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Molecular Characterization of *Borrelia* Species as Causative Agents for Tick-Borne Relapsing Fever from *Ixodidae* and *Argasidae* in Palestine

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DEDICATION

This thesis is dedicated to my beloved parents, wife, and siblings, whose unwavering support, encouragement, and love have been my greatest sources of strength. To my mentors and supervisors, who have offered guidance and inspiration throughout this journey, and to all those who believed in me even when I doubted myself. This work is a testament to your faith in my abilities.

Abbas Khader Hussein Masalma

Declaration

I certify that this thesis submitted for the degree of Master is the result of my own research, except where otherwise acknowledged, and that this thesis (or any part of the it) has not been submitted for a higher degree to any other university or Institution.

Signed:

Abbas Khader Hussein Masalma

Date: 09 – 01 - 2025

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Abstract

Introduction: Tick-Borne Relapsing Fever (TBRF) is primarily caused by several *Borrelia* species that are transmitted through the bites of ticks. It is mainly characterized by multiple recurrences of nonspecific signs and symptoms, including fever, headache, myalgia, arthralgia, and even some neurologic complications. These *Borrelia* species are mostly vectored by hard ticks like *Rhipicephalus* genus and soft ticks like *Ornithodoros tholozani* which is known as the main vector of *B. persica* in Palestine. However, Molecular based data on *Borrelia* species and their hosts in Palestine are very scarce as *B. persica* is the only *Borrelia* species that had been studied.

Objectives: This study aimed to investigate the molecular epidemiology of *Borrelia* species in ectoparasite hard and soft ticks, collected from different animal hosts throughout Palestine.

Methodology: Ticks samples were collected from different animal hosts residing in different districts through Palestine, all ticks were identified at the genus and species levels based on taxonomic keys. DNA extraction was carried out, then screening for the presence of Borrelial DNA was carried out by PCR targeting flagellin (*fla B*) gene. All positive samples were confirmed by 16sRNA – PCR. PCR products were loaded onto agarose gel for band detection at 250 bp for *fla B* and 523 bp for *16sRNA*, positive samples were sequenced and analyzed for BLAST identification. Statistical analysis was carried out using IBM SPSS v27.0 software.

Results: Among all tick samples: 86% (n= 734) were identified as Ixodidae (hard ticks) and 14% (n= 117) were Argasidae (soft ticks). Overall, 76% of the identified hard ticks belonged to the genus *Rhipicephalus* (92% *R. sanguineous* and 5.5% *R. Turanicus*), 12% of *Haemaphysalis* ticks (80% *H. parva* and 20% *H. adleri*), 11% were *Hyalomma* ticks (84% *H. dromedarri*, 6% *H. impeltatum*, and 5% *H. eagyptium*). All collected soft ticks (n= 117) were identified as *Ornithodoros tholozani*. Out of 734 hard ticks, 2% (n= 13) were detected to be positive for *Borrelia* DNA by PCR. Sequence analysis revealed the presence of *B. persica* in 77% (n=10) of positive samples, while 15% (n=2) were *B. turcicae*, and 8% (n=1) was *B. Lonestari*. On the other hand, 9% of soft ticks (n=17) were found to harbor *B.persica*.

Conclusions: Our results highlight the presence of *Borrelia persica* among hard ticks despite its' usual presence among soft ticks, this could indicate an incidental acquisition or ecological overlaps. *B. persica* genotype was also observed in soft ticks. Notably, in Palestine, *R. sanguineus* is the major hard ticks which vector *Borrelia* species among hard ticks, while *O. tholozani* is the major one among soft ticks.

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Table of Abbreviations

Abbreviation	Full Word
TBD	Tick – borne disease
TBRF	Tick-borne relapsing fever
B	*Borrelia*
RH	*Rhipicephalus*
Hy	*Hyalomma*
O	*Ornithodoros*
PCR	Polymerase chain reaction
DNA	Deoxyribonucleic acid
rRNA	Ribosomal Ribonucleic acid
Bp	Base pair
µL	Microliter
µM	Micromolar
℃	Celsius
PBS	Phosphate buffered saline
EDTA	Ethylene diamin tetraacetic acid
BLAST	Basic local alignment search tool
BioEdit	Biological sequence alignment editor
SNP	Single nucleotide polymorphism

CHAPTER ONE

INTRODUCTION

Ticks are small obligate ectoparasitic arachnids, they are considered as the world's first vector of medical and veterinary significance, their potential arises from their significant economic influence on veterinary health which results a burden on human health by the pathogenic organisms they can transmit during its life cycle (Moraga-Fernández et al., 2023). Ticks are external parasites that don't share the biological and ecological features of other parasitic Diptera like mosquitos. However, ticks particularly survive by hematophagy on the blood of different types of mammals, birds, and even reptiles and amphibians, they can vector a high diversity of pathogens including bacteria which makes them as the second most significant carriers of various pathogens like *Borrelia* or *Babesia*. To date, the factors that modify the ticks activity and distribution are host, climate, urbanization, environmental greening, and socio-economical changes, exposing animals and people at risk of tick bites leading to tick-borne diseases (TBD) (Stachurski et al., 2021; Rashid, M., et al. 2019).

Ticks are being a special fecund of research because of their vectorial capacity of some deleterious pathogens that could affect both animals and human beings, in addition to poor knowledge about the molecular nexus between ticks and some particular pathogens transmittable by them. Interestingly, broad studies have been on the rise in the last years to understand the biology and ecology of ticks as well as their somewhat complex relationships with abiotic conditions (vegetation and climate).

Moreover to evaluate the risk of tick-borne pathogen transmission to animals and human to reveal the surveillance of tick-borne pathogen, most studies highlight on pathogen detection in field-collected ticks (Díaz-Sánchez et al., 2023).

1.1 Ticks Classification and Taxonomy

Tick taxonomy has usually been determined by morphological characteristics, but this can be challenging due to the possibility of cryptic speciation, in addition to some polymorphisms (Moraru et al. 2018, Lado et al. 2018, Dantas-Torres 2018). Recently, some studies had approved the usefulness of what is called integrative taxonomy to overcome the dilemma of tick classifications despite the possibility of cryptic speciation (Lado et al. 2021).

Ticks are majorly classified into three main families; one is *Nutalliellidae* which only encompasses the species of *Nuttalliella Namaqua.* The other two large families are hard ticks (*Ixodidae*) and the soft ticks (*Argasidae*), both comprise a total of about 850 species (Tabor, A. E. 2024).

It is possible to differentiate between *Argasidae* and *Ixodidae* based on their biological and morphological features (Chong, S. T., et al., 2020). Ixodidae are so called hard ticks because of the hard shield or scutum they characterized by; it protects them from minatory factors. Unlike *Argasidae* which are called soft ticks because they lack this shield (Fig. 1.1).

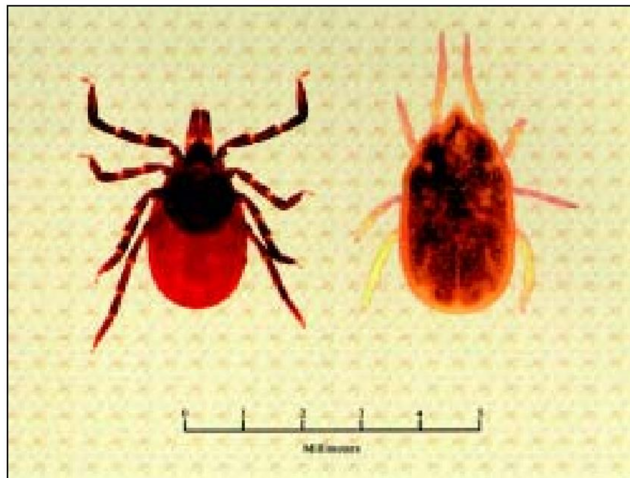

Figure 1.1: Dorsal view of a female *Ixodes scapularis* (family *Ixodidae*, hard ticks) (left), and a female *Ornithodoros hermsi* (family *Argasidae,* soft ticks), (right) (Schwan et al., 2002).

Taxonomically, there are some obvious distinctions between the *Ixodidae* and the *Argasidae* (Narcis, S. F. 2024). The tick is made up of an oval-shaped, flattened body called the idiosoma and a capitulum, or head. Although larvae hatch from the egg with only six legs, adult ticks and nymphs typically have eight legs. The dorsal surface of hard ticks has a hardened plate known as the scutum. (table 1.1). On females, this scutum, which makes up around one-third of the dorsal surface, can help distinguish between different species of ticks. On males, their ability to feed is impacted by the scutum, which covers the entire dorsal surface. The chelicerae and hypostome, which are the tick's mouthparts that emerge on the capitulum, are used to pierce and save the tick from its host. During feeding, ticks release chemicals that aid in their attachment to their host,

serve as an anesthetic to reduce bite pain, and stop blood clotting. Since ticks are efficient feeders and consistent once attached, they are able to transmitting disease (Maqbool, M., et al. 2022). (Fig. 1.2, 1.3).

Table 1.1: Main Morphological Differences Between Adult of *Argasidae* and *Ixodidae.* (Santiago et al., 2017).

Ixodidae	Argasidae
With dorsal scutum	Without dorsal scutum
Evident sexual dimorphism	Not evident sexual dimorphism (with few exceptions)
Capitulum visible dorsally	Capitulum not visible dorsally
Coxae with spurs	Coxae without spurs
Festoon present or absent	Festoon never present
Porose area of female present (with one exception)	Porose area of female never present
Spiracular plates present	Spiracular plates absent

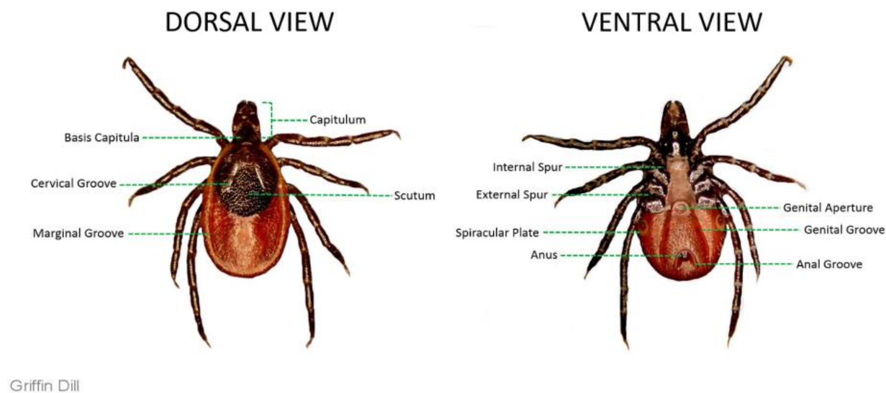

Figure 1.2: Tick – External Morphology. Tick Biology Tick Lab – University of Maine Cooperative Extension (2019). Retrieved from https://extension.umaine.edu/ticks/tick-biology.

