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Article *in* Ticks and Tick-borne Diseases · March 2016

DOI: 10.1016/j.ttbdis.2016.03.003

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Ticks and Tick-borne Diseases xxx (2016) xxx–xxx

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Molecular detection of *Theileria*, *Babesia*, and *Hepatozoon* spp. in ixodid ticks from Palestine

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ARTICLE INFO

Article history:
Received 16 December 2015
Received in revised form 1 March 2016
Accepted 2 March 2016
Available online xxx

Keywords:
Hepatozoon
Babesia
Theileria
Ticks

ABSTRACT

Ixodid ticks transmit various infectious agents that cause disease in humans and livestock worldwide. A cross-sectional survey on the presence of protozoan pathogens in ticks was carried out to assess the impact of tick-borne protozoa on domestic animals in Palestine. Ticks were collected from herds with sheep, goats and dogs in different geographic districts and their species were determined using morphological keys. The presence of piroplasms and *Hepatozoon* spp. was determined by PCR amplification of a 460–540 bp fragment of the 18S rRNA gene followed by RFLP or DNA sequencing. A PCR-RFLP method based on the 18S rRNA was used in order to detect and to identify *Hepatozoon*, *Babesia* and *Theileria* spp. A total of 516 ticks were collected from animals in six Palestinian localities. Five tick species were found: *Rhipicephalus sanguineus* sensu lato, *Rhipicephalus turanicus*, *Rhipicephalus bursa*, *Haemaphysalis parva* and *Haemaphysalis adleri*. PCR-based analyses of the ticks revealed *Theileria ovis* (5.4%), *Hepatozoon canis* (4.3%), *Babesia ovis* (0.6%), and *Babesia vogeli* (0.4%). *Theileria ovis* was significantly associated with ticks from sheep and with *R. turanicus* ticks ($p < 0.01$). *H. canis* was detected only in *R. sanguineus* s.l. and was significantly associated with ticks from dogs ($p < 0.01$).

To our knowledge, this is the first report describing the presence of these pathogens in ticks collected from Palestine. Communicating these findings with health and veterinary professionals will increase their awareness, and contribute to improved diagnosis and treatment of tick-borne diseases.

1. Introduction

Ixodid (hard) ticks are vectors of numerous diseases among animals and humans throughout the world (Harrus and Waner, 2011; Inokuma et al., 2003). *Hepatozoon* spp. and the piroplasms which include *Babesia* and *Theileria* spp. are tick-borne intracellular parasites that infect vertebrates. Some piroplasm species cause major economic losses to the livestock industry in Asia, resulting in weight loss, decreased meat and milk production, abortions and death (Zintl et al., 2003). Infections vary in severity from sub-clinical to acute with fever, anemia, severe lethargy, and circulatory shock (Homer et al., 2000; Zintl et al., 2003). Several species of piroplasms are transmitted to sheep and goats by ixodid ticks. Ovine theileriosis is caused by several species of *Theileria* and transmitted by ticks of the genera *Amblyomma*, *Haemaphysalis*, *Hyalomma* and *Rhipicephalus* (Bishop et al., 2004). While bovine theileriosis has been extensively studied, ovine theileriosis has received less scientific attention (Gao et al., 2002). *Hepatozoon canis* is a protozoan transmitted by the *Rhipicephalus sanguineus* sensu lato group and present in Europe, Asia, America and Africa (Aydin et al., 2015; Criado-Fornelio et al., 2003; Duscher et al., 2013; Ewing and Panciera, 2003; Karagenc et al., 2006; Maia et al., 2014; Najm et al., 2014; Tolnai et al., 2015). This parasite is transmitted to dogs by the ingestion of ticks containing mature oocysts. *H. canis* infections range from being sub-clinical to severe.

To date, there are no data on the presence and geographic distribution of hard ticks, and the extent of tick infection with piroplasm pathogens in Palestine. We therefore aimed in this study to collect ixodid ticks found on small ruminants and dogs in different

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http://dx.doi.org/10.1016/j.ttbdis.2016.03.003
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Table 1
Different tick species collected from studied animals in different areas of Palestine.

