among *A. platys* and related strains cluster and formed a separate sub-cluster closely related to another sub-cluster formed by two *A. platys*-like variants isolated from Tunisian cattle. This novel sub-cluster was relatively distant to a sub-cluster that includes all sequence types isolated from Italian ruminants and cats, ticks from Costa Rica and Italy, and all variants isolated from sheep and goats from Tunisia (Fig. 5).

4. Discussion

A fast, rapid and specific detection of pathogenic bacteria like *A. platys*, the causative agent of infectious canine cyclic thrombocytopenia, is essential for estimating the endemicity and the spread of this tick-borne disease during control programs. Until 2017, the specific detection of pathogenic *A. platys* or strains genetically related to *A. platys* in animals and their infesting ticks required post-amplification sequencing (Ait Lbacha et al., 2017; Belkahia et al., 2015; Campos-Calderón et al., 2016; Liu et al., 2012; Zobba et al., 2014, 2015). Consequently, Ben Said et al. (2017) developed a diagnostic approach based on the *groEL* PCR combined to restriction enzyme digestion with the potential of discriminating among *A. platys* and genetically related strains. By using this method, the same authors detected specifically *A. platys*-like strains in Tunisian sheep, goats and cattle, and discriminated them from *A. platys* strains without a sequencing step. In addition, an *in silico* analysis, carried out by Ben Said et al. (2017) and in our study, proved that this approach also allows specific detection of *A. platys*-like strains in Italian sheep, goats, cattle, red deer and cats (Zobba et al., 2014, 2015) and even in *Rhipicephalus* ticks i.e *R. sanguineus* s.l. and *R. bursa* removed from dogs in Costa Rica and ruminants in Italy, respectively (Campos-Calderón et al., 2016; Chisu et al., 2018).

Therefore, and since we have evidence of the infection of Tunisian camels with *A. platys*-like strains (Belkahia et al., 2015), we initially proposed to use this method to investigate the occurrence and the prevalence of these strains genetically related to *A. platys* in dromedaries and their ticks in several Tunisian regions without going through a sequencing step. However, this approach based on the restriction action of the *Stu*I enzyme could not discriminate the camel *A. platys*-like strains from the *A. platys* strains. This finding was confirmed by sequencing of the *groEL* amplicons isolated from infected dromedaries.

As a result, we have selected another restriction enzyme (*Hind*III) that has the power to discriminate between camel *A. platys*-like strains and *A. platys* strains preferentially found in dogs and their infesting ticks. This method has the advantage of potentially allowing the search of co-infecting pathogenic *A. platys* and *A. platys*-like strains in the same dromedary or tick sample. This is especially useful when dealing with

Table 3
Designation and information about sequencing and RFLP data of the seven *groEL* genetic variants of *Anaplasma* sp. closely related to *A. platys* identified in camels and infesting ticks in this study.

Anaplasma spp.	Host or possible vector	Genotype	Isolate	Geographical location	GenBank accession no.	BLAST analysis	RS of StuI[1]	RFLP by StuI[2]	RS of HindIII[1]	RFLP by HindIII[2]
A. platys	*Canis canis*	AplDog Lara	Lara	Sardinia, Italy	AY848753	100% A. platys	+	+	+	+
	Canis canis	Santiago 17	Santiago 17	Santiago, Chile	EF201806	100% A. platys	+	NP	NA	NA
	Canis lupus familiaris	Canine1	Nice	Sardinia, Italy	KP090270	99% A. platys	+	NP	NA	NA
	Camelus bactrianus[3]	WScam11	WScam11	Xinjiang, China	KR011925	100% A. platys	+	NP	NA	NA
A. platys-like	*Ovis aries*	Ovine7	208	Sardinia, Italy	KC335250	92% A. platys	–	NP	NA	NA
		ApllikeOv1	N1Ov30	Nabeul, Tunisia	KX650613	92% A. platys	–	–	NA	NA
	Bos taurus	Bovine6	306	Sardinia, Italy	KC335256	93% A. platys	–	NP	NA	NA
		ApllikeBv1	A1Bv6	Ariana, Tunisia	KX650588	85% A. platys	–	–	NA	NA
	Capra hircus	Caprine4	324	Sardinia, Italy	KC335260	92% A. platys	–	NP	NA	NA
		ApllikeCp1	Bz1Cp6	Bizerte, Tunisia	KX650594	92% A. platys	–	–	NA	NA
	Cervus elaphus	Cervus1	1	Sardinia, Italy	KC335246	93% A. platys	–	NP	NA	NA
	Felis catus	Feline1	Cuoricino	Sardinia, Italy	KP090273	92% A. platys	–	NP	NA	NA
	Rhipicephalus sanguineus[4]	CR1[5]	CR1[5]	Costa Rica	KU534871	93% A. platys	–	NP	NA	NA
	Rhipicephalus bursa[6]	Rbur2	72	Sardinia, Italy	KY709324	93% A. platys	–	NP	NA	NA
	Camelus dromedarius	ApllikegroelCd1	GbCd001	Gabes, Tunisia	MH510090	87% A. platys	+	+	–	–
			KrCd108	Kairouan, Tunisia	MH510091	87% A. platys	+	+	–	–
		ApllikegroelCd2	KrCd105	Kairouan, Tunisia	MH510092	87% A. platys	+	+	–	–
		ApllikegroelCd3	KrCd107	Kairouan, Tunisia	MH510093	87% A. platys	+	+	–	–
		ApllikegroelCd4	KrCd112	Kairouan, Tunisia	MH510094	87% A. platys	+	+	–	–
			KrCd116	Kairouan, Tunisia	MH510095	87% A. platys	+	+	–	–
		ApllikegroelCd5	DzCd354	Douz, Tunisia	MH510096	87% A. platys	+	+	–	–
		ApllikegroelCd6	DzCd070	Douz, Tunisia	MH510097	87% A. platys	+	+	–	–
	Hyalomma dromedarii[7]	NS	DzHd030M1	Douz, Tunisia	NS	NS	+	+	–	–

Abbreviations: NP not performed; NA not analyzed; NS not sequenced.

[1] Presence (+) or absence (-) of the restriction site of *Stu*I or *Hind*III enzyme during *in silico* analysis.

[2] Restriction (+) or not (-) of the PCR product with *Stu*I or *Hind*III enzyme during RFLP assay.

[3] Camels belonging to *Camelus bactrianus* species infested by *Rhipicephalus sanguineus* sensu lato ticks.

[4] *Rhipicephalus sanguineus* sensu lato tick.