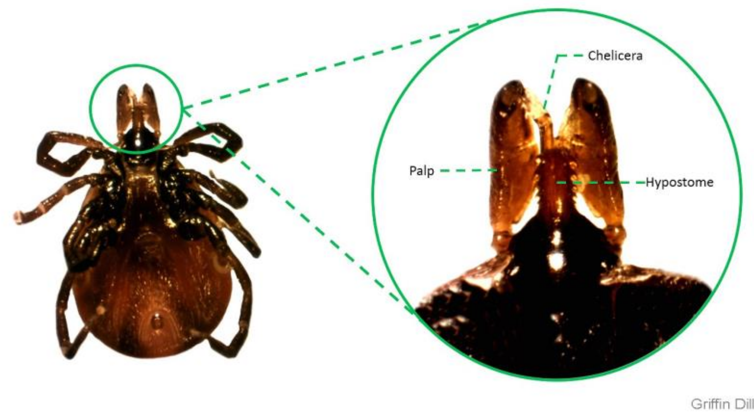

Figure 1.3: Tick – Tick Mouthparts. Tick Biology Tick Lab – University of Maine Cooperative Extension (2019). Retrieved from https://extension.umaine.edu/ticks/tick-biology.

1.2 Ticks Life Cycle

Generally, tick species have a three-host life cycle, meaning that at each active life stage (larva, nymph, and adult), they feed on a different individual host. Upon hatching, Six-legged larvae, also known as seed ticks, search for and start feeding on their first host. It is usually during the larval stage when the tick gets infected with disease-causing organisms. Once fully engorged, the larval tick drops from its host and molts to a nymph. The nymph will then locate and feed on its second host, and molt into an adult. (Ergunay, K., et al. 2024). The adult tick nourishes upon a third and final host. The mature female tick will lay a single batch of several thousand eggs in the leaf litter after mating and becoming completely engorged, then die. (Fig 1.4). Depending on the tick species, the entire life cycle lasts anywhere from one to three years. The winter tick, which has a one-host life cycle and lives its whole existence on a single host, is an exception to this three-host life cycle (Hoffman, T., et al. 2023).

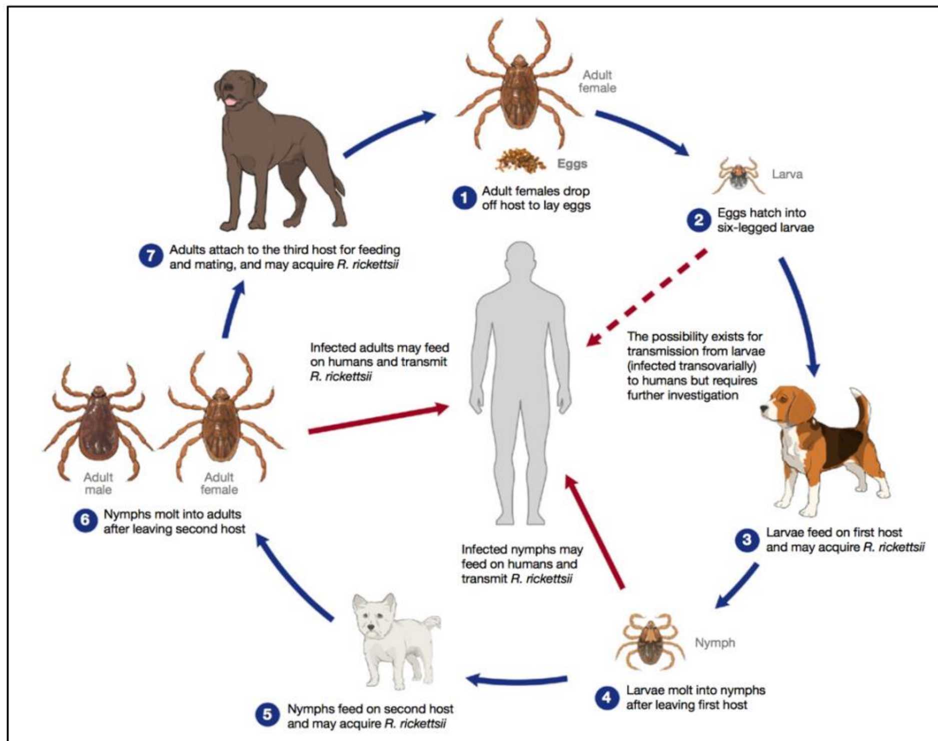

Figure 1.4: Life cycle of *Rh. sanguineous* (species of hard ticks) and the example transmission of tick-borne disease (*Rickettsia*). The Centers for Disease Control and Prevention (CDC, 2024), Retrieved from https://www.cdc.gov/ticks/about/tick-lifecycles.html.

1.3 Ticks Distribution in Palestine

There were several kinds of ticks from both main families (*Ixodidae* and *Argasidae*) had been identified throughout Palestinian districts, cities, and villages (Ereqat S. et al., 2016). It was also revealed that the same kinds of ticks are present in different neighboring countries with different environmental conditions (Tirosh-Levy, S. et al., 2018; Obaidat, M. et al., 2020; Darwiche, W. et al. 2023). Some identified common ticks in different Palestinian region are *Rh. sanguineus*, *Rh. turanicus* and *H. parva*. (Ereqat S. et al., 2016).

Ticks are ectothermic, they are more active in warmer conditions which enhance host-seeking behavior, development, and reproduction (Fieler, A. M. et al., 2021). Ticks need a relative humidity of more than 80% to survive and avoid dryness. In winter and dry periods, ticks may enter in a state of inactivity because of unfavorable temperature and humidity (Tian, Y. et al., 2022).

1.4 Lyme Borreliosis

Lyme borreliosis is one of the most common tick-borne illnesses throughout the world, with around 200,000 cases every year in Europe (Marques et al. 2021). The term "*Borrelia*" refers to a bacterium that incorporates of more than 30 bacterial species of arthropod vector-borne spirochetes that could be categorized into two main categories. The first one of spirochetes

includes 19 *Borrelia species* that are approved since 1984 (Wang G., 2024; Trevisan, G., et al., 2021). The term '*Borrelia burgdorferi'* has been collectively used to refer to all *Borrelia species* and isolates in this bunch, which is transmitted by the *Ixodes* ticks and is pathologically related to Lyme borreliosis (LB) or Lyme disease (Kurokawa at al. 2020). The second category of spirochetes includes 25 validated or approved *Borrelia* species transmitted by *Argasidae* with some exceptions (louse-borne *B. recurrentis* and *Borrelia lonestari*). *Borrelia* species in the second major category are related to human relapsing fever (RF). All Borrelia species and isolates in this category have been referred to as "relapsing fever Borrelia." (Rauer, S. et al., 2020; Trevisan, G. et al., 2021).

1.5 Tick -borne Borreliosis as a public health issue in Palestine

Ticks play an important role in transmitting infectious diseases in the middle east countries. Emerging infectious diseases are being a global public health issue (Cunningham, A. et al., 2017). The incidence of tick-borne borreliosis has raised globally, especially in the Mediterranean countries. In Palestine, the studies regarding public health concerns of tick borne borreliosis are still scarce. However, the Palestinian environment and ecosystem are similar to those in neighboring countries such as Jordan, Lebanon, and Egypt. Factors such as climate change, deforestation, and increased human exposure to tick habitats contribute to the rise in cases (Perveen, N. et al., 2021).

Climate change contributes to tick vector expansion by warmer temperatures that have expanded tick habitats into new areas, raising the risk of transmission. This is one way that climate change contributes to tick vector expansion. Furthermore, tick populations are boosted by ecosystem changes such as decreased predator populations and an increase in deer.

The clinical diagnosis of borreliosis is being skeptical, because its symptoms resemble other diseases signs like seasonal influenza and other viral infections. However accurate diagnosis relies on clinical signs, history of tick exposure and related animals, and confirmatory laboratory tests such are serology and PCR.

Antibiotics are one of the treatment choices in these cases, with increasing antimicrobial resistance among causative bacterial pathogens, the occurrence of tick-borne diseases has been increased with considerable domestic animal, wildlife and human mortality (Kullberg, B. J. et al., 2020). However, incomplete or delayed antibiotic treatment may lead to persistent symptoms, a case often known as post-treatment Lyme disease syndrome.

1.6 Study Objectives

Ticks are considered as one of the most important particular vectors of relapsing fever or tick-borne relapsing fever (TBRF) affecting human, it is characterized by recurring episodes of fever, headache, nausea, muscle and joints aches, it is strictly caused by certain particular species of tick-borne bacteria *Borrelia* spirochetes. The purpose of this study was to identify and genetically describe *Borrelia species* in hard and soft ticks that were obtained from various animal hosts. (Dogs, sheep, camel, turtle, wolf, rodents, badger, goat, rock hyrax and porcupine) in the west bank, Palestine.

Moreover, our study's results are likely to reveal a better understanding of how *Borrelia species* are distributed in targeted regions. Therefore, the main objectives of this study were:

i. To perform a taxonomical classification of different ticks from both groups soft and hard ticks collected from different Palestinian districts.
ii. To investigate the most prevalent tick kinds distributed in Palestine among both soft and hard ticks.
iii. To genetically characterize *Borrelia species* detected in hard and soft ticks collected from different animal hosts.

1.7 Research Significance

Our study's findings are hopeful to contribute to better understanding how *Borrelia* are maintained in nature and planning for preventive measures against tick-borne borreliosis in our target regions.

1.8 Literature Review

Tick-borne relapsing fever (TBRF) is a bacterial infection that is primarily caused by different spirochetes species of the genus *Borrelia* (Jakab, Á. et al. 2022), it is mainly transmitted to human via the bites of infected specific species of soft ticks (*Argasidae*) of the genus *Ornithodoros* (Kazim, A. R., et al. 2021). However, TBRF should be considered in patients presented with recurrent fever, this fever period is sudden and commonly followed by a febrile period, the first period is characterized by headache, myalgia, arthralgia, nick stiffness, and nausea (Maxwell, S. P., et al., 2022). Neurologic complications including, delirium, meningismus, facial palsy, and rediculopathy may occur and are accompanied with the second fever episode (Trouillas, P., & Franck, M. 2023).