Tick species (number & % of total ticks collected)	Animal host (number of ticks collected from each host species)	Area					
		Jenin	Jericho	Nablus	Qalqilia	Ramallah	Tubas
Rhipicephalus sanguineus s.l. (*n* = 305, 59.1%)	Dog (*n* = 250)	63	33	20	89	16	29
	Goat (*n* = 13)	0	0	0	0	0	13
	Sheep (*n* = 42)	0	0	11	0	0	31
Rhipicephalus bursa (*n* = 15, 2.9%)	Goat (*n* = 1)	0	0	0	0	0	1
	Sheep (*n* = 14)	0	0	3	0	0	11
Rhipicephalus turanicus (*n* = 142, 27.5%)	Dog (*n* = 6)	2	3	0	1	0	0
	Sheep (*n* = 136)	0	0	111	0	0	25
Haemaphysalis parva (*n* = 40, 7.7%)	Dog (*n* = 35)	15	0	3	9	7	1
	Goat (*n* = 3)	0	0	0	0	0	3
	Sheep (*n* = 2)	0	0	0	0	0	2
Haemaphysalis adleri (*n* = 14, 2.7%)	Dog (*n* = 13)	5	0	1	6	1	0
	Sheep (*n* = 1)	0	0	1	0	0	0
Total ticks	516	85	36	150	105	24	116

districts of Palestine to study the protozoan pathogens found in them by molecular techniques.

2. Materials and methods

2.1. Tick collection and identification

Ticks were collected from small ruminant herds composed of 1–20 animals in six rural districts of Palestine between January and September of 2014. Ticks were collected from all sheep and goats present in the same herd and all dogs accompanying the herd. The ticks were taken off from the skin of animals and collections were carried out once in each sampled herd. All ticks found on each animal were collected and immediately introduced into 70% ethanol and kept at −20 °C until taxonomic identification based on morphological criteria was applied (Feldman-Muhsam, 1951, 1954; Pegram et al., 1987). Ticks were then used for DNA extraction for the detection of tick-borne protozoal pathogens.

2.2. DNA extraction of the collected ticks

DNA was extracted from each tick using a DNA extraction kit (QIAGEN GmbH, 40724Hilden, Germany) following the manufacturer's instructions. Each tick was crushed individually with a disposable sterile scalpel in a micro tube. After digestion with proteinase K (20 μg/ml), samples were applied to columns for absorption and washing of DNA. DNA was eluted in 100 μl of buffer and stored at 4 °C until used as template for PCR amplification.

2.3. Polymerase chain reaction

The PCR reactions for detection of piroplasmid and *Hepatozoon* spp. in ticks were performed using primers BJ1: 5′-GTC TTG TAA TTG GAA TGA TGG-3′ and BN2: 5′-TAG TTT ATG GTT AGG ACT ACG-3′ which amplify a fragment of 460–540 bp of the 18S rRNA gene of the genus *Babesia* as described previously (Casati et al., 2006) with the following modification: the PCR reactions were performed in a total volume of 25 μl using PCR-Ready Supreme™ mix (Syntezza Bioscience, Jerusalem) including the primers at 1 μM final concentration. The PCR amplification program performed by a thermocycler (Mastercycler Personal, Eppendorf) included an initial denaturation step of 5 min at 94 °C, followed by 35 cycles of denaturation at 94 °C for 45 s, annealing at 55 °C for 30 s, and elongation at 72 °C for 45 s and final extension 72 °C for 5 minutes. Five microliters of the PCR products were analyzed on 2% Tris-acetate-EDTA buffer (TAE 1X) agarose gels and visualized under UV transillumination.

2.4. DNA sequencing

PCR products were sequenced using an ABI 3730xl DNA Analyzer (Hylab Co., sequencing service). The products were sequenced in both directions with the same primers as for PCR. The chromatograms were checked and the sequences were assembled by the Bio-edit software. The 18S rRNA sequences were aligned using the Multalin Multiple sequence alignment tool (http://multalin.toulouse.inra.fr/multalin/). In order to verify whether the size of the amplified fragment of the detected microbial species in ticks is sufficient to discriminate the different species, the DNA sequences were compared with the GenBank database by the nucleotide sequence homology search facilitated by the National Centre for Biotechnology Information (NCBI) using the BLAST analysis database (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The species were identified based on being the first match by BLAST and having >97% identity with this match.

2.5. PCR-RFLP

For species identification, the partial 18S rRNA DNA sequences of *T. ovis, He. canis, B. ovis and B. vogeli* obtained in the present study and reference strains from GenBank were mapped for restriction site polymorphisms using the NEBcutter V2.0 program available at http://tools.neb.com/NEBcutter2/index.php. The restriction enzyme, XapI (ApoI), originating from the bacteria *Xylophilus ampelinus* was selected because it was indicated to produce distinguishable fragment sizes for some of the species. The PCR-amplified products were digested with the ApoI restriction enzyme (Thermo, Germany) according to the manufacturer's recommendations. Each digestion reaction was set up in 15 μl volume containing 1.5 μl of the 10X reaction buffer and 1 μl of restriction enzyme. Digested PCR products were analyzed on 2% TAE agarose gels and visualized under UV transillumination.