[5] The complete genotype's and isolate's name is groELAplatyslikeCR1.

[6] *Rhipicephalus bursa* ticks collected from a goat and a mouflon in Italy.

[7] *Hyalomma dromedarii* tick collected from one *Camelus dromedarius* camel in this study.

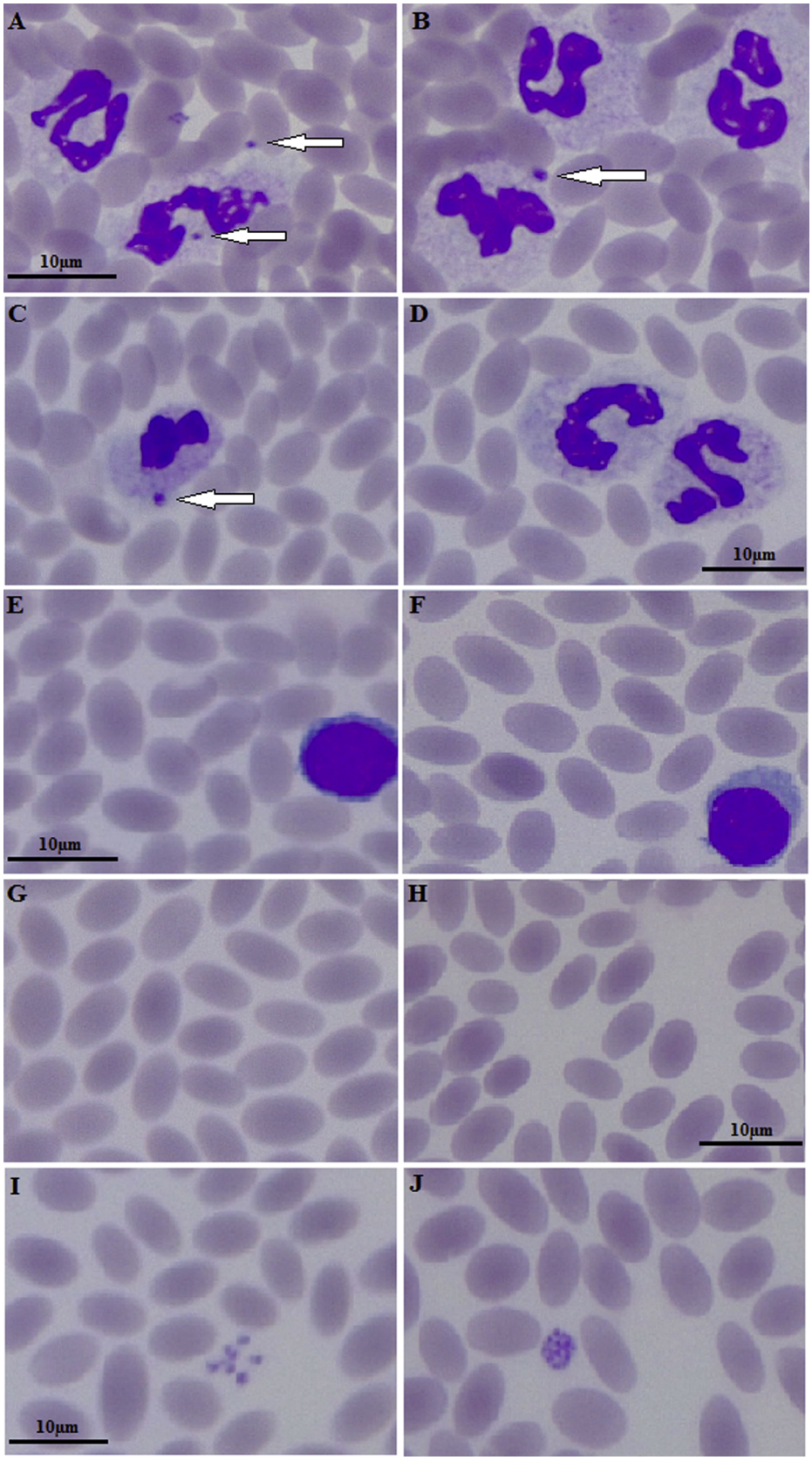

Fig. 3. Microscopic examination of blood smears from three positive *Anaplasma platys*-like camel samples stained with Giemsa. A to C: Inclusions of *A. platys*-like strains in camel neutrophil granulocytes are visible; D: Neutrophil granulocytes of uninfected camels; E, G and I: Monocytes, erythrocytes and platelets of camels infected with *A. platys*-like strains, respectively and F, H and J: Monocytes, erythrocytes and platelets of camels uninfected with *A. platys*-like strains, respectively.

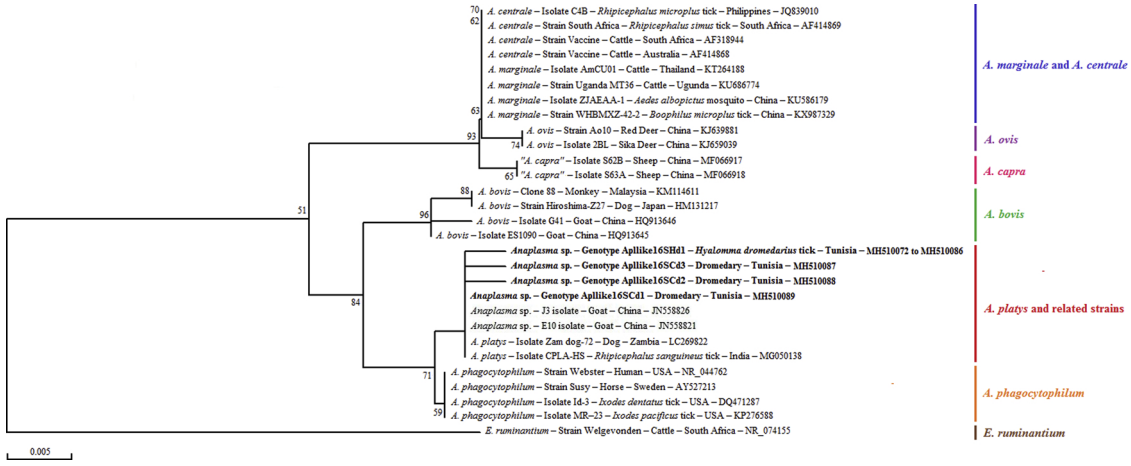

Fig. 4. Representative Neighbor-Joining tree based on multiple sequence alignments of the partial 16S rRNA nucleotide sequences (305 bp) of revealed strains genetically related to *A. plays* with *Anaplasma* strains isolated from animals and ticks published in GenBank.
Legend: Numbers in nodes represent the percentage of 1000 bootstrap reiterations supporting the nodes (only percentages greater than 50% are shown). The host or vector, the genotype, strain or isolate identification, country of origin, and GenBank accession number are indicated in the tree for each sequence. Sequences newly obtained in the current study are highlighted in bold and marked with asterisks. One *Ehrlichia ruminantium*16S rRNA partial sequence was added as an out-group.

ticks, which can be vectors of these two bacteria, if they infest camels and dogs sharing in the same area. In addition, the proof of (i) the presence of *A. platys*-like strains in ticks infesting dogs in Costa Rica (Campos-Calderón et al., 2016) and (ii) the detection of the canine *A. platys* strains in Bactrian camels (*Camelus bactrianus*) and their ticks in China (Li et al., 2015) makes this adapted RFLP method necessary and interesting during field epidemiological surveys, especially in low income countries where, in most cases, camels cohabit with watch dogs.