Tick - borne borreliosis comprises a collection of diseases that are widely dispersed and proliferating quickly due to different species of *Borrelia* that have a veterinary and public

health significance. *Borrelial* diseases, their corresponding tick hosts, and their geographic ranges are listed in detail in (Table 1.2).

Table 1.2: Listing of some *Borrelial* pathogens, their tick hosts, and geographic

Borrelia spp.	Host	Vector	Distribution	Disease	References
B. yangtze	Rodents	*Ixodes granulates Ixodes nipponensis*	China	Unspecified	Hu, X. et al. (2023)
B. valaisiana	Birds, Rodents	*Ixodes ricinus, Ixodes nipponensis, Ixodes columnae*	Asia and Europe	Lyme borreliosis	Ušanović, L. et al. (2024)
B. afzelii	Rodents	*Ixodes ricinus, Ixodes persulatus*	Asia and Europe	Lyme borreliosis	Porcelli S. et al. (2024)
B. burgdorferi sensu stricto	Rodents, birds	*Ixodes scapularis, Ixodes pacificus, I. ricinus*	Europe and USA	Lyme borreliosis	Mancilla-Agrono, L. et al. (2022)
B. bavariensis	Birds, rodents	*Ixodes ricinus, Ixodes persulatus*	Asia and Europe	Lyme borreliosis	Hunfeld, K. et al. (2022)
B. lonestari	Rodents, birds	*Amblyomma americanum*	Southeastern USA	Lyme-like illness?	Margos, G., at al. (2020)
B. hispanica	Rodents	*Ornithodoros erraticus*	Spain, Portugal, Morocco, Algeria, Tunisia	TBRF	Margos, G., et al. (2020)
B. persica	Rodents, bats	*Ornithodoros tholozani*	Middle East, Central Asia	TBRF	Koutantou, M., et al. (2024)
B. theileri	Ruminants, horses	*Rhipicephalus decoloratus Rhipicephalus evertsi Boophilus micropus*	South Africa, Australia, Europe, North America	Bovine borreliosis	Hyung HJ., et al. (2024)
B. turicatae	Rodents	*Ornithodoros turicatae*	USA and Mexico	TBRF	Mays S. E et al. (2024)
B. turcica	Unknown	*Hyalomma aegyptium*	Turkey		Hepner, S. et al., (2020)

It is nearly inconceivable to separate between distinctive fevers caused by *Borrelia*, and to distinguish between diverse species of *Borrelia* by their morphology. LB *Borrelia* do not have a particular parasite vector relationship including a single tick species. Therefore, the ability to recognize and distinguish between various *Borrelia* species is being primarily based on molecular characterizations of their features (Ortega, N., et al., 2024). Some studies applied the metagenomic next-generation sequencing and PCR for the detection on tick-borne diseases from ticks like *Rhipicephalus* (Intirach, J. 2024). There are several molecular approaches have been designed and widely applied for the purpose of classification and identification of different *Borrelia species*, these approaches include: DNA-DNA homology analysis, DNA sequencing of some conserved genes like 16 S rRNA, randomly amplified polymorphic DNA analysis, and PCR and next-generation sequencing.

DNA-DNA Homology is a molecular approach has been used as a reference technique for species determination of *Borrelia*, studies revealed that the phylogenetic analysis of *Borrelia species* generally would comprise strains with at least 70% or more DNA- DNA similarities (Kumar, S., et al., 2018), it was recognized that *Borrelia* isolates within the same species have 70-100% DNA homology, while those isolated from different species have only 30-65% DNA homology (Lemieux, J. E. 2024). Though the DNA-DNA homology varies from 13% to 44% between different diseases caused by *Borrelia* like LB and RF (Wachter, J., et al. 2021).

Regarding the DNA sequencing of some conserved genes (16s RNA), studies revealed that the rRNA gene cluster of *Borrelia* contains a single copy of a 16 S rRNA sequence (Landesman, W. J. et al., 2019). A PCR-based RFLP technique has been developed and is frequently used for Borrelia identification and characterization based on the unique rRNA gene. (Šušnjar, J., et al., 2023).

Randomly amplified polymorphic DNA (RAPD) analysis, uses a low-stringency polymerase chain reaction amplification with a single primer of specified sequence to produce strain-specific arrays of anonymous DNA fragments. It is reported that this technique is a powerful tool for *Borrelia burgdorferi* strain and species identification (Vinodhkumar, O. R. et al., 2024.; Teodorowicz, P., & Weiner, M.2020).

CHAPTER TWO

MATERIALS AND METHODS

2.1 Samples Collection.

A total of tick samples (n=851) was collected from different Palestinian regions and districts (n= 10 districts). These regions include: Jenin, Tulkarm, Tubas, Nablus, Salfit, Ramallah, Jericho, Qalquilya, Jerusalem and Hebron. The whole ticks' samples were collected randomly in winter and early spring seasons between January 2014 to April 2014, this period is the favorable period for most ticks to thrive considering suitable temperature and humidity. The collection was performed by host sampling and sometimes by direct hand collection. However, the overall collected ticks including hard and soft ticks were gathered from different animal hosts, include: Dogs, sheep, camel, turtle, wolf, rodents, badger, goat, rock hyrax, porcupine, and some from horse. All ticks were microscopically identified to the genus and species level using standard taxonomic keys (Filipe Dantas-Torres. 2018), split into different Eppendorf tubes containing 99% ethyl alcohol and kept frozen at −20°C until DNA extraction.

2.2 Ticks Identification

Ticks' identification was performed using a stereomicroscope, they were categorized based on published identification taxonomic and structural differences keys by sex and species. The genus, species, gender, and developmental stage were determined according to taxonomic keys (Beard, D., *et al.* 2021). *Rhipicephalus* was identified depending on their main morphological features. it is a medium sized (unfed adult females are 4-5 mm long) yellowish-brown to reddish-brown tick with a dark, inornate brown scutum. The larvae have six legs while the nymphs and adults have eight legs. It has festoons, distinctive eyes and the basis capitula is hexagonal (Fig. 2.1, 2.2). Then the ticks have been identified to the species level on the basis of their morphological parameters such as basis capitula lateral angle which are blunt in *R. turnicus*, and sharp in *R. sanguinus*.

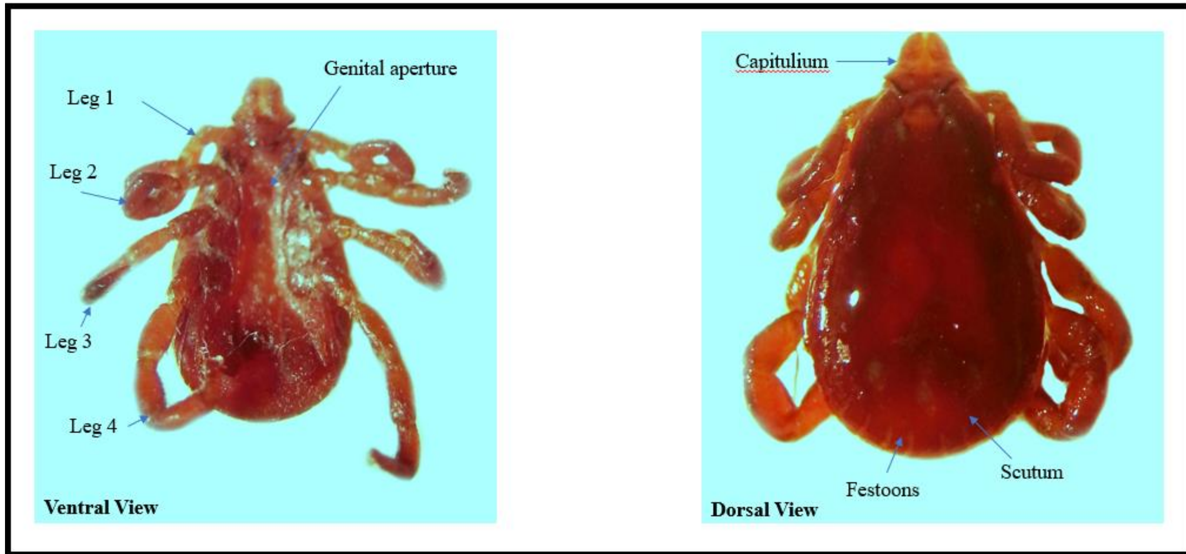

Figure 2.1: Some morphological features of male *Rhipicephalus* tick: dorsal view and ventral view. The arrow indicates the scutum which covers part of dorsal view and other parameters (Photo taken by Abbas Khader).

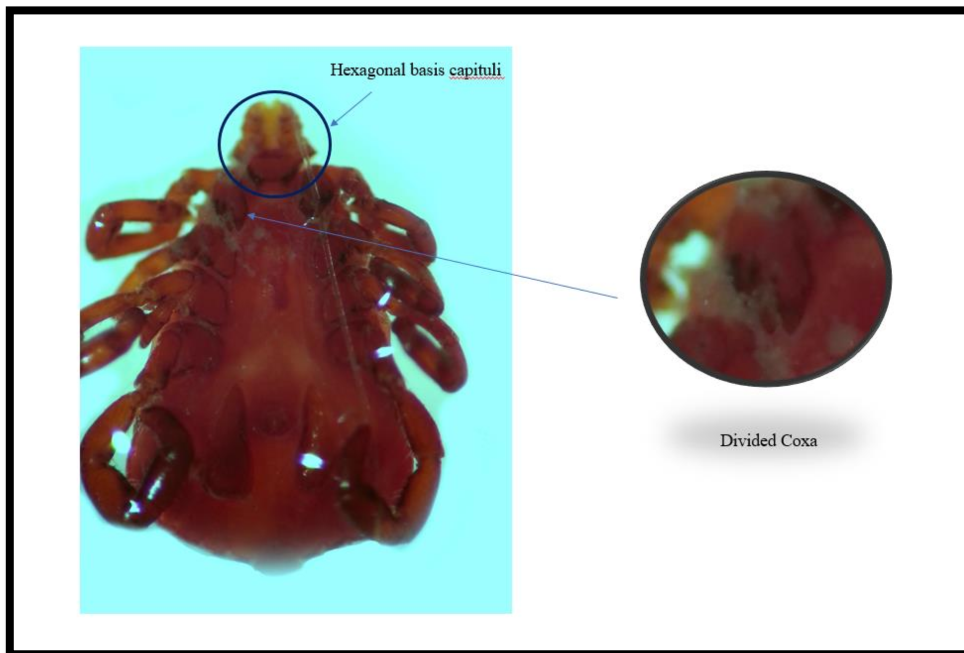

Figure 2.2: Ventral view of male *Rhipicephalus* tick, showing the divided coxa and the hexagonal basis capitula. (Photo taken by Abbas Khader).