2.6. Phylogenetic analysis

Phylogenetic analyses of the 18S rRNA sequences were performed by the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) applying the neighbor joining and maximum likelihood algorithms. Phylogenetic tree analysis was conducted by the MEGA 6 program using UPGMA program. The reliability of internal

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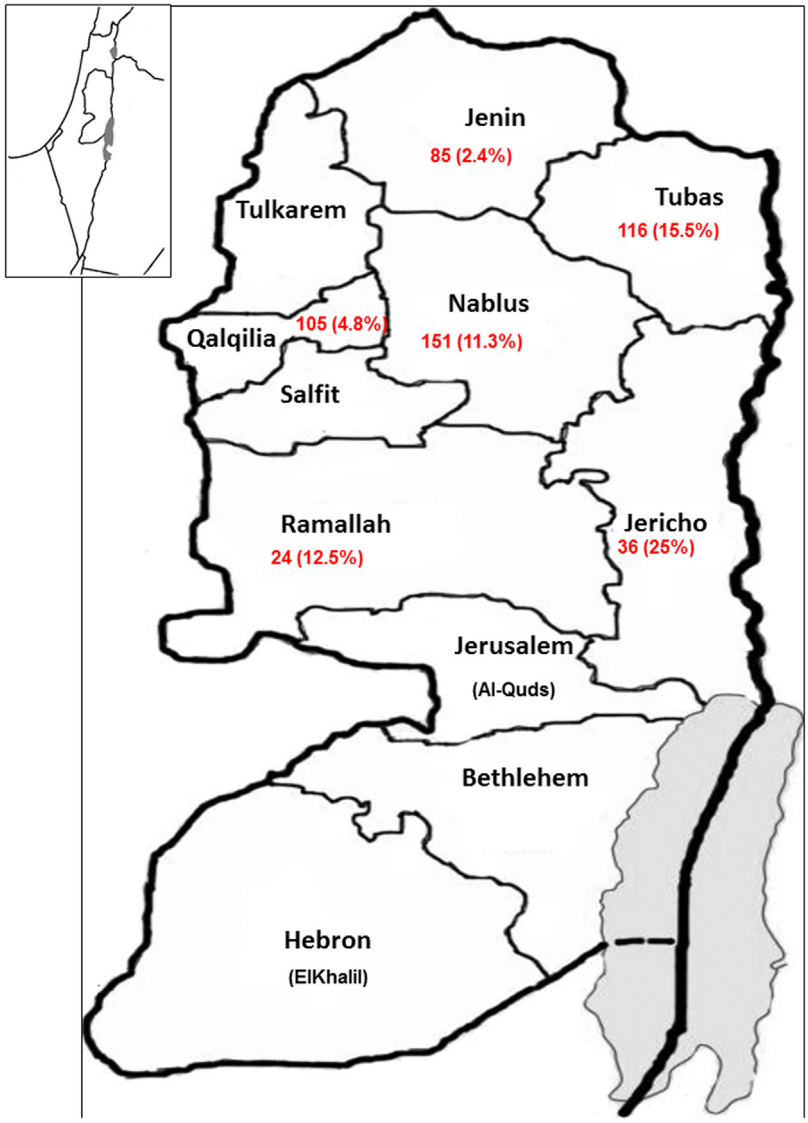

Fig. 1. Map describing the districts of Palestine studied and the number of ticks collected in each district. The overall infection rates with tick-borne pathogens identified are in brackets.

branches was assessed by bootstrapping with 1000 pseudoreplicates. Nodes with bootstrap support less than 70% were collapsed.

2.7. Statistical analysis

Statistical analysis of the epidemiological data was carried out using the SPSS V.17.0 program. Two-tailed *t*-test and Pearson's correlation were performed. *p*-value <0.05 was considered statistically significant.