This report confirmed the infection of Tunisian camels by *A. platys*-like strains with an overall prevalence estimated at 5.6%. This rate was similar to that reported in *C. dromedarius* camels from Iran (6%) (Sharifiyazdi et al., 2016), and lower than those estimated in the same species from Morocco (39.62%) (Ait Lbacha et al., 2017), Saudi Arabia (26%) (Bastos et al., 2015) and from other Tunisian regions (17%) (Belkahia et al., 2015). This discrepancy may probably results from differences in adopted methods of sample analysis, tick control programs, farm management, husbandry practices, wildlife reservoir hosts, and/or abiotic factors (Ben Said et al., 2017, 2018).

In this report, *A. platys*-like prevalence is significantly higher in dromedaries from arid and semi-arid areas (13%) compared to Saharan area (3%) (Table 1, $P < 0.001$). This result could be explained by the fact that, dromedaries in arid and semi-arid areas are kept with other ruminants in common shelters. This practice can increase the transmission of this bacterium by ticks to these camels compared to those from Saharan area raised usually separately (Gharbi et al., 2013). Furthermore, seasonal analysis showed that *A. platys*-like strains infect camels throughout the year, and the number of infected animals increased during the autumn season. Since *Anaplasma* spp. were mainly transmitted by ticks, these results may be partially explained by those reported by Gharbi et al. (2013) who found that *H. impeltatum* and *H. dromedarii* ticks remained active during all seasons among Tunisian camel herds, and by the data of Hniche (2006) showing that the maximum infestation by *H. impeltatum* of camels located in south-western Tunisia was observed in the autumn season.

Additionally, the current study confirmed the presence of *A. platys*-like strains, for the first time, in *H. dromedarii* collected from an uninfected camel and provides evidence that this tick species may be one of the main vectors of *A. platys*-like strains in Tunisia. However, its vector competence needs to be proved by further experimental assays. Notably, *A. platys* and/or related strains (reported as *A. platys*) has been isolated from *R. bursa* feeding on goats from Turkey (Aktas et al., 2009) and *Rhipicephalus* spp. infesting cattle in Malaysia (Tay et al., 2014). More recently, *A. platys*-like strains were also reported in one *R. sanguineus* s.l. infesting a dog from Costa Rica (Campos-Calderón et al., 2016) and in two *R. bursa* specimens collected from goats and mouflon in Italy (Chisu et al., 2018).

The analysis of the 16S rRNA gene is considered a sensitive molecular tool for the *Anaplasma* spp. typing in phylogenetic studies (Belkahia et al., 2017a,b; Ben Said et al., 2015a,b, 2017; Lew et al., 2003). Sequencing of 305 bp of the 16S rRNA gene isolated from randomly selected the *Anaplasmataceae*-positivecamels revealed three distinct genotypes isolated from dromedaries and one genotype isolated from *H. dromedarii* tick. Nucleotide alignment and sequence identity comparison (Table 2) of the 16S rRNA partial sequences obtained in this study confirmed the evidence of strains genetically related to *A. platys*. Indeed, these genotypes shared identity greater than 99% with the 16S rRNA sequences of the canine *A. platys* and related strains found in Chinese goats (Liu et al., 2012, Table 2). Phylogenetic analysis of 16S rRNA partial sequences performed with *Anaplasma* sequences in this study and selected sequences of *Anaplasma* species retrieved in GenBank confirmed the conclusions obtained from the BLAST analysis (Table 2 and Fig. 4). Notably, phylogenetic tree based on 305 bp of the 16S rRNA genes shows that *Anaplasma* genotypes identified from Tunisian dromedaries and the only one infesting tick, cluster with *A. platys* and related strains (Fig. 4).

In order to further characterize the diversity of camel *A. platys*-like strains, sequencing of 476 bp of the *groEL* gene amplified from randomly selected positive camels was performed. Used frequently in the phylogeny of the *Anaplasmataceae*, this gene provides a better intraspecific differentiation (Ben Said et al., 2017; Zobba et al., 2014, 2015). Nucleotide alignments and comparison of sequence identity of *groEL* genotypes obtained in this study confirm that these analyzed samples (eight positive camels) are infected with different *A. platys*-like strains (Tables 3 and 4). Phylogenetic analysis showed that all revealed sequences formed separately the fourth sub-cluster belonging to *A. platys* and related strains group (Fig. 5). This sub-cluster was close to subcluster 3 formed by ApllikeBv1 and ApllikeBv2 variants isolated from Tunisian cattle (Ben Said et al., 2017) and relatively distant from subclusters 1 and 2 formed, respectively, by (i) the *A. platys* strains infecting dogs from Italy and Chile (Abarca et al., 2007; Alberti et al., 2005), and (ii) *A. platys*-like strains infecting Italian ruminants and cats, Tunisian small ruminants and ticks from Italy and Costa Rica (Ben Said et al., 2017; Campos-Calderón et al., 2016; Chisu et al., 2018; Zobba et al., 2014, 2015) (Fig. 5).

In this study, microscopic examination of blood smears revealed that camels positive to *A. platys*-like strains harbored morulae

Table 4
Identity (on the left) and genetic distance (on the right) between *groEL* sequences (476 bp) from *Anaplasma platys*-like strains isolated from Tunisian camels, and *A. platys* and related strains infecting domestic and wild animals found in GenBank.