Hyalomma genus was distinguished by dense punctuations, dense circumspiracular setae, and shape of the spiracular plate, see (Fig 2.3) for more *Hyalomma* features.

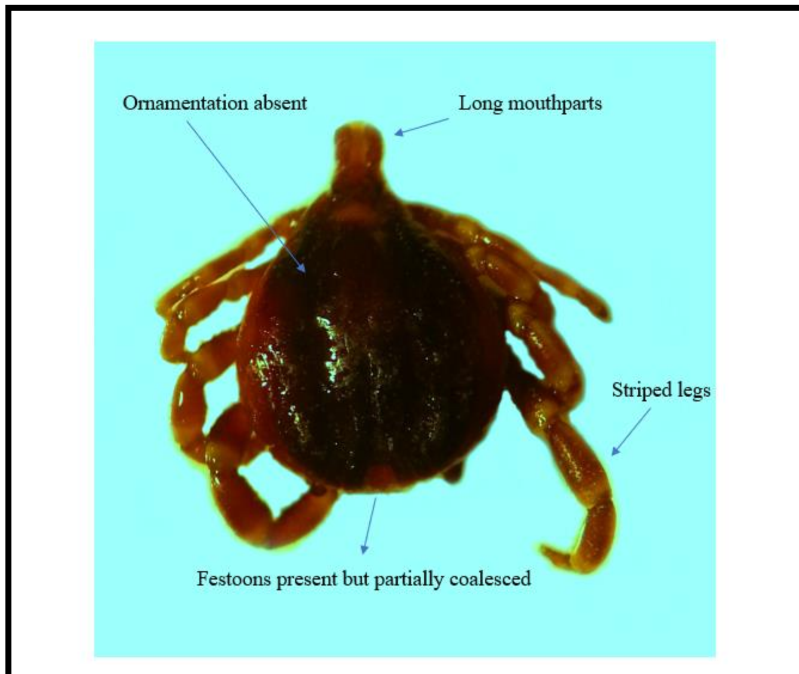

Figure 2.3: Morphological characteristics of *Hyalomma*: Dorsal view of male *Hyalomma dromedarii*. (Photo taken by Abbas Khader).

Small ticks with short mouthparts are a defining characteristic of the genus *Haemaphysalis*. The palps of certain species appear triangular because to lateral extending posterolateral angles on the second segment. Their rectangular foundation capituli, lack of adanal plates on the males, and absence of eyes and festoons in all stages allow them to be clearly identified from other genera that contain small tick species. Few species are commercially significant and infest domestic livestock (Alan A. *et al*. 2019).

For *Ornithodoros* species, they possess hypostomes that are well developed and very similar between males, females, and nymphal instars. The integument is comprised of both discs and mammilla which create various patterns that are continuous from dorsal to ventral surfaces, see (Fig 2.4).

Figure 2.4: *Ornithodoros tholozani* – Soft ticks. (Photo taken by Abbas Khader).

2.3 DNA Extraction

The DNA extraction was performed in a clean isolated area, all ticks were individually washed with phosphate-buffered saline (PBS) before extraction, air dried for 10 min on tissue paper and separately sliced into small pieces by a sterile scalpel blade then manually homogenized with a sterile micro pestle (Bhatia and Baersch, 2024; Ghodrati et al., 2024). The sliced sample was re-suspended in 180 µl of ATL buffer and 20 µl of proteinase K, then incubated at 56˚C overnight, then 200µl of AL binding buffer was added and incubated at 70°C for 10 min. followed by DNA extraction according to manufacturer's instructions using QIAamp animal blood and tissue Kit procedure (QIAGEN GmbH, Hilden, Germany). DNA concentration was measured by Nanodrop (Thermo Scientific NanoDrop 1000) and kept frozen at -20 until further use.

2.4 PCR and DNA Amplification

Molecular detection of Borrelia species in the collected samples was performed by polymerase chain reaction (PCR) using different sets of established primers (Table 2.1). This detection was run by amplifying partial fragments of flagellin gene (*fla B*). Flagellin gene was used as a screening gene for detecting *Borrelia* presence in the whole samples, then 16sRNA was used to confirm the positivity of positive samples to avoid the false diagnosis of *Borrelia* infection.

The PCR reactions were performed in a total volume of 25 µl using ready mix which contains 1 µM of each set of primers and 5 µl of the extracted DNA. All PCR Positive

samples were subjected to a second PCR using the primers Rec4-F and Rec9-R, designed to amplify a 523 bp fragment of the 16srRNA gene specific for *Borrelia* to confirm infection and rule out the possibility of double infection or risks of errors. The PCR amplification was set up within a 25 µl reaction mixture containing 5 µl of DNA template and 20 µl of master mix. After that, thermal cycler procedure included a denaturation step of 5 min at 95ºC, each of 35 cycles consisted of denaturation at 95ºC for 20s, annealing at 50ºC for 30s, extension at 72ºC for 2 min with a final extension step of 6 min at 72ºC. Nuclease-free water was used as negative control in each run. Confirmed positive samples were sent for DNA sequencing.

Table 2.1: Targeted genes and sequences of the oligonucleotide primers. Flagellin gene (Forward: Bor250, Reverse: Flagellin) and 16sRNA gene (Forward: Rec4, Reverse: Rec9).

Target Gene	Primer Name	Sequence 5` - 3`	Amplic on Size	Annealing Temp.	Reference
(16SrRNA)	Rec4 (F)	5'-ATGCTAGAAACTGCATGA-3'	552 bp	45 °C	Ras NM., et al. (1996); Rafinejad J., et al. (2011)
	Rec9 (R)	5'-TCGTCTGAGTCCCATCT-3'			
Flagellin gene	Bor 250 (F)	5`-GGAATGCAACCTGCAAAAAT-3`	250 bp	50 °C	Radulović, Ž. Et al. (2010)
	Flagelli n R	5`-GCATCAACAGCAGTTGTAACATT-3`			

2.5 Gel Electrophoresis

A 2% agarose gel (Agarose LE, Analytical gradient, Promega, Spain) was loaded with all PCR products. 2g of agarose was dissolved in 100 ml of 1X Tris-acetate EDTA buffer (TAE) (40 mM Tris acetate and 1 mM EDTA) to prepare the gel. After using a microwave to completely dissolve the agarose in an Erlenmeyer flask for approximately one minute, 3.5µl of 10 mg/ml (0.35µg/ml) of ethidium bromide was added for DNA staining. The gel tray in the casting chamber (Bio-Rad Laboratories Inc., USA) was filled with the gel. Onto the gel, 5 µl of PCR products were added. Each run used a 100 bp DNA marker ladder (Thermo Scientific Lithuania). For 45 minutes, the gel was operated at 100V. The gel images were captured using MiniLumi 1.4 gel 15 documentation system from (DNR Bio-Imaging Systems Ltd.).

2.6 DNA Sequence Analysis

After detecting the amplified samples by electrophoresis, the confirmed positive samples were confirmed by sequencing from both directions (forward and reverse). The obtained sequences were arranged and aligned using BioEdit sequence alignment editor software. Sequence analysis and arrangement was done by the Sequence Manipulation Suite which was used to generate reverse complement (RC) sequences, then multiple sequence alignment (http://multalin.toulouse.inra.fr/multalin/cgi-bin/multalin.pl) was used to obtain full sequences. Consequently, the NCBI BLAST software (https://blast.ncbi.nlm.nih.gov) (Fig. 2.6) was used for *Borrelia* genus and species identification, and then to compare the obtained DNA sequences in our study to the reference sequences deposited in the database.

2.7 Statistical Analysis

Statistical analysis for collected data was carried out using the IBM SPSS v27.0 software. Different variables were considered to be analyzed, the frequency of the collected ticks, by animal host, genus, developmental stage, and species, and the infected ticks.

2.8 Research Ethics Statement

The animals were spread around the West Bank, living on various farms. The project's objectives and the sampling procedure were explained orally to the animal owners prior to the ticks being sampled. Every owner verbally consented to the collection of ticks from their animals.

CHAPTER THREE

RESULTS

3.1 Ticks Sampling.

Our study was targeting both hard ticks and soft ticks, for hard ticks, a total of 734 ticks (*Ixodidae*) were collected from 6 different animal hosts residing in 10 different regions through the west bank of Palestine. 9 ticks (1.2%) from Salfeet, 15 ticks (2%) from Tulkarim, 26 ticks (3.5) from Jerusalem, 34 ticks (4.6%) from Hebron, 72 ticks (9.8%) from Jenin, 90 ticks (12.3%) from Nablus, 93 ticks (12.7%) from Tubas, 107 ticks (14.6%) from Ramallah, 127 ticks (17.3%) from Jericho, 161 hard ticks (21.9%) were obtained from Qalqilya.

For soft ticks (*argasidae*), a total of 117 ticks were collected from 3 different vicinities in west bank of Palestine, 8 ticks (6.8%) obtained from Hebron, 13 ticks (11.1%) from Nablus, and 96 ticks (82.1%) from Tubas.

Overall, 625 hard ticks were collected from dogs, 9 hard ticks were sampled from sheep, 18 hard ticks were from goat, 77 hard ticks were from camel, 4 hard ticks were from turtle, and 1 tick were collected from wolf (Table 3.1). While for soft ticks, 83 soft ticks were sampled from rock hyrax, 11 soft ticks were collected from fox, 12 soft ticks were from badger, 6 soft ticks were from rodents, 2 soft ticks were from dogs, and 3 soft ticks were gathered from porcupines (Table 3.2).

Table 3.1: Distribution of collected hard ticks by animal host.

Host	No. of collected ticks	%
Dog	625	85.1%
Sheep	9	1.2%
Goat	18	2.5%
Camel	77	10.5%
Turtle	4	0.5%
Wolf	1	0.1%
TOTAL	**734**	

Table 3.2: Distribution of collected soft ticks by animal host.

Host	No. of collected ticks	%
Rock Hyrax	83	70.9%
Fox	11	9.4%
Badger	12	10.3%
Rodents	6	5.1%
Dogs	2	1.7%
Porcupine	3	2.6%
TOTAL	**117**	

3.2 Ticks Classification.

Using a stereomicroscope (Dissecting microscope), the collected ticks were entirely isolated and identified based on morphological features and characteristics. However, the classification was at the levels of genus, species, and developmental stage. 90 hard ticks were identified as *Haemaphysalis*, 563 of the whole hard ticks were *Rhipicephalus*, and 81 of hard ticks were from *Hyalomma* genus. Moreover, one genus from soft ticks were identified, 117 soft ticks were *Ornithodoros (Pavlovskyella)* (Table 3.3).