3. Results

3.1. Tick identification

A total of 516 partially fed hard ticks were collected from 189 animals (117 dogs, 66 sheep and 6 goats) in herds composed from 1–20 animals, living in six different districts. Collection included: 150 ticks from Nablus, 116 from Tubas, 105 from Qalqilia, 85 from Jenin, 36 from Jericho, and 24 from Ramallah (Fig. 1). The most abundant tick genera were *Rhipicephalus* (462/516, 89.5%) and *Haemaphysalis* (54/516, 10.5%) (Table 1). Three hundred and five (305) *R. sanguineus* s.l. ticks were collected from all the six districts, 142 *Rhipicephalus turanicus* from five districts, and 40 *Haemaphysalis parva* were collected from five districts. Only 15 *Rhipicephalus bursa* were collected from sheep sampled in the Nablus and Tubas districts. Fourteen *Haemaphysalis adleri* were collected from dogs and sheep. *Ha. adleri* was identified according to Feldman-Muhsam (1951, 1954). The majority of collected ticks were in the adult stage (females: 247/516, 47.9% and males: 237/516, 45.9%) and only a small part (32/516, 6.2%) were nymphs.

3.2. Pathogen detection and identification

Pathogens were detected in 25 ticks from dogs which comprised 8.2% of the total number of ticks collected from dogs, and in 29 ticks collected from sheep which comprised 14.9% of the ticks from sheep (Table 2). No pathogens were detected in ticks from goats. The animals carrying the infected ticks were apparently healthy and did not show any overt disease manifestations.

PCR and sequencing of DNA of all positive PCR products ($n = 54$) from the study identified the presence of one *Theileria* spp. (*T. ovis*), two *Babesia* spp. (*B. ovis* and *B. vogeli*) and one *Hepatozoon* spp. (*He. canis*). Overall, a total of 54 ticks (10.5%) were positive for pathogens which included: *T. ovis* (28/516, 5.4%), *He. canis* (21/516, 4.1%), *B.*

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Table 2
Pathogens found in the different tick hosts according to the animal host of ticks.

Pathogen	Tick host	Animal host of infected ticks			
		Dog	Sheep	Goat	Total
Theileria ovis	*Rhipicephalus sanguineus* s.l.	1	6	0	7
	Rhipicephalus bursa	0	5	0	5
	Haemaphysalis parva	0	0	0	0
	Haemaphysalis adleri	0	0	0	0
	Rhipicephalus turanicus	0	16*	0	16
	Total *Theileria ovis*	1	27	0	28
Babesia ovis	*Rhipicephalus sanguineus* s.l.	1 (1§)	0	0	1
	Rhipicephalus bursa	0	1	0	1
	Haemaphysalis parva	1	0	0	1
	Haemaphysalis adleri	0	0	0	0
	Rhipicephalus turanicus	0	0	0	0
	Total *Babesia ovis*	2	1	0	3
Hepatozoon canis	*Rhipicephalus sanguineus* s.l.	20 (1§)*	1	0	21
	Rhipicephalus bursa	0	0	0	0
	Haemaphysalis parva	0	0	0	0
	Haemaphysalis adleri	0	0	0	0
	Rhipicephalus turanicus	0	0	0	0
	Total *Hepatozoon canis*	20	1	0	21
Babesia vogeli	*Rhipicephalus sanguineus* s.l.	2	0	0	2
	Rhipicephalus bursa	0	0	0	0
	Haemaphysalis parva	0	0	0	0
	Haemaphysalis adleri	0	0	0	0
	Rhipicephalus turanicus	0	0	0	0
	Total *Babesia vogeli*	2	0	0	2
Total infected ticks	54	25	29	0	54

§ nymphal tick.
* $p < 0.01$, prevalence compared to presence of the specified pathogen in other tick species.

vogeli (2/516, 0.4%) and *B. ovis* (3/516, 0.6%). The pathogens found in the different tick species are shown in Table 2. Out of 117 ticks collected from dogs, 13.6%, 1.6%, 1.6% and 0.8%, were positive for *He. canis*, *B. ovis*, *B. vogeli* and *T. ovis*, respectively. Of 66 ticks from sheep, 33.3% were positive for *T. ovis* and 1.5% for *B. ovis*. In general, piroplasms were the most frequently detected pathogens with a positivity rate of 6.4%.

The rate of *He. canis* DNA detection was significantly higher in male ticks (15/21, 71.4%) compared to female (5/21, 23.8%) and nymphal ticks (4.7%, $p < 0.01$). The presence of *Babesia*/Theileria DNA was not significantly different in female (16/28, 57%) than in male (12/28, 43%) and nymphal ticks (3.5%).