	Anaplasma sp. (Genotype[1])	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	*A.* sp. (AplikegroelCd1)	100/0	0.004	0.006	0.002	0.004	0.004	0.126	0.116	0.120	0.122	0.095	0.126	0.128	0.122	0.122	0.118	0.128	0.126	0.134	0.126
2	*A.* sp. (AplikegroelCd2)	99.6	100/0	0.011	0.006	0.008	0.008	0.130	0.120	0.124	0.126	0.095	0.130	0.132	0.126	0.126	0.122	0.130	0.130	0.139	0.130
3	*A.* sp. (AplikegroelCd3)	99.4	98.9	100/0	0.004	0.006	0.002	0.124	0.113	0.118	0.120	0.092	0.124	0.126	0.120	0.120	0.116	0.124	0.124	0.132	0.124
4	*A.* sp. (AplikegroelCd4)	99.8	99.4	99.6	100/0	0.002	0.002	0.124	0.113	0.118	0.120	0.092	0.124	0.126	0.120	0.120	0.116	0.124	0.124	0.132	0.124
5	*A.* sp. (AplikegroelCd5)	99.6	99.2	99.4	99.8	100/0	0.004	0.126	0.116	0.120	0.122	0.095	0.126	0.128	0.122	0.122	0.128	0.126	0.126	0.134	0.126
6	*A.* sp. (AplikegroelCd6)	99.6	99.2	99.8	99.8	99.6	100/0	0.122	0.112	0.116	0.118	0.090	0.122	0.124	0.118	0.118	0.124	0.122	0.122	0.130	0.122
7	*A.* sp. (Ovine7)	87.4	87.0	87.6	87.6	87.4	87.8	100/0	0.017	0.013	0.011	0.139	0.006	0.023	0.017	0.011	0.013	0.080	0.080	0.088	0.080
8	*A.* sp. (ApllikeOv1)	88.4	88.0	88.7	88.7	88.4	88.8	98.3	100/0	0.004	0.011	0.137	0.015	0.019	0.013	0.011	0.008	0.076	0.076	0.084	0.076
9	*A.* sp. (Bovine6)	88.0	87.6	88.2	88.2	88.0	88.4	98.7	99.6	100/0	0.006	0.132	0.011	0.015	0.008	0.006	0.004	0.071	0.071	0.080	0.071
10	*A.* sp. (ApllikeBv1)	87.8	87.4	88.0	88.0	87.8	88.2	98.9	98.9	99.4	100/0	0.134	0.008	0.017	0.011	0.000	0.006	0.074	0.074	0.082	0.074
11	*A.* sp. (Caprine4)	90.5	90.5	90.8	90.8	90.5	91.0	86.1	86.3	86.8	86.6	100/0	0.139	0.141	0.134	0.134	0.130	0.143	0.143	0.151	0.143
12	*A.* sp. (ApllikeCp1)	87.4	87.0	87.6	87.6	87.4	87.8	99.4	98.5	98.9	99.2	86.1	100/0	0.021	0.015	0.008	0.011	0.078	0.078	0.086	0.078
13	*A.* sp. (Cervus1)	87.2	86.8	87.4	87.4	87.2	87.6	97.7	98.1	98.5	98.3	85.9	97.9	100/0	0.015	0.017	0.011	0.078	0.078	0.086	0.078
14	*A.* sp. (Feline1)	87.8	87.4	88.0	88.0	87.8	88.2	98.3	98.7	99.2	98.9	86.6	98.5	98.5	100/0	0.011	0.004	0.071	0.071	0.080	0.071
15	*A.* sp. (CR1[2])	87.8	87.4	88.0	88.0	87.8	88.2	98.9	98.9	99.4	100	86.6	99.2	98.3	98.9	100/0	0.006	0.074	0.074	0.082	0.074
16	*A.* sp. (Rbur2)	88.2	87.8	88.4	88.4	88.2	88.7	98.7	99.2	99.6	99.4	87.0	98.9	98.9	99.6	99.4	100/0	0.067	0.067	0.076	0.067
17	*A. platys* (Dog Lara)	87.4	87.0	87.6	87.6	87.4	87.8	92.0	92.4	92.9	92.6	85.7	92.2	92.2	92.9	92.6	93.3	100/0	0.000	0.008	0.000
18	*A. platys* (Santiago 17)	87.4	87.0	87.6	87.6	87.4	87.8	92.0	92.4	92.9	92.6	85.7	92.2	92.2	92.9	92.6	93.3	100	100/0	0.008	0.000
19	*A. platys* (Canine1)	86.6	86.1	86.8	86.8	86.6	87.0	91.2	91.6	92.0	91.8	84.9	91.4	91.4	92.0	91.8	92.4	99.2	99.2	100/0	0.008
20	*A. platys* (WScam11)	87.4	87.0	87.6	87.6	87.4	87.8	92.0	92.4	92.9	92.6	85.7	92.2	92.2	99.9	92.6	93.3	100	100	99.2	100/0

Abbreviations: *A.* sp (AplikegroelCd1-6): Six different *Anaplasma* sp. (*A. platys*-like) genotypes represented eight *A. platys*-like isolates infected Tunisian camels in this study (GenBank accession numbers MH510090 to MH510097, respectively); *A.* sp (Ovine7, Bovine6, Caprine4, Cervus1 and Feline1): *Anaplasma* sp. (*A. platys*-like strains) isolated from Italian sheep, cattle, deer and cat, respectively (GenBank accession numbers KC335250, KC335256, KC335246 and KP090273, respectively); *A.* sp (ApllikeOv1, ApllikeBv1 and ApllikeCp1): *Anaplasma* sp. (*A. platys*-like strains) isolated from Tunisian sheep, cattle and goat, respectively (GenBank accession numbers KX650613, KX650588 and KX650594, respectively); *A.* sp (CR1 and Rbur2): *Anaplasma* sp. (*A. platys*-like strains) isolated from *Rhipicephalus sanguineus* sensu lato and *R. bursa* ticks, respectively (GenBank accession numbers KU534871 and KY709324, respectively); *A. platys* (Dog Lara, Santiago 17, Canine1 and WScam11): specific *Anaplasma platys* isolated from dog located in Italy, Chile and Italy, and from Chinese camels, respectively (GenBank accession numbers AY848753, EF201806, KP090270 and KR011925, respectively).

[1] Genotype, genetic variant, sequence type or strain.

[2] The complete genotype's name is groELAplatyslikeCR1.

Table 5
Identification of nucleotide variable positions in the alignment of the *groEL* camel *A. platys*-like sequences with canine *A. platys* reference sequence.