Table 3.3: Distribution of identified ticks by genus.

Hard Ticks		
Genus	**No. of hard ticks**	**%**
Rhipicephalus	563	76.7%
Haemaphysalis	90	12.3%
Hyalomma	81	11.0%
Soft Ticks		
Genus	**No. of soft ticks**	**%**
Ornithodoros	117	100%

All hard ticks (n=734) were further identified up to the species level, the most prevalent species were *R. Sanguineus* (n= 521; 71.0%), and *R. Turanicus* (n= 31; 4.2%). Among *Haemephysalis*, there are *H. Parva* (n= 72; 9.8%) and H. adleri (n= 18; 2.5%). Additionally, *Hyalomma* were identified as *Hy. Dromedarii* (n= 68; 9.3%), *Hy. Impeltatum* (n=5; 0.7%), and *Hy. Aegyptium* (n= 4; 0.5%). However, there are 15 ticks of *Rhipicephalus* and *Hyalomma* ticks were identified only to the genus level designated *R. spp*. (n= 11) and *Hy. spp* (n=4). Among soft ticks (n=117), all ticks were *O.Tholozani* (n=117; 100%) (Table 3.4).

Table 3.4: Distribution of identified ticks by species.

Hard Ticks		
Species	**No. Of Hard Ticks**	**%**
H. Parva	72	9.8%
H. Adleri	18	2.5%
R. Sanguineus	521	71.0%
R. Turanicus	31	4.2%
Hy. Dromedarii	68	9.3%
Hy. Aegyptium	4	0.5%
Not identified	11 (*Rhipicephalus*)	2.0%

	4 (*Hyalomma*)	
Hy. Impeltatum	5	0.7%
Total	**734**	
Soft Ticks		
Species	**No. Of Soft Ticks**	**%**
O. Tholozani	117	100%

Both hard and soft ticks were microscopically identified by their developmental stage, three stages were detected, male, female, and nymph. From the hard ticks, male (n=368; 50.1%), female (n=298; 40.6%), and nymph (n= 63; 8.6%). There are five hard ticks (0.7%) which their developmental stage was not possible to be determined as they were partially damaged during handling. From soft ticks, male (n=11; 9.4%), female (n=26; 22.2%), and nymph (80; 68.4%) (Table 3.5).

Table 3.5: Distribution of identified ticks by their developmental stage.

Hard Ticks			**Soft Ticks**		
Dev. Stage	**No.**	**%**	**Dev. Stage**	**No.**	**%**
Male	368	50.1%	Male	11	9.4%
Female	298	40.6%	Female	26	22.2%
Nymph	63	8.6%	Nymph	80	68.4%
Unknown	5	0.7%	Unknown	0	0
Total	734		**Total**	117	

3.3 Amplified gene for *Borrelia* identification.

In order to identify the presence of *Borrelia* in our samples, we used to screen for the band of 250 bp (Positive) with negative control by targeting the gene *fla B* which encodes the flagellin polypeptide, primer was used to target a 250 bp sequence by Bor250 (F) 5`-GGAATGCAACCTGCAAAAAT-3` and flagellin (R) 5`-GCATCAACAGCAGTTGTAACATT-3`. A negative control was used in each PCR run. Positive samples were confirmed by targeting *16sRNA* gene using Rec4 (F) 5'-ATGCTAGAAACTGCATGA-3' and Rec9 (R) 5'-TCGTCTGAGTCCCATCT-3'.

3.4 Molecular detection of *Borrelia*.

All 734 hard tick samples and 117 soft tick samples were entirely screened for the presence of *borrelial* DNA using *fla B* – PCR and 16SrRNA gene to confirm the positive samples. The sample was considered positive if a fragment of (*fla B*) 250 bp and (*16srRNA*) 552 bp was detected on 2% agarose gel and (Fig. 3.3, Fig. 3.4).

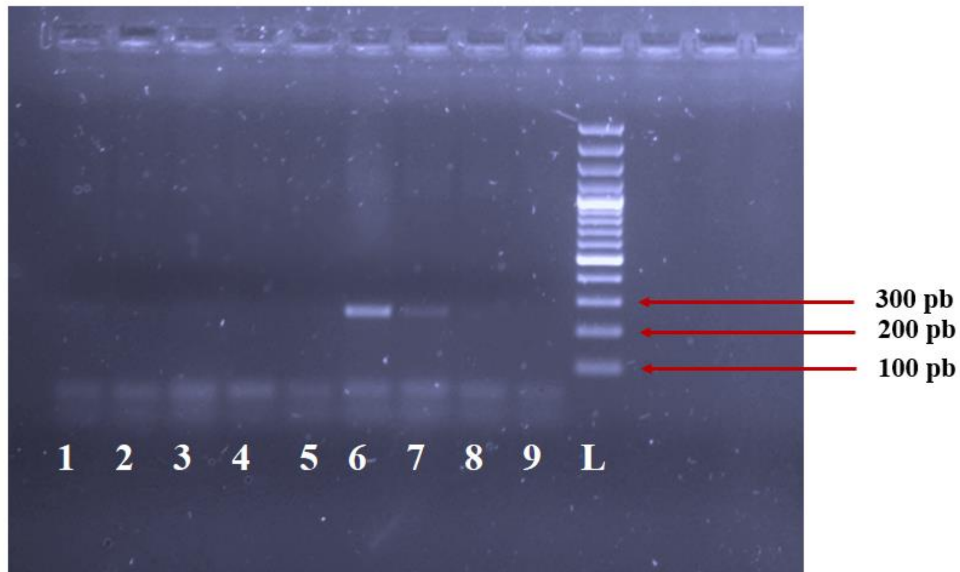

Figure 3.1: Molecular detection of *Borrelia* using *fla B* primers, a 250 bp for samples 6 and 7 were considered positives. 9 is negative control, L is a DNA ladder (marker).

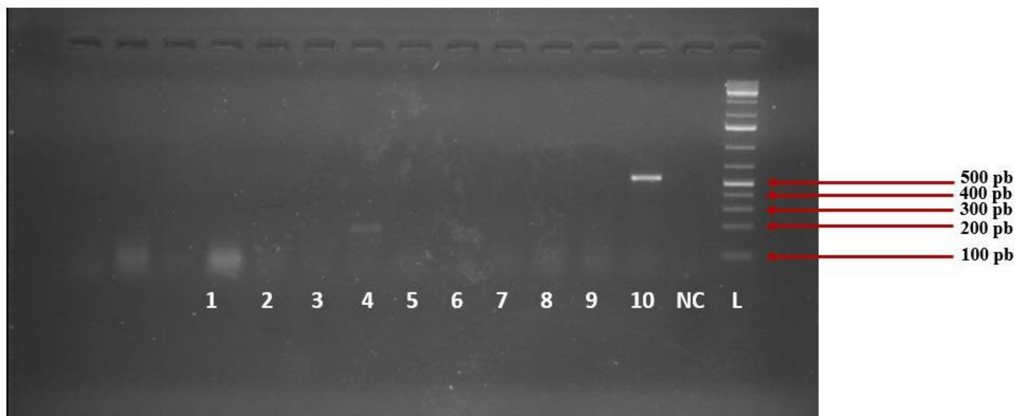

Figure 3.2: Molecular detection of *Borrelia* using 16sRNA primers, a 552 bp for sample 10 was confirmed positive (*B. Lonestari*). NC is negative control; L is a DNA

Among 734 tested hard ticks, 13 ticks (1.8%) were positives while 721 ticks (98.2%) were negative for *Borrelia*. Moreover, among 117 tested soft ticks, 9 ticks (7.7%) were

positive for *Borrelia*, while 108 ticks (92.3%) were negative. All positive samples were sequenced in order to verify the identity of the amplified products. Based on NCBI BLAST analysis for positive sequenced samples from both hard and soft ticks, among hard ticks, 10 ticks (76.9%) were infected with *Borrelia Persica*, 2 ticks (15.3%) were infected with *Borrelia turcica*, and 1 tick (7.6%) was infected with *Borrelia lonestari*. Moreover, among soft ticks all positive ticks (n=9 ticks, 100%) were found to be infected with *Borrelia persica* (Table 3.6).

Table 3.6: Distribution of identified *Borrelia species* ticks by their vector, host, and district.

Borrelia spp.	Vector tick specifications	Animal Host	District
HARD TICKS			
B. persica	*H. parva*, Female	Dog	Qalqilia
B. persica	*H. parva*, Male	Dog	Qalqilia
B. turcica	*Hy. eagyptium*, Male	Turtle	Tubas
B. turcica	*Hy. eagyptium*, Male	Turtle	Tubas
B. persica	*Rh. Sanguineus*, Male	Dog	Qalqilia
B. persica	*Rh. Sanguineus*, Female	Dog	Qalqilia
B. persica	*Rh. Sanguineus*, Male	Dog	Ramallah
B. persica	*Rh. Sanguineus*, Female	Dog	Ramallah
B. persica	*H. parva*, Male	Dog	Ramallah
B. persica	*Rh. Sanguineus*, Female	Dog	Ramallah
B. persica	*Rh. Sanguineus*, Nymph	Dog	Nablus
B. persica	*Rh. Sanguineus*, Male	Dog	Nablus
B. lonestari	*Rh. Sanguineus, unspecified*	Dog	Jerusalem

SOFT TICKS			
B. persica	*Ornithodoros tholozani,* Nymph	Rock Hyrax	Tubas
B. persica	*Ornithodoros tholozani,* Nymph	Rock Hyrax	Tubas
B. persica	*Ornithodoros tholozani,* Nymph	Rock Hyrax	Tubas
B. persica	*Ornithodoros tholozani,* Nymph	Rock Hyrax	Tubas
B. persica	*Ornithodoros tholozani,* Nymph	Rock Hyrax	Tubas
B. persica	*Ornithodoros tholozani,* Nymph	Rock Hyrax	Tubas
B. persica	*Ornithodoros tholozani,* Nymph	Rock Hyrax	Tubas
B. persica	*Ornithodoros tholozani,* Nymph	Rock Hyrax	Tubas
B. persica	*Ornithodoros tholozani,* Nymph	Rock Hyrax	Tubas

We examined the association between different host animals and the presence of a positive or negative *Borrelia* status across 734 cases. Among the hosts, dogs represent the majority, with 614 (85.2%) testing negative and 11 (84.6%) testing positive for *Borrelia*, accounting for 85.1% of all cases. Other host animals included sheep (1.2% negative, 0% positive), goats (2.5% negative, 0% positive), camels (10.7% negative, 0% positive), turtles (0.3% negative, 15.4% positive), and wolves (0.1% negative, 0% positive). A statistically significant correlation was found using the Pearson Chi-Square test between host type and *Borrelia* infection status, χ^2 (5, N = 734) = 55.37, p < .001. (Table 3.7).