Overall, 12.0% of the ticks tested were pathogen-positive: 11.1% of the *R. sanguineus* s.l. and 12.7% of the *R. turanicus* were positive for pathogens. All *T. ovis* infections were detected in *R. bursa*, *R. sanguineus* s.l. and *R. turanicus* from sheep. *T. ovis* was significantly associated with ticks from sheep and also with *R. turanicus* ticks ($p < 0.01$). The DNA of *He. canis* was detected only in *R. sanguineus* s.l. and *He. canis* was significantly associated with ticks from dogs and also with *R. sanguineus* ticks ($p < 0.01$). The DNA of *B. ovis* was detected in *R. bursa Ha. parva* and *R. sanguineus* s.l. while the DNA of *B. vogeli* was detected in *R. sanguineus* s.l. None of the studied pathogens were found in *Ha. adleri* (Table 1).

3.3. DNA Sequencing and RFLP

The 18S rRNA PCR yielded a product of approximately 465–542 bp (Fig. 2a). A 542 bp band was amplified from *He. canis* positive samples. It was clearly distinguished from bands amplified for the other pathogen species with *T. ovis* and *B. vogeli* producing a 489 bp band and *B. ovis* producing a 465 bp band. PCR products

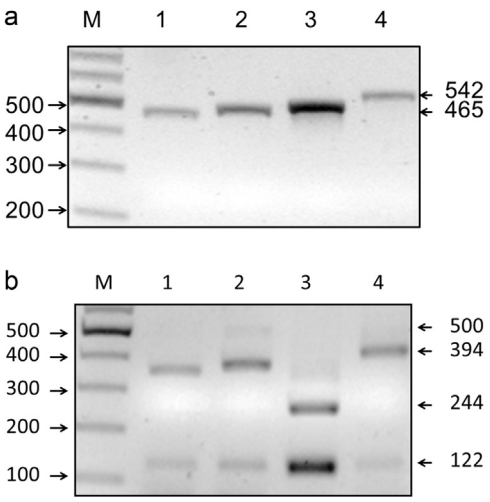

Fig. 2. (A) Agarose gel electrophoresis of PCR products from representative DNA positive samples amplifying a fragment of the 18S rRNA gene. Lane M, 100 bp DNA ladder, the thicker band is 500 bp; lane 1 *B. ovis*; lane 2, *B. vogeli*; lane 3, *T. ovis*, lane 4, *H. canis*. (B) RFLP analysis of PCR products of 18SrRNA gene following restriction with the ApoI restriction enzyme. Lane M, 100 bp DNA ladder; lane 1 *B. ovis*; lane 2, *B. vogeli*; lane 3, *T. ovis*, lane 4, *He. canis*.

digested with ApoI showed three fragments of 394, 122 and 26 bp for *He. canis*, four fragments of 244, 121, 115 (both latter bands observed as one heavy band) and 26 bp for *T. ovis*, but only two fragments of 367 and 122 bp for *B. vogeli* and two fragments of 343 and 122 bp for *B. ovis* (Fig. 2b). The lower band of 26 bp was not visualized on agarose gel for all species. Specifically, RFLP was able to distinguish between piroplasms and *He. canis*, since both species *T. ovis* and *B. vogeli* showed the same PCR size product. RFLP of PCR positive samples of *He. canis*, *B. vogeli*, *B. ovis* and *T. ovis*, were run in parallel and used as a positive control in each digestion reaction.

Twenty of the 54 (37%) sequenced PCR products were analyzed blindly, validated and confirmed the PCR RFLP profiles described above. Representative confirmed partial sequences of the 18S rRNA gene of two *He. canis* samples from ticks collected from dogs, two *B. vogeli* from dog ticks, two *B. ovis* from ticks found on a sheep and a dog, and one *T. ovis* from a sheep tick identified in the present study were deposited in GenBank under accession nos. KT587789, KT587790, KT587791, KT587792, KT587793, KT587794 and KT587795, respectively.

3.4. Phylogenetic analysis

Three phylogenetic trees of *Hepatozoon*, *Babesia* and *Theileria*, were constructed from the 18S rRNA gene sequences generated in this study with comparison to selected sequences available in GenBank.

The first tree (Fig. 3A) represents all the sequences of *He. canis* generated in this study ($n = 21$). Three clades were formed with most of the study sequences falling into two clades. One clade included sequences from other countries such as Israel, Sudan, Austria and Spain, while two clades included only sequences from Palestine.

Concerning *Babesia* sequences, the second phylogenetic tree analysis (Fig. 3B) found two major clades for *Babesia* species and showed evidence of two monophyletic clades, one consisting of *B. vogeli* with *B. canis* and the other including *B. ovis* with well-supported separation among them. The comparison of the 18S rRNA *B. ovis* sequences revealed 99% homology between Palestinian sequences and those from Spain (AY150058.1), Turkey (JF923656.1) and Albania (KF681514.1).