	***groEL* variable positions**																														
Genotypes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**
	5	5	5	5	5	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	7	7	7	7	7	7	7	7	7	**7**
	7	8	9	9	9	0	0	0	2	2	3	4	5	5	5	6	7	7	8	8	8	9	0	1	2	2	2	3	3	4	4	**5**
	8	4	0	6	9	2	8	9	0	6	2	4	0	3	6	5	1	7	0	3	6	8	7	7	2	5	8	1	4	0	6	**3**
A. platys (AY848753)	C	T	C	C	T	A	G	A	G	G	G	A	G	G	A	A	G	T	G	G	C	T	C	C	C	C	C	T	G	A	A	**T**
ApllikeCd1	T	-	-	T	C	C	A	-	A	A	A	C	A	A	G	-	A	A	A	A	T	A	A	T	G	A	A	C	A	T	C	**C**
ApllikeCd2	T	C	T	T	C	C	A	-	A	A	A	C	A	A	G	-	A	A	A	A	T	A	A	T	G	A	A	C	A	T	C	**C**
ApllikeCd3	T	-	-	T	C	C	A	-	A	A	A	C	A	A	G	G	A	A	A	A	T	A	A	T	G	A	A	C	A	T	C	**C**
ApllikeCd4	T	-	-	T	C	C	A	-	A	A	A	C	A	A	G	-	A	A	A	A	T	A	A	T	G	A	A	C	A	T	C	**C**
ApllikeCd5	T	-	-	T	C	C	A	C	A	A	A	C	A	A	G	-	A	A	A	A	T	A	A	T	G	A	A	C	A	T	C	**C**
ApllikeCd6	T	-	-	T	C	C	A	-	A	A	A	C	A	A	G	-	A	A	A	A	T	A	A	T	G	A	A	C	A	T	C	**C**

	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
	7	7	7	7	7	7	7	8	8	8	8	8	8	8	8	8	8	8	9	9	9	9	9	9	9	9	9	0	0	0
	5	6	6	7	8	8	9	0	1	2	2	3	6	6	7	8	8	9	9	0	2	2	2	3	4	5	5	5	6	3	3	4
	5	4	8	9	5	8	1	0	8	1	4	3	0	9	2	1	7	0	6	2	0	3	6	2	8	0	6	9	5	1	7	0
A. platys (AY848753)	A	G	C	G	T	T	C	A	C	T	T	G	A	T	T	C	T	C	A	T	G	A	T	A	C	T	C	A	T	C	A	G
ApllikeCd1	G	A	T	A	C	G	G	C	A	C	C	A	T	C	C	G	C	T	C	C	T	G	C	G	T	C	T	T	C	A	G	C
ApllikeCd2	G	A	T	A	C	G	G	C	A	C	C	A	T	C	C	G	C	T	C	C	T	G	C	G	T	C	T	T	C	A	G	C
ApllikeCd3	G	A	T	A	C	G	G	C	A	C	C	A	T	C	C	G	C	T	C	C	T	G	C	G	T	C	-	T	C	A	G	-
ApllikeCd4	G	A	T	A	C	G	G	C	A	C	C	A	T	C	C	G	C	T	C	C	T	G	C	G	T	C	T	T	C	A	G	-
ApllikeCd5	G	A	T	A	C	G	G	C	A	C	C	A	T	C	C	G	C	T	C	C	T	G	C	G	T	C	T	T	C	A	G	-
ApllikeCd6	G	A	T	A	C	G	G	C	A	C	C	A	T	C	C	G	C	T	C	C	T	G	C	G	T	C	-	T	C	A	G	-

Variable positions conserved among camel strains are colored in blue; Numbers represent the nucleotide position with respect to the OVI isolate from South Africa for *A. ovis* (GenBank accession number AF441131) (Lew et al., 2003); Nucleotide position 753 shows the substitution of T by C permitting the discrimination between specific *A. platys* and camel *A. platys*-like strains by *Hind*III enzyme.