Table 3.7: the association between host animals and the presence of *Borrelia* status

		BORRELIA				**Total**		**X²**	**P-value**
		NEGATIVE		**POSITIVE**					
		n	%	n	%	n	%		
HOST	DOG	624	99.8%	1	0.16%	625	85.1%	55.373	<0.001*
	SHEEP	9	1.2%	0	0.0%	9	1.2%		
	GAOT	18	2.5%	0	0.0%	18	2.5%		
	CAMEL	77	10.7%	0	0.0%	77	10.5%		
	TURTLE	2	0.3%	2	15.4%	4	0.5%		
	WOLF	1	0.1%	0	0.0%	1	0.1%		

*Significant at p-value <0.05.

3.5 Sequence Analysis.

To verify the nucleotide sequence identity, and to compare the sequences of our positive samples among hard and soft ticks to the reference sequences in gene bank, the sequences were aligned to each other. As noted in the multiple alignment figure (Fig 3.5), a 100% homology was noted between our *Borrelia Persica* sequence from hard tick and the reference sequence of flagellin gene.

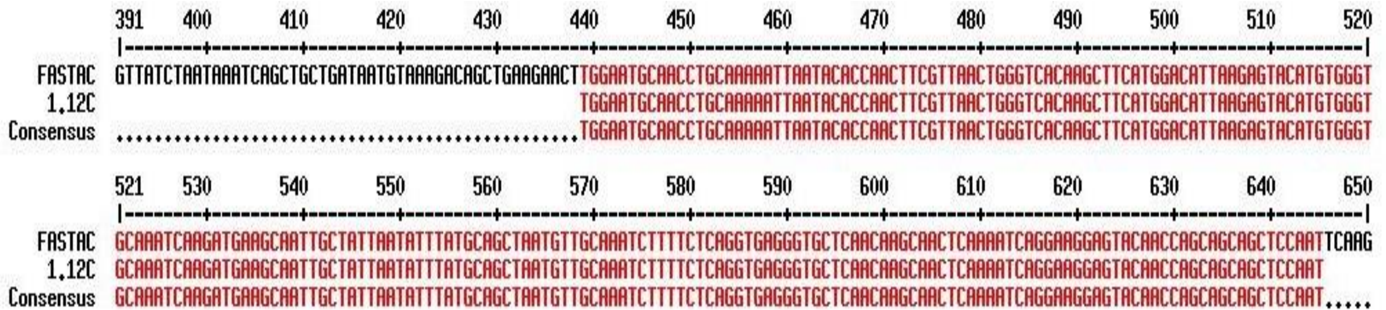

Figure 3.3: Multiple alignment of *Borrelia Persica* detected in hard tick and reference strain in the gene bank.

Regarding *B.turcica* which was also detected in the hard ticks, a multiple alignment was also performed between our sequence and the reference sequence in the gene bank, few single nucleotide polymorphism (SNP) was noted in the consensus sequence as shown in the figure below (Fig 3.6).

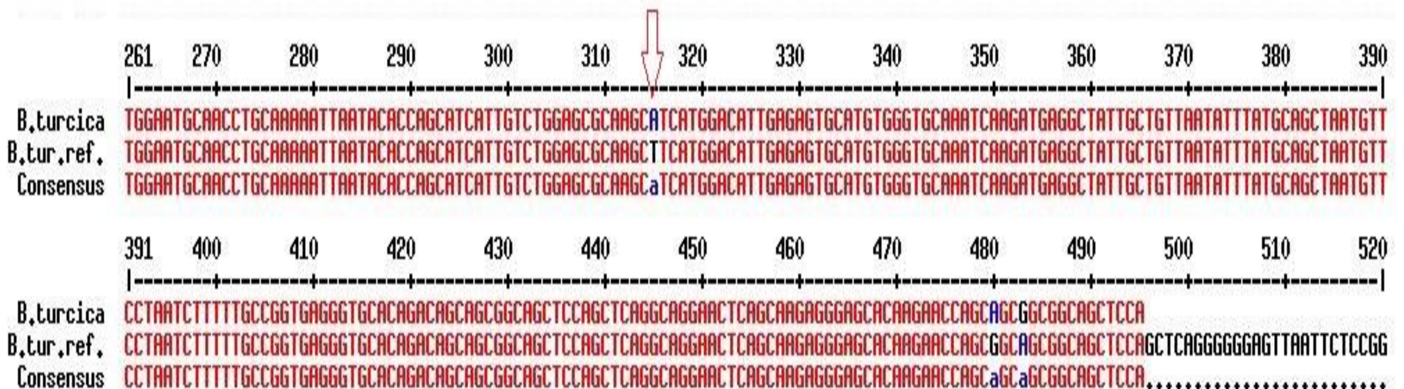

Figure 3.4: Multiple alignment of *Borrelia turcica* detected in hard tick and reference strain in the gene bank, the arrows refer to a few SNPs were noted.

Borrelia persica was detected in both hard and soft ticks, a sequence from both kinds were aligned, a 100% homology between two sequences was noted (Fig 3.7).

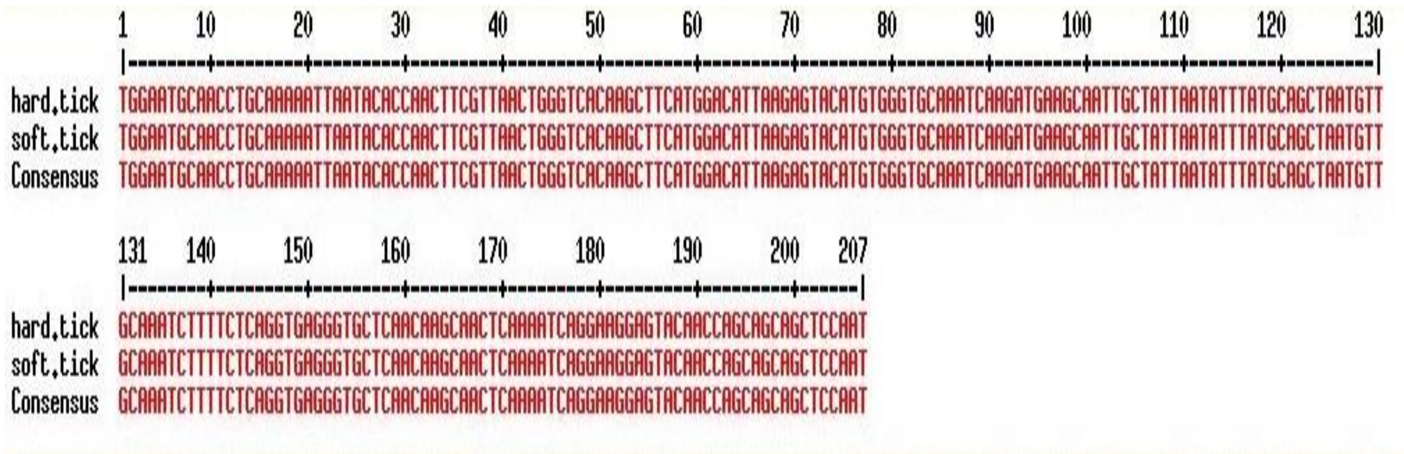

Figure 3.5: Multiple alignment of *B. persica* from hard ticks with *B. Persica* from soft ticks.

CHAPTER FOUR

DISCUSSION

Tick-borne borrelial diseases like Lyme borreliosis and relapsing fever are crucial issues which globally affect the health of both humans and different kinds of animals (Stachurski, F. *et al*. 2021). The symptoms of these diseases are usually similar to other diseases like influenza and are non-specific, which include neural, dermal, joint manifestations, and erythema migrans-like rash (Stanek, G. *et al.* 2018). In Palestine, each *Borrelia* species is transmitted within a geographical range by a specific species of soft ticks of the genus *Ornithodoros* (Argasidae) (Shwartz, D., *et al.,* 2023). Previous literature revealed the molecular characterization of Borrelia persica in the predominant soft ticks in Palestine (Shwartz, D., *et al.*, 2024). However, the molecular based data on borrelia species among hard ticks in Palestinian districts are scarce.

Unlike other bacteria, *borrelia* has a flagella which are inserted at each end of the protoplasmic cylinder and uniquely extend along its axis, overlapping each other in the middle. The flagella of *borreliae* are different from those of other spirochaetes which makes it distinguished for diagnostic uses, they are not surrounded by an outer sheath layer, and comprised of a single flagellin protein. In our study, we were able to define the association between borrelia as a causative agent of TBRF and its animal host, and molecularly characterize it among hard ticks and soft ticks by targeting the *fla B* that encodes flagellin protein. Notably, the studies on the characterization of borrelia vectored by hard ticks distributed in different Palestinian districts are still scant.

Regarding the soft ticks relapsing fever, it is well studied that *B. persica* is associated with the genus *Ornithodoros*, especially *O. tholozani* (Bankole, A. *et al*. 2023). However, the presence of *Borrelia persica* in hard ticks is unusual since it is predominantly associated with soft ticks like *Ornithodoros tholozani*. Accordingly, and in some cases, it could be possible for hard ticks to occasionally acquire and harbor *Borrelia* species under certain ecological or environmental circumstances. *Rhipicephalus* and *Heamaphysalis* from Hard ticks might act as an accidental host for *B. persica*, yet the common characteristics that make such these ticks as effective vectors is the behavior of actively seeking out hosts and the generality of host preference (Sakamoto, J. M. 2018). If these ticks feed on the same hosts as soft ticks infected with *B. persica*, they could acquire the spirochete during blood meals. This is particularly plausible in areas where the

distribution of soft and hard ticks' overlaps. As there are some studies focused on species interactions, tick-host niche overlap, and the percentage of the environmental niche where tick-borne illnesses may spread due to interactions and overlapping environmental characteristics (Estrada-Peña, A. *et al.* 2016). On the other hand, some tick species would rather spend their whole life cycle inside their hosts' nests or burrows. However, some species will quickly look for non-typical hosts if their chosen host is no longer available. When an unwary person comes into contact with a hungry tick, this can result in unexpected illness outbreaks. Accordingly, the changes in host availability, habitat disruption, or overlapping tick populations could lead to interspecies transmission. In our study, the detection of *borrelia persica* in hard ticks may be a sign of an accidental acquisition or adaptation that permits transmission. These results highlight the need for more studies on the ecology of *Borrelia* and tick vector competence to determine whether these hard ticks are capable vectors or just unintentional carriers. In this study, *B. persica* was detected in *H. parva* and *R. sanguineus* ticks both hosted by dogs. In this context, there are studies indicating that *H. parva* ticks can carry unusual *Borrelia* species. Notably, *Borrelia burgdorferi sensu stricto*, typically associated with Lyme disease and commonly found in *Ixodes* ticks, has been detected in *H. parva* (Orkun et al., 2014). This suggests that *H. parva* may serve as a vector for *Borrelia* species not typically associated with it. Additionally, research has identified *Borrelia miyamotoi*, a relapsing fever *Borrelia* species found in *Haemaphysalis inermis* ticks. While this finding pertains to a different *Haemaphysalis* species, it underscores the potential for *Haemaphysalis* ticks to harbor various *Borrelia* species (Heglasová et al., 2020).