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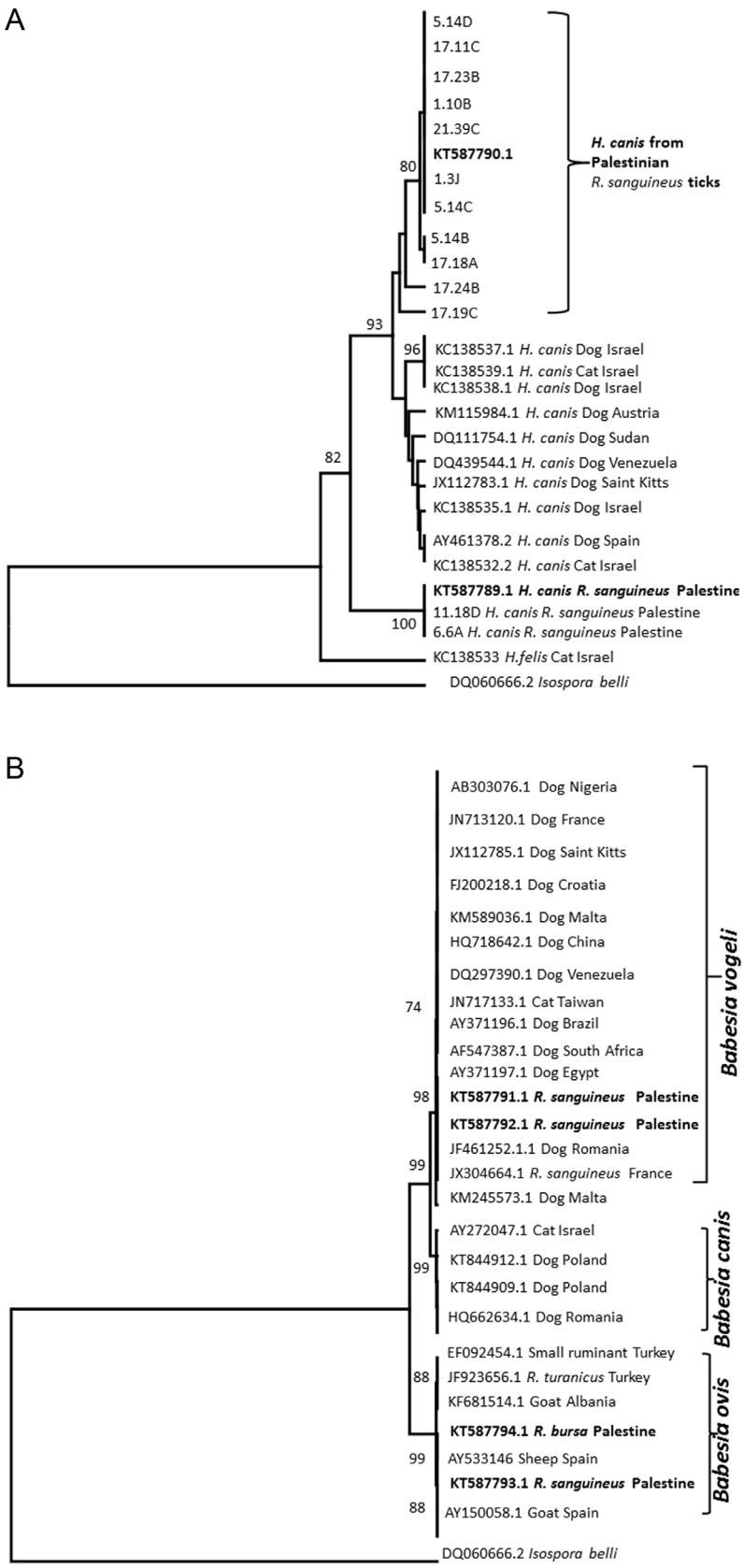

Fig. 3. Neighbor joining phylogram trees. Phylogenetic analysis was constructed by the UPGMA method with bootstrap of 1000 replications using Mega 6 program. The Embank accession numbers, species of infected animals and country of origin from which the sequences were derived are included for each sequence. New GenBank accessions for sequences derived from the present study are highlighted in bold letters. The number of identical sequences is in brackets. Selected reference piroplasm and *He. canis* sequences from GenBank are also shown. *Isospora belli* and *Theileria cervi* were been used as out-groups. (A) Phylogenetic tree based on partial sequences of the 18S rRNA gene of *Hepatozoon canis* comparing sequences generated in this study to sequences from other countries. (B) Phylogenetic tree based on partial sequences of the 18S rRNA gene of *Babesia* species comparing sequences generated in this study to sequences from other countries. (C) Phylogenetic tree based on partial sequences of the 18S rRNA gene of Theileria comparing sequences generated in this study to sequences from other countries.