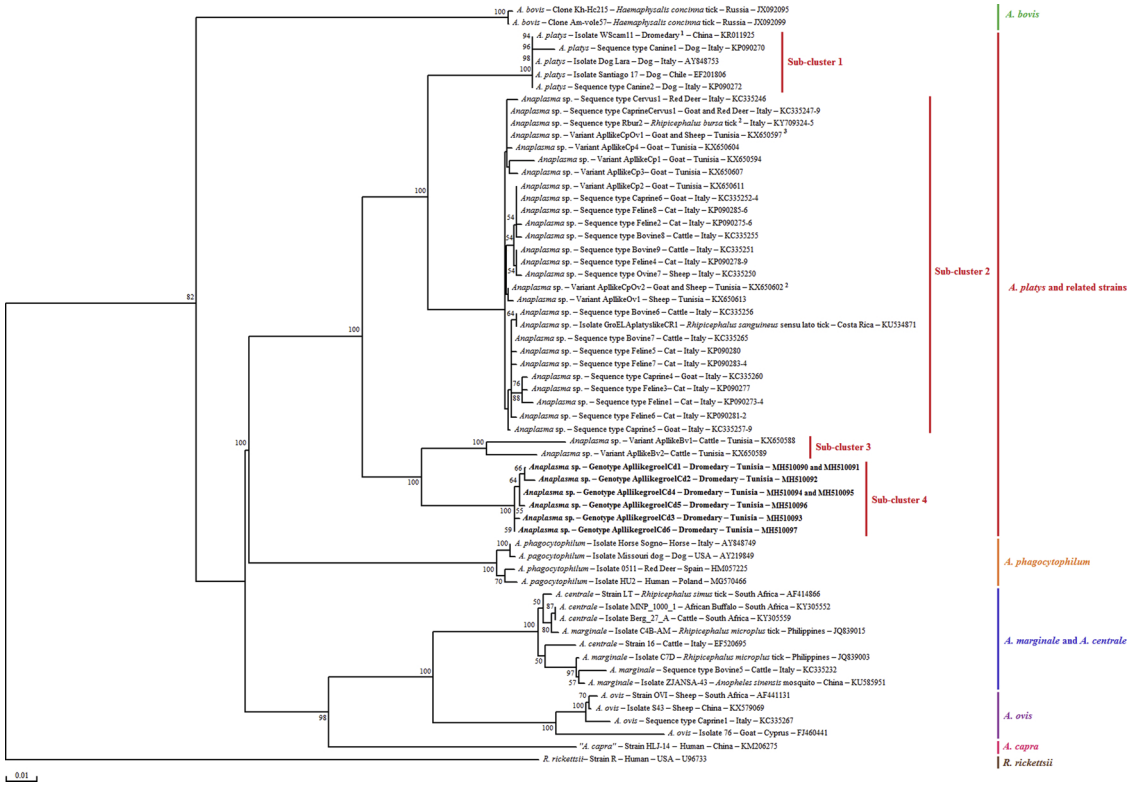

Fig. 5. Phylogenetic tree of *Anaplasma* spp. inferred with *groEL* partial sequences (476 bp) using the neighbor-joining method showing the position of revealed sequences isolated from Tunisian camels and their infesting ticks.
Legend: Genotypes from this study represented in bold and marked with asterisks. Numbers associated with nodes represent the percentage of 1000 bootstrap reiterations supporting the nodes (only percentages greater than 50% were represented). The host or vector, the genotype, strain or isolate name, the country of origin and the GenBank accession number are indicated. One *Rickettsia rickettsii groEL* partial sequence was added as an out-group. [1]: Camels belonging to *Camelus bactrianus* species infested by *R. sanguineus* s. l. ticks; [2]: *R. bursa* ticks collected from a goat and a mouflon in Italy; [3]: ApllikeCpOv1 sequence was deposited in the GenBank (KX650590 to KX650593, KX650595 to KX650601, KX650603, KX650605, KX650606 and KX650608 to KX650610). [4] ApllikeCpOv2 variant was also deposited under accession number KX650612; [5]: *Rhipicephalus sanguineus* sensu lato ticks collected from dog in Costa Rica.

exclusively in neutrophil granulocytes (Fig. 3). This is in line with previous findings of Zobba et al. (2014) indicating the presence of *A. platys*-like strains in neutrophil granulocytes of other ruminants (cattle, sheep and goats). However, in cats, this bacterium was identified in platelets (Zobba et al., 2015). Probably due to the cellular specificities and associated mechanisms of infection, *A. platys*-like strains were found to be adapted to neutrophils in ruminants including camels, while the pathogenic *A. platys* remains bound to platelets in canids.

Infection with *A. platys* causes canine infectious cyclic thrombocytopenia in dogs associated to thrombocytopenia, anemia and/or leucopenia (Zobba et al., 2014, 2015). In this study, *A. platys*-like positive camels did not show any symptom related to *A. platys* infection. This finding was previously observed in ruminants from China, Italy, Tunisia, Algeria and Morocco (Ait Lbacha et al., 2017; Belkahia et al., 2015; Ben Said et al., 2017; Dahmani et al., 2015; Liu et al., 2012; Zobba et al., 2014) and cats from Italy (Zobba et al., 2015).

5. Conclusion

Our report indicates a natural infection with *A. platys*-like strains of camels (*C. dromedarius*) and their associated ticks (*H. dromedarii*) from Tunisia. The RFLP assay, adapted in this study, allows fast and specific detection of *A. platys* and *A. platys*-like strains in camels and their infesting ticks and has the intrinsic potential of revealing co-infections with these two types of bacteria in the same sample. Further studies are required to investigate the clinical significance of these strains genetically related to *A. platys* and to explore their zoonotic potential and their epidemiology in other hosts and reservoirs.

Ethics approval

Animal restraint and blood sampling were performed in accordance with the European legislation regarding ethics and animal welfare. Blood samples were, professionally, collected by veterinarians from restrained camels during their monitoring or treatment to avoid animal suffering. Informed verbal consents were obtained from the camel owners participating in this study.

Authors' contributions

RS, MBS and LM conceived the idea. RS, MBS, MD and HBY performed the experiments. RS and MBS adapted PCR-RFLP method and performed risk factor analysis and phylogenetic study. RS and MBS wrote the manuscript and RS, MBS and LM finalized it. All authors read and approved the final manuscript.

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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.2019.07.004.

References

Abarca, K., Lopez, J., Perret, C., Guerrero, J., Godoy, P., Veloz, A., Valiente-Echeverria, F., Leon, U., Gutjahr, C., Azocar, T., 2007. *Anaplasma platys* in dogs, Chile. Emerg. Infect. Dis. 13, 1392–1395.

Ait Lbacha, H., Zouagui, Z., Alali, S., Rhalem, A., Petit, E., Ducrotoy, M.J., Boulouis, H.J., Maillard, R., 2017. "*Candidatus* Anaplasma camelii" in one-humped camels (*Camelus dromedarius*) in Morocco: a novel and emerging *Anaplasma* species? Infect. Dis. Poverty 6, 1.

Aktas, M., Altay, K., Dumanli, N., Kalkan, A., 2009. Molecular detection and identification of *Ehrlichia* and *Anaplasma* species in ixodid ticks. Parasitol. Res. 104, 1243–1248.

Alberti, A., Sparagano, O.A., 2006. Molecular diagnosis of granulocytic anaplasmosis and infectious cyclic thrombocytopenia by PCR-RFLP. Ann. N. Y. Acad. Sci. 1081, 371–378.

Alberti, A., Zobba, R., Chessa, B., Addis, M.F., Sparagano, O.A., Pinna Parpaglia, M.L., Cubeddu, T., Pintori, G., Pittau, M., 2005. Equine and canine *Anaplasma phagocytophilum* strains isolated on the island of Sardinia (Italy) are phylogenetically related to pathogenic strains from the United States. Appl. Environ. Microbiol. 71, 6418–6422.

Allsopp, M., Visser, E.S., du Plessis, J.L., Vogel, S.W., Allsopp, B.A., 1997. Different organisms associated with heart water as shown by analysis of 16S ribosomal RNA gene sequences. Vet. Parasitol. 71, 283–300.

Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W., Lipman, D.J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25, 3389–3402.

Arraga-Alvarado, C.M., Qurollo, B.A., Parra, O.C., Berrueta, M.A., Hegarty, B.C., Breitschwerdt, E.B., 2014. Case report: molecular evidence of *Anaplasma platys* infection in two women from Venezuela. Am. J. Trop. Med. Hyg. 91, 1161–1165.

Bastos, A.D.S., Mohammed, O.B., Bennett, N.C., Petevinosa, C., Alagaili, A.N., 2015. Molecular detection of novel *Anaplasmataceae* closely related to *Anaplasma platys* and *Ehrlichia canis* in the dromedary camel (*Camelus dromedarius*). Vet. Microbiol. 179, 310–314.

Belkahia, H., Ben Said, M., Sayahi, L., Alberti, A., Messadi, L., 2015. Detection of novel strains genetically related to *Anaplasma platys* in Tunisian one-humped camels (*Camelus dromedarius*). J. Infect. Dev. Ctries 9, 1117–1125.

Belkahia, H., Ben Said, M., El Mabrouk, N., Saidani, M., Cherni, C., Ben Hassen, M., Bouattour, A., Messadi, L., 2017a. Spatio-temporal variations and genetic diversity of *Anaplasma* spp. In cattle from the North of Tunisia. Vet. Microbiol. 208, 223–230.