Borrelia turcica, is a member of the reptile-associated *Borrelia* clade, was originally isolated from the hard tick *Hyalomma aegyptium* in Istanbul in the Northwestern Turkey and Greece (Sabrina Hepner et al. 2020). Reptiles are its main animal host but still not well identified all over the Mediterranean countries (Hepner, S., et al., 2019). In Palestine, this study identified that *borrelia turcica* is a member of an expanding Borrelia clade associated primarily with turtle and reptile hosts and vectored by hard ticks *Hyalomma aegyptium*, these finding are consistent with previous literature in middle east.

Borrelia lonestari was first detected in the lone star tick *Amblyomma ameicanum* hosted by rodents and birds, the detection of *B. lonestari* in patients with febrile illness demonstrates it's potential as a human pathogen (Barbour AG., at al. 1996; Burkot TR., et al. 2001). However, in this study we found a high sequence mimicry of a bacteria that is genetically very close to *B. lonestari* performed by multiple alignment (appendix C4). This bacterium was detected only in one tick of our collected samples and is vectored by *Rhipicephalus sanguineous* hosted by dog. We need further samples to be collected from the same area and further investigations to confirm the presence of this kind of borrelia clade in Palestine.

Borrelia persica is a borrelial species that can cause tick-borne relapsing fever in Palestine, some Mediterranean countries and Asia. Relapsing fever is associated with severe illness and potentially death in both humans and animals. Since *B. persica* infection has rarely been described in wild animals in Palestinian regions, one of our aims was to evaluate the prevalence of infection with *B. persica* among soft ticks in Palestine. Studies on *B. persica* in Palestine revealed that the prevalence of *B. persica* infection was unexpectedly high, suggesting that this

infection is widespread in some wild animal species in all regions of Palestine (Shwartz, D. et al. 2023). In this study, we had found that *Borrelia persica* is the main borrelia species found in soft ticks obtained from different animal hosts distributed in Palestine. Rock hyrax was the major animal host of the *B. persica* infected ticks. Moreover, our findings are consistent with the literature.

Tick-borne borreliosis is being a growing public health issue, it demands a multidisciplinary approach to be overcome. Public awareness, preventive measures, and improved diagnostic tools are essential to relieve its effects (Rochlin, I., & Toledo, A. 2020). Furthermore, ticks are highly adaptable to specific environmental conditions, but their survival depends on crucial factors such as temperature, humidity, and habitat availability. An imbalance in these factors, particularly due to climate changes or human activities, are changing the tick's survival and distribution. However, managing these factors is essential for preventing tick-borne borreliosis and controlling tick populations.

The crosstabulation examines the association between different host animals and the presence of a positive or negative *Borrelia* status across 734 cases. Among the hosts, dogs represent the majority, with 614 (85.2%) testing negative and 11 (84.6%) testing positive for borrelia, accounting for 85.1% of all cases. Other host animals included sheep (1.2% negative, 0% positive), goats (2.5% negative, 0% positive), camels (10.7% negative, 0% positive), turtles (0.3% negative, 15.4% positive), and wolves (0.1% negative, 0% positive). A Pearson Chi-Square test revealed a statistically significant relationship between host type and borrelia infection status, $\chi^2(5, N = 734) = 55.37, p < .001$. In summary, dogs were the predominant host, and the association between host type and infection status was statistically significant. However, the analysis was limited by the low expected counts in some categories, which may influence the robustness of the findings. In summary, dogs were the predominant host, and the association between host type and infection status was statistically significant. However, the analysis was limited by the low expected counts in some categories, which may influence the robustness of the findings.

4.1 Conclusion

I. For the first time, our study emphasizes the presence of B*orrelia persica*, *Borrelia turcica*, and *B. Lonestari* in hard ticks in Palestine.

II. Dogs represent the vast majority among animal hosts that harbor *Rhipicephalus sanguineous* in Palestine.

III. Our results are consensus with previous literature, we revealed that *Ornothodoros tholozani* is the predominant soft ticks distributed in Palestine and infected mainly with *borrelia persica*.

4.2 Recommendations

I. Further studies are recommended to reveal ecological overlaps and vector-host dynamics among ticks in Palestine.

II. Increase the level of education and awareness of ticks and tick -borne diseases among farmers and veterinarians in Palestine.

III. Larger samples to confirm the distribution of *B. lonestari* in Palestine.

4.3 Study Limitations

I. Number of animal hosts among both soft and hard ticks, and limited understanding of host behavior. broader range of hosts should be considered in future studies, because understanding the full host spectrum reveals how ticks interact with the ecosystem and adapt to environmental changes. This will help to refine control strategies, and decrease the risks of tick-borne diseases to public health.

II. Some of our samples were collected in winter (January), a period when ticks enter in their dormant state to conserve energy and survive cold temperatures. The pathogen prevalence in ticks collected during winter may differ from other seasons, offering insights into the year-round dynamics of tick-borne borreliosis.

III. The number of diseased animals which were used to collect ticks from was limited. Because diseased animals often serve as reservoirs or amplifiers of tick-borne borreliosis making them critical targets for ick sampling and *Borrelia* surveillance.

IV. Not all geographic areas in Palestine were covered in collection process. This is essential for better understanding regional tick dynamics, ecology and reducing the risks of tick-borne borreliosis in Palestinian regions.

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Appendix A: Representative sequences of *Borrelia* and it’s species among hard ticks (*Ixodidae*).

Sequence A1: *Borrelia persica* isolate flagellin (*flaB*) gene, partial cds identified on BLAST.

>1.12 C
TGGAATGCAACCTGCAAAAATTAATACACCAACTTCGTTAACTGGGTCACAAGC
TTCATGGACATTAAGAGTACATGTGGGTGCAAATCAAGATGAAGCAATTGCTAT
TAATATTTATGCAGCTAATGTTGCAAATCTTTTCTCAGGTGAGGGTGCTCAACAA
GCAACTCAAAATCAGGAAGGAGTACAACCAGCAGCAGCTCCAAT

Sequence A2: *Borrelia persica* isolate flagellin (*flaB*) gene, partial cds identified on BLAST.

>1.12D
TGGAATGCAACCTGCAAAAATTAATACACCAACTTCGTTAACTGGGTCAC*AAGCT*
*TC*ATGGACATTAAGAGTACATGTGGGTGCAAATCAAGATGAAGCAATTGCTATT
AATATTTATGCAGCTAATGTTGCAAATCTTTTCTCAGGTGAGGGTGCTCAACAAG
CAACTCAAAATCAGGAAGGAGTACAACCAGCAGCAGCTCCAATTCAA

Sequence A3: *Borrelia turcica* flagellin (*flaB*) gene, partial cds, strain IST4, identified on BLAST.

>2.12A
TGGAATGCAACCTGCAAAAATTAATACACCAGCATCATTGTCTGGAGCGCAAGC
ATCATGGACATTGAGAGTGCATGTGGGTGCAAATCAAGATGAGGCTATTGCTGT
TAATATTTATGCAGCTAATGTTCCTAATCTTTTTGCCGGTGAGGGTGCACAGACA
GCAGCGGCAGCTCCAGCTCAGGCAGGAACTCAGCAAGAGGGAGCACAAGAACC
AGCAGCGGCGGCAG*C*TCCA

Sequence A4: *Borrelia turcica* flagellin (*flaB*) gene, partial cds, strain IST4, identified on BLAST.

>2.12C
TGGAATGCAACCTGCAAAAATTAATACACCAGCATCATTGTCTGGAGCGCAAGC
ATCATGGACATTGAGAGTGCATGTGGGTGCAAATCAAGATGAGGCTATTGCTGT
TAATATTTATGCAGCTAATGTTCCTAATCTTTTTGCCGGTGAGGGTGCACAGACA
GCAGCGGCAGCTCCAGCTCAGGCAGGAACTCAGCAAGAGGGAGCACAAGAACC
AGCAGCGGCGGCAG*C*TCCA

Sequence A5: *Borrelia persica* isolate flagellin (*flaB*) gene, partial cds identified on BLAST.

>4.13C
TGGAATGCAACCTGCAAAAATTAATACACCAACTTCGTTAACTGGGTCACAAGC
TTCATGGACATTAAGAGTACATGTGGGTGCAAATCAAGATGAAGCAATTGCTAT
TAATATTTATGCAGCTAATGTTGCAAATCTTTTCTCAGGTGAGGGTGCTCAACAA
GCAACTCAAAATCAGGAAGGAGTACAACCAGCAGCAGCTCCAAT

Sequence A6: *Borrelia persica* isolate flagellin (*flaB*) gene, partial cds identified on BLAST.

>4.17A
TGGAATGCAACCTGCAAAAATTAATACACCAACTTCGTTAACTGGGTCACAAGC
TTCATGGACATTAAGAGTACATGTGGGTGCAAATCAAGATGAAGCAATTGCTAT
TAATATTTATGCAGCTAATGTTGCAAATCTTTTCTCAGGTGAGGGTGCTCAACAA
GCAACTCAAAATCAGGAAGGAGTACAACCAGCAGCAGCTCCA

Sequence A7: *Borrelia persica* isolate flagellin (*flaB*) gene, partial cds identified on BLAST.

>5.4C
TGGAATGCAACCTGCAAAAATTAATACACCAACTTCGTTAACTGGGTCACAAGC
TTCATGGACATTAAGAGTACATGTGGGTGCAAATCAAGATGAAGCAATTGCTAT
TAATATTTATGCAGCTAATGTTGCAAATCTTTTCTCAGGTGAGGGTGCTCAACAA
GCAACTCAAAATCAGGAAGGAGTACAACCAGCAGCAG

Sequence A8: *Borrelia persica* isolate flagellin (*flaB*) gene, partial cds identified on BLAST.