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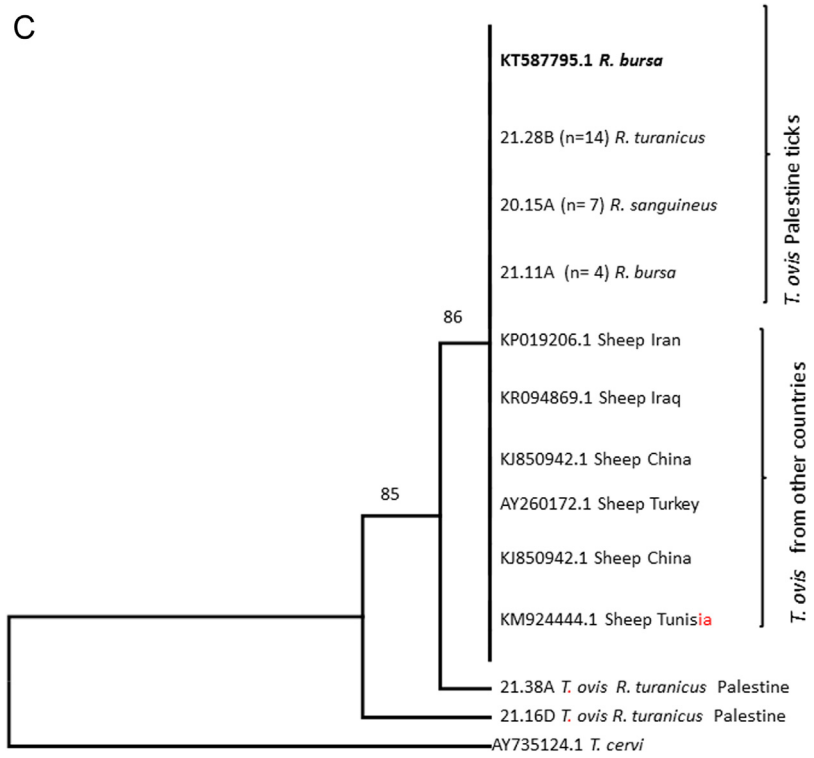

Fig. 3. (*continued*)

The third phylogenetic tree consisted of all *T. ovis* sequences generated in this study (Fig. 3C). Here, the *18S rRNA gene* sequences of *T. ovis* ($n = 26$) described herein formed well supported clades of sequences that were identical to each other and all revealed 100% homology to the *T. ovis* reference sequence (accession no. KP019206.1) deposited in GenBank with the exception of two sequences (21.16D and 21.38A).

4. Discussion

Ticks are vectors of important pathogens of human and animals which cause theilerosis (Jalali et al., 2014), hepatozoonosis (Piratae et al., 2015) and babesiosis (Theodoropoulos et al., 2006). Several tick species, including *R. sanguineus* s.l., *R. turanicus* and additional tick species have been identified in neighboring countries (Harrus et al., 2011a,b; Keysary et al., 2011; Salant et al., 2014). In this study, we found that *R. sanguineus* s.l. and *R. turanicus* are frequently found on dogs, sheep and goats often without obvious host specificity. Among five tick species examined for *Theileria*, *Babesia* and *Hepatozoon* spp., four (*R. bursa*, *R. turanicus*, *R. sanguineus* s.l. and *Ha. parva*) were found to be infected by one or more pathogen species. The rate of *He. canis* detection was significantly higher in male ticks, this could be due to the locomotion capacity of males seeking a new host (Solano-Gallego et al., 2012), while the presence of *Babesia*/Theileria was not significantly different in between the sexes.

Our finding that ticks from sheep have a higher infection rate of *Theileria* sp. compared to ticks from goats is in agreement with results reported directly from the blood of these animal species in a study from Ethiopia (Gebrekidan et al., 2014). This may suggest that different tick species are involved in the transmission of *Theileria* sp. in sheep from Palestine.

About two thirds (57%) of the positive ticks which were infected with *T. ovis* were of the species *R. turanicus*. However, the results of this study and the detection of *T. ovis* in *R. turanicus* could suggest that it is a possible vector responsible for transmission of *T. ovis* as proposed previously (Razmi et al., 2013). Neither *B. ovis* nor *B. vogeli* or *He. canis* infection was detected in this tick species.