Belkahia, H., Ben Said, M., El Mabrouk, N., Saidani, M., Cherni, C., Ben Hassen, M., Bouattour, A., Messadi, L., 2017b. Seasonal dynamics, spatial distribution and genetic analysis of *Anaplasma* species infecting small ruminants from Northern Tunisia. Infect. Genet. Evol. 54, 66–73.

Ben Dhia, M., Smiti, N., Gadouar, T., 1999. Situation actuelle et perspective de développement de l'élevage du dromadaire en Tunisie au cours du VIII^{ème} Plan. Options Medit. 13, 9–17.

Ben Said, M., Belkahia, H., Alberti, A., Zobba, R., Bousrih, M., Yahiaoui, M., Daaloul-Jedidi, M., Mamlouk, A., Gharbi, M., Messadi, L., 2015a. Molecular survey of *Anaplasma* species in small ruminants reveals the presence of novel strains closely related to *A. phagocytophilum* in Tunisia. Vector Borne Zoonotic Dis. 15, 580–590.

Ben Said, M., Belkahia, H., Karaoud, M., Bousrih, M., Yahiaoui, M., Daaloul-Jedidi, M., Messadi, L., 2015b. First molecular survey of *Anaplasma bovis* in small ruminants from Tunisia. Vet. Microbiol. 179, 322–326.

Ben Said, M., Belkahia, H., El Mabrouk, N., Saidani, M., Alberti, A., Zobba, R., Cherif, A., Mahjoub, T., Bouattour, A., Messadi, L., 2017. *Anaplasma platys*-like strains in ruminants from Tunisia. Infect. Genet. Evol. 49, 226–233.

Ben Said, M., Belkahia, H., Messadi, L., 2018. *Anaplasma* spp. in North Africa: a review on molecular epidemiology, associated risk factors and genetic characteristics. Ticks Tick Borne Dis. 9, 543–555.

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.

Breitschwerdt, E.B., Hegarty, B.C., Qurollo, B.A., Saito, T.B., Maggi, R.G., Blanton, L.S., Bouyer, D.H., 2014. Intravascular persistence of *Anaplasma platys*, *Ehrlichia chaffeensis*, and *Ehrlichia ewingii* DNA in the blood of a dog and two family members. Parasit. Vectors 7, 298.

Campos-Calderón, L., Ábrego-Sánchez, L., Solórzano-Morales, A., Alberti, A., Tore, G., Zobba, R., Jiménez-Rocha, A.E., Dolz, G., 2016. Molecular detection and identification of Rickettsiales pathogens in dog ticks from Costa rica. Ticks Tick Borne Dis. 7, 1198–1202.

Chisu, V., Zobba, R., Lecis, R., Sotgiu, F., Masala, G., Foxi, C., Pisu, D., Alberti, A., 2018. GroEL typing and phylogeny of *Anaplasma* species in ticks from domestic and wild vertebrates. Ticks Tick Borne Dis. 9, 31–36.

Chochlakis, D., Ioannou, I., Sharif, L., Kokkini, S., Hristophi, N., Dimitriou, T., Tselentis, Y., Psaroulaki, A., 2009. Prevalence of *Anaplasma* sp. in goats and sheep in Cyprus. Vector Borne Zoonotic Dis. 9, 457–463.

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.

Djiba, M.L., Mediannikov, O., Mbengue, M., Thiongane, Y., Molez, J.F., Seck, M.T., Fenollar, F., Raoult, D., Ndiaye, M., 2013. Survey of *Anaplasmataceae* bacteria in sheep from Senegal. Trop. Anim. Health Prod. 45, 1557–1561.

Dumler, J.S., Barbet, A.F., Bekker, C.P., Dasch, G.A., Palmer, G.H., Ray, S.C., Rikihisa, Y., Rurangirwa, F.R., 2001. Reorganization of genera in the families Rickettsiaceae and *Anaplasmataceae* in the order Rickettsiales: unification of some species of *Ehrlichia* with *Anaplasma*, *Cowdria* with *Ehrlichia* and *Ehrlichia* with *Neorickettsia*, descriptions of six new species combinations and designation of *Ehrlichia equi* and 'HGE agent' as subjective synonyms of *Ehrlichia phagocytophilum*. Int. J. Syst. Evol. Microbiol. 51, 2145–2165.

Ehounoud, C.B., Fenollar, F., Dahmani, M., N'Guessan, J.D., Raoult, D., Mediannikov, O., 2017. Bacterial arthropod-borne diseases in West Africa. Acta Trop. 171, 124–137.

Elghali, A., Hassan, S.M., 2009. Ticks (Acari: ixodidae) infesting camels (*Camelus*