Ovine babesiosis caused by *B. ovis* is an important tick-borne hemoparasitic disease of small ruminants (Schnittger et al., 2003) often leading to loss in productivity and in some cases mortality (Bai et al., 2002; Erster et al., 2016; Esmaeilnejad et al., 2014a,b; Rjeibi et al., 2014). The two 18S rRNA gene *B. ovis* sequences in the present study (from ticks feeding on a sheep and a dog, respectively) had high similarity to a *B. ovis* sequence deposited in GenBank (AY150058.1), from an infected goat from Spain. The results presented in this study have also demonstrated the existence of *B. ovis* in *R. bursa* collected from sheep. Our results agree with other studies that reported *B. ovis* in *Rhipicephalus* spp. including *R. bursa*, *R. sanguineus*, and *R. turanicus* (Erster et al., 2016; Rjeibi et al., 2014). *R. bursa* plays an important role as a natural vector of *B. ovis* and has been reported as the only vector for *B. ovis* that can transovarially transmit this *Babesia* species to sheep and goat (Altay et al., 2008).

Ovine theileriosis has been reported in various countries such as Ethiopia, Iran and China (Gebrekidan et al., 2014; Iqbal et al., 2013; Liu et al., 2008). In our study, the only species of *Theileria* found in ticks from Palestinian sheep was *T. ovis* with a high infection rate. Contrary to a report from Tunisia (M'Ghirbi et al., 2008), which detected *T. ovis* in sheep and goats, this species was only present in ticks from sheep in our study. This could be because both the number of goats and the number of ticks obtained from them was very low. However, this species of *Theileria* reportedly causes subclinical infections in small ruminants (Jalali et al., 2014).

Theileria ovis from sheep in this study showed low genetic diversity. The reported sequences for the 18S ssu rRNA gene (489 bp length) from five countries (Tunisia, Turkey, Iraq, Iran and China) had 100% homology with our sequences, except for two, and clustered together in a clade clearly distinct from *T. cervi*.

Canine babesiosis is a clinically important disease affecting dogs. The most common piroplasm in dogs in the Mediterranean region

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and some other areas is *B. vogeli* (Harrus et al., 2011b; Solano-Gallego and Baneth, 2011). In this study, *B. vogeli* was detected in *R. sanguineus* ticks which are recognized as the main global vector for this infection (Harrus et al., 2011b).

Based on these results, it can be concluded that *T. ovis* and *He. canis* have a high prevalence in ticks collected from sheep and dogs in Palestine, respectively. *He. canis* is present in many regions of the world (Criado-Fornelio et al., 2003; Duscher et al., 2013; Ewing and Panciera, 2003) and although most canine *He. canis* are considered sub-clinical, they could also be severe in cases with high parasitemia (Baneth et al., 2001; Baneth and Weigler, 1997). The infection rate of *He. canis* was high in *R. sanguineus* ticks collected from dogs in our study. This study demonstrated that *He. canis* and its vector *R. sanguineus* (Baneth et al., 2001) are present in all the studied areas except for Jenin in which *He. canis* was not detected.

We found one tick infected with *He. canis* which was collected from a sheep and *B. ovis* was detected in one *Ha. parva* collected from dog. This was unexpected, because these parasites are considered not to infect these animal hosts. These detections might be related to the possibility that these ticks fed on other hosts in a previous life stage or as adult males and became infected before taking an additional blood meal on the host on which they were found. The mere presence of pathogen DNA in ticks does not necessarily mean that they are biological vectors, as any pathogen in the blood meal could make the tick positive without the tick necessarily transmitting it to a susceptible animal host in the next blood meal.

5. Conclusions

This is the first report describing the presence of several tick-borne pathogens of veterinary importance in Palestine. The RFLP method based on digestion of the18S rRNA amplicon with the restriction enzyme *ApoI* proved useful for discriminating not only between the piroplasms *T. ovis* and *B. vogeli*, but also to confirm *He. canis* infection. It was possible to rely on the sizes of the upper fragments (394 bp for *He. canis*, 244 bp for *T. ovis*, 367 bp for *B. vogeli* and the 343 bp for *B. ovis*) to distinguish between these pathogens. The results from this study suggest that vector-borne infections of dogs and small ruminants are common, widespread and in need of further investigation in Palestine in order to establish effective measures to control their vector ticks.

Acknowledgments

This study was supported financially by grant 2014.52146 funded by the Netherlands Ministry of Foreign Affairs, The Hague, Netherlands and USAID grant MERC TA-MOU-12-M32-038. We thank Mr. Samir Sawalha, Taher Zaid and Ahmad Abdelkader for their kind help during sample collection.

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