dromedarius) in Northern Sudan. Onderstepoort J. Vet. Res. 76, 177–185.
Fard, S.R., Fathi, S., Asl, E.N., Nazhad, H.A., Kazeroni, S.S., 2012. Hard ticks on one-humped camel (*Camelus dromedarius*) and their seasonal population dynamics in southeast, Iran. Trop. Anim. Health Prod. 44, 197–200.
Gharbi, M., Moussi, N., Jedidi, M., Mhadhbi, M., Sassi, L., Darghouth, M.A., 2013. Population dynamics of ticks infesting the one-humped camel (*Camelus dromedarius*) in central Tunisia. Ticks Tick Borne Dis 4, 488–491.
Hniche, M., 2006. Identification des tiques et dépistage de l'infection piroplasmique des bovins au sein d'un élevage mixte de bovins et de dromadaire dans le sud ouest de la Tunisie. Thèse Doc. Méd. Vét. E.N.M.V., Sidi Thabet.
INM, 2018. Institut National de Météorologie. Données climatiques mensuelles, Tunisia Accessed 30 August 2018. http://www.meteo.tn/donnees/.html.
Lew, A.E., Gale, K.R., Minchin, C.M., Shkap, V., de Waal, D.T., 2003. Phylogenetic analysis of the erythrocytic *Anaplasma* species based on 16S rDNA and GroEL (HSP60) sequences of *A. marginale*, *A. centrale*, and *A. ovis* and the specific detection of *A. centrale* vaccine strain. Vet. Microbiol. 92, 145–160.
Li, Y., Yang, J., Chen, Z., Qin, G., Li, Y., Li, Q., Liu, J., Liu, Z., Guan, G., Yin, H., Luo, J., Zhang, L., 2015. *Anaplasma* infection of Bactrian camels (*Camelus bactrianus*) and ticks in Xinjiang, China. Parasit. Vectors 8, 313.
Li, Y., Chen, Z., Liu, Z., Liu, J., Yang, J., Li, Q., Li, Y., Luo, J., Yin, H., 2016. Molecular survey of *Anaplasma* and *Ehrlichia* of red deer and sika deer in Gansu, China in 2013. Transbound. Emerg. Dis. 63, 228–236.
Lima, M.L., Soares, P.T., Ramos, C.A., Araújo, F.R., Ramos, R.A., Souza, I.I., Faustino, M.A., Alves, L.C., 2010. Molecular detection of *Anaplasma platys* in a naturally-infected cat in Brazil. Braz. J. Microbiol. 41, 381–385.
Lin, M., Kikuchi, T., Brewer, H.M., Norbeck, A.D., Rikihisa, Y., 2011. Global proteomic analysis of two tick-borne emerging zoonotic agents: *Anaplasma phagocytophilum* and *Ehrlichia chaffeensis*. Front. Microbiol. 2, 24.
Liu, Z., Ma, M., Wang, Z., Wang, J., Peng, Y., Li, Y., Guan, G., Luo, J., Yin, H., 2012. Molecular survey and genetic identification of *Anaplasma* species in goats from central and southern China. Appl. Environ. Microbiol. 78, 464–470.
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.
Lorusso, V., Wijnveld, M., Latrofa, M.S., Fajinmi, A., Majekodunmi, A.O., Dogo, A.G., Igweh, A.C., Otranto, D., Jongejan, F., Welburn, S.C., Picozzi, K., 2016a. Canine and ovine tick-borne pathogens in camels, Nigeria. Vet. Parasitol. 228, 90–92.
Lorusso, V., Wijnveld, M., Majekodunmi, A.O., Dongkum, C., Fajinmi, A., Dogo, A.G., Thrusfield, M., Mugenyi, A., Vaumourin, E., Igweh, A.C., Jongejan, F., Welburn, S.C., Picozzi, K., 2016b. Tick-borne pathogens of zoonotic and veterinary importance in Nigerian cattle. Parasit. Vectors 9, 217.
M'ghirbi, Y., Ghorbel, A., Amouri, M., Nebaoui, A., Haddad, S., Bouattour, A., 2009. Clinical, serological, and molecular evidence of ehrlichiosis and anaplasmosis in dogs in Tunisia. Parasitol. Res. 104, 767–774.
Machado, R.Z., Teixeira, M.M., Rodrigues, A.C., André, M.R., Gonçalves, L.R., Barbosa da Silva, J., Pereira, C.L., 2016. Molecular diagnosis and genetic diversity of tick-borne *Anaplasmataceae* agents infecting the African buffalo *Syncerus caffer* from Marromeu Reserve in Mozambique. Parasit. Vectors 9, 454.
Maggi, R.G., Mascarelli, P.E., Havenga, L.N., Naidoo, V., Breitschwerdt, E.B., 2013. Co-infection with *Anaplasma platys*, *Bartonella henselae* and *Candidatus* Mycoplasma haematoparvum in a veterinarian. Parasit. Vectors 6, 103.
Moslam, M., Megdiche, F., 1989. L'élevage camelin en Tunisie. Options Médit 2, 33–36.
OIE, 2016. World Organisation for Animal Health. Animal Health Situation, Country: Tunisia, Year: 2016. World Animal Health Information Database (WAHIS Interface) - Version 1. 2016. Accessed 30 December 2017. http://web.oie.int/wahis.
Parola, P., Cornet, J.-P., Sanogo, Y.O., Miller, R.S., Thien, H.V., Gonzalez, J.-P., Raoult, D., Telford, S.R., Wongsrichanalai, C., 2003. Detection of *Ehrlichia* spp., *Anaplasma* spp., *Rickettsia* spp., and other Eubacteria in ticks from the Thai-Myanmar Border and Vietnam. J. Clin. Microbiol. 41, 1600–1608.
Saitou, N., Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4, 406–425.
Salakij, C., Lertwatcharasarakul, P., Salakij, J., Nunklang, K., Rattana kunuprakarn, J., 2012. Molecular characterization of *Anaplasma platys* in a domestic cat from Thailand. Comp. Clin. Pathol. 21, 345–348.
Sarih, M., M'ghirbi, Y., Bouattour, A., Gern, L., Baranton, G., Postic, D., 2005. Detection and identification of *Ehrlichia* spp. In ticks collected in Tunisia and Morocco. J. Clin. Microbiol. 43, 1127–1132.
Sharifiyazdi, H., Jafari, S., Ghane, M., Nazifi, S., Sanati, A., 2016. Molecular investigation of *Anaplasma* and *Ehrlichia* natural infections in the dromedary camel (*Camelus dromedarius*) in Iran. Comp. Clin. Path. 26, 99–103.
Tamura, K., Nei, M., 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol. Biol. Evol. 10, 512.
Tay, S.T., Koh, F.X., Kho, K.L., Ong, B.L., 2014. Molecular survey and sequence analysis of *Anaplasma* spp. In cattle and ticks in a Malaysian farm. Trop. Biomed. 31, 769–776.
Thrusfield, M., 2005. Veterinary Epidemiology, third ed. Blackwell Publishing, Oxford, UK, pp. 305–330.
Walker, A.R., Bouattour, A., Camicas, J.-L., Estrada-Peña, A., Horak, I.G., Latif, A.A., Pegram, R.G., Prestom, P.M., 2013. Ticks of domestic animals in Africa: a guide to identification of species. Bioscience Reports. Part 4: Species of Ticks. pp. 45–221.
Zeleke, M., Bekele, T., 2004. Species of ticks on camels and their seasonal population dynamics in eastern Ethiopia. Trop. Anim. Health Prod. 36, 225–231.
Zobba, R., Anfossi, A.G., Pinna Parpaglia, M.L., Dore, G.M., Chessa, B., Spezzigu, A., Rocca, S., Visco, S., Pittau, M., Alberti, A., 2014. Molecular investigation and phylogeny of *Anaplasma* spp. in Mediterranean ruminants reveal the presence of neutrophil-tropic strains closely related to *A. platys*. Appl. Environ. Microbiol. 80, 271–280.
Zobba, R., Anfossi, A.G., Visco, S., Sotgiu, F., Dedola, C., Pinna Parpaglia, M.L., Battilani, M., Pittau, M., Alberti, A., 2015. Cell tropism and molecular epidemiology of *Anaplasma platys*-like strains in cats. Ticks Tick Borne Dis. 6, 272–280.