>5.7A
ATTGGAGCTGCTGCTGGTTGTACTCCTTCCTGATTTTGAGTTGCTTGTTGAGCACC
CTCACCTGAGAAAAGATTTGCAACATTAGCTGCATAAATATTAATAGCAATTGCT
TCATCTTGATTTGCACCCACATGTACTCTTAATGTCCATGAAGCTTGTGACCCAGTTA
ACGAAGTTGGTGTATTAATTTTTGCAGGTTGCATTCCA

Sequence A9: *Borrelia persica* isolate flagellin (*flaB*) gene, partial cds identified on BLAST.

>5.8A
TGGAATGCAACCTGCAAAAATTAATACACCAACTTCGTTAACTGGGTCACAAGC
TTCATGGACATTAAGAGTACATGTGGGT.GCAAATCAAGATGAAGCAATTGCTAT
TAATATTTATGCAGCTAATGTTGCAAATCTTTTCTCAGGTGAGGGTGCTCAACAA
GCAACTCAAAATCAGGAAGGAGTACAACCAGCAGCAGCTCCAAT

Sequence A10: *Borrelia persica* isolate flagellin (*flaB*) gene, partial cds identified on BLAST.

>5.15A
TGGAATGCAACCTGCAAAAATTAATACACCAACTTCGTTAACTGGGTCACAAGC
TTCATGGACATTAAGAGTACATGTGGGTGCAAATCAAGATGAAGCAATTGCTATT
AATATTTATGCAGCTAATGTTGCAAATCTTTTCTCAGGTGAGGGTGCTCAACAAG
CAACTCAAAATCAGGAAGGAGTACAACCAGCAGCAG

Sequence A11: *Borrelia Lonstari* isolate flagellin (*flaB*) gene, partial cds identified on BLAST.

>A2-7
GAACTAACGCTGGCAGTGCGTCTTAAGCATGCAAGTCAAACGGAATGTAGCAAT
ACATTCAGTGGCGAACGGGTGAGTAACGCGTGGATAATCTACCTACGAGATGGG
GATAACTATTAGAAATGGTAGCTAATACCGAATAAAGTCAATTGAGGTGTCAAT
TGATGAAATGAAGCCTTTAAAGCTTCGCTTGTAGATGAGTCTGCGTCTTATTAGC
TAGTTGGTAGGGTAAGAGCCTACCAAGGCTATGATAAGTAACCGGCCTGAGAGG
GTGATCGGTCACACTGGAACTGAGATACGGTCCAGACTCCTACGGGAGGCAGCA
GCTAAGAATCTTCCGCAATGGGCGAAAGCCTGACGGAGCGACACTGCGTGAACG
AAGAAGGTCGAAAGATTGTAAAGTTCTTTTATAAATGAGGAATAAGTTTTGTAG
GAAATGACAAAGTGATGACGTTAGTTTATGAATAAGCCCCGGCTAATTACGTGC
CAGCAGCCGCGGTAATACGTAAGG

Sequence A12: *Borrelia persica* isolate flagellin (*flaB*) gene, partial cds identified on BLAST.

>8.1C
TGGAATGCAACCTGCAAAAATTAATACACCAACTTCGTTAACTGGGTCACAAGC
TTCATGGACATTAAGAGTACATGTGGGTGCAAATCAAGATGAAGCAATTGCTATT
AATATTTATGCTCTCAGGTGAGGGTGCTCAACAAGCAACTCAAAATCAGGAAGG
AGTACAACCAGCAGCAGCTCCAATTCA

Sequence A13: *Borrelia persica* isolate flagellin (*flaB*) gene, partial cds identified on BLAST.

>14.10B
TGGAATGCAACCTGCAAAAATTAATACACCAACTTCGTTAACTGGGTCACAAGC
TTCATGGACATTAAGAGTACATGTGGGTGCAAATCAAGATGAAGCAATTGCTATT
AATATTTATGCAGCTAATGTTGCAAATCTTTTCTCAGGTGAGGGTGCTCAACAAG
CAACTCAAAATCAGGAAGGAGTACAACCAGCAGCAGCTCCA

Appendix B: Representative sequences of *Borrelia* and it's species among soft ticks (*argasidae*).

Sequence B1: *Borrelia persica* isolate flagellin (*flaB*) gene, partial cds identified on BLAST.

>5.5.75
TGGAATGCAACCTGCAAAAATTAATACACCAACTTCGTTAACTGGGTCACAAGC
TTCATGGACATTAAGAGTACATGTGGGTGCAAATCAAGATGAAGCAATTGCTAT
TAATATTTATGCAGCTAATGTTGCAAATCTTTTCTCAGGTGAGGGTGCTCAACAA
GCAACTCAAAATCAGGAAGGAGTACAACCAGCAGCAGCTCCAAT

Sequence B2: *Borrelia persica* isolate flagellin (*flaB*) gene, partial cds identified on BLAST.

>5.5.91
TGGAATGCAACCTGCAAAAATTAATACACCAACTTCGTTAACTGGGTCACAAGC
TTCATGGACATTAAGA*A*GTACATGTGGGTGCAAATCAAGATGAAGCAATTGCTA
TTAATATTTATGCAGCTAATGTTGCAAATCTTTTCTCAGGTGAGGGTGCTCAACA
AGCAACTCAAAATCAGGAAGGAGTACAACCAGCAGCAGCTCCAAT

Sequence B3: *Borrelia persica* isolate flagellin (*flaB*) gene, partial cds identified on BLAST.

>5.5.103

TGGAATGCAACCTGCAAAAATTAATACACCAACTTCGTTAACTGGGTCACAAGC
TTCATGGACATTAAGA*A*GTACATGTGGGTGCAAATCAAGATGAAGCAATTGCTA
TTAATATTTATGC*C*AGCTAATGTTGCAAATCTTTTCTCAGGTGAGGGTGCTCAAC
AAGCAACTCAAAATCAGGAAGGAGTACAACCAGCAGCAGCTCCAAT

Sequence B4: *Borrelia persica* isolate flagellin (*flaB*) gene, partial cds identified on BLAST.
>5.5.95
TGGAATGCAACCTGCAAAAATTAATACACCAACTTCGTTAACTGGGTCACAAGC
TTCATGGACATTAAGAGTACATGTGGGTGCAAATCAAGATGAAGCAATTGCTAT
TAATATTTATGCAGCTAATGTTGCAAATCTTTTCTCAGGTGAGGGTGCTCAACAA
GCAACTCAAAATCAGGAAGGAGTACAACCAGCAGCAGCTCCAA

Sequence B5: *Borrelia persica* isolate flagellin (*flaB*) gene, partial cds identified on BLAST.

>5.5.94
TGGAATGCAACCTGCAAAAATTAATACACCAACTTCGTTAACTGGGTCACAAGC
TTCATGGACATTAAG*C*AGTACATGTGGGTGCAAATCAAGATGAAGCAATTGCTA
TTAATATTTATGCAGCTAATGTTGCAAATCTTTTCTCAGGTGAGGGTGCTCAACA
AGCAACTCAAAATCAGGAAGGAGTACAACCAGCAGCAGCTCCAAT

Sequence B6: *Borrelia persica* isolate flagellin (*flaB*) gene, partial cds identified on BLAST.

>5.5.106
TGGAATGCAACCTGCAAAAATTAATACACCAACTTCGTTAACTGGGTCACAAGC
TTCATGGACATTAAGAAGTACATGTGGGTGCAAATCAAGATGAAGCAATTGCTA
TTAATATTTATGCAGCTAATGTTGCAAATCTTTTCTCAGGTGAGGGTGCTCAACA
AGCAACTCAAAATCAGGAAGGAGTACAACCAGCAGCAGCTCCAA

Sequence B7: *Borrelia persica* isolate flagellin (*flaB*) gene, partial cds identified on BLAST.

>5.5.69
ATTGGAGCTGCTGCTGGTTGTACTCCTTCCTGATTCATTGAGTTGCTTGTTGAGCACC
CTCACTTGCTGAGAAAAGATTTGCAACATTAGCTGCATAAATATTAATAGCAATTGCTT
CATCTTGATTTGCACCCACATGTACTCTTAATGTCCATGAAGCTTGTGACCCAGTTAA
CGAAGTTGGTGTATTAATTTTTGCAGGTTGCATTCCA

Sequence B8: *Borrelia persica* isolate flagellin (*flaB*) gene, partial cds identified on BLAST.

>5.5.88
TGGAATGCAACCTGCAAAAATTAATACACCAACTTCGTTAACTGGGTCACAAGC
TTCATGGACATTAAGAAGTACATGTGGGTGCAAATCAAGATGAAGCAATTGCTA
TTAATATTTATGCAGCTAATGTTGCAAATCTTTTCTCAGGTGAGGGTGCTCAACA
AGCAACTCAAAATCAGGAAGGAGTACAACCAGCAGCAGCTCCAAT

Sequence B9: *Borrelia persica* isolate flagellin (*flaB*) gene, partial cds identified on BLAST.

>5.5.84
TGGAATGCAACCTGCAAAAATTAATACACCAACTTCGTTAACTGGGTCACAAGC
TTCATGGACATTAAGAAGTACATGTGGGTGCAAATCAAGATGAAGCAATTGCTA
TTAATATTTATGCAGCTAATGTTGCAAATCTTTTCTCAGGTGAGGGTGCTCAACA
AGCAACTCAAAATCAGGAAGGAGTACAACCAGCAGCAGCTCCAAT

Appendix C: BLAST analysis of flagellin (*fla B*) gene sequences obtained in this study compared with reference DNA sequences deposited in the Gene Bank.

C1: BLAST of *Borrelia persica fla B* against reference strains sequences in the gene bank. Sequences producing significant alignment. (Bacteria, coverage and identity are marked with red).

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident
Borrelia persica isolate CBkc7 flagellin (flaB) gene, partial cds	Borrelia persica	1386	1386	100%	0.0	100.00%
Borrelia persica isolate C1015B flagellin (flaB) gene, partial cds	Borrelia persica	1380	1380	100%	0.0	99.87%
Borrelia persica isolate FL1 flagellin (flaB) gene, partial cds	Borrelia persica	1375	1375	100%	0.0	99.73%
Borrelia persica isolate TGd1 flagellin (flaB) gene, partial cds	Borrelia persica	1352	1352	97%	0.0	100.00%
Borrelia persica strain H1370 flagellin (flaB) gene, partial cds	Borrelia persica	1349	1349	97%	0.0	100.00%
Borrelia persica strain H1369 flagellin (flaB) gene, partial cds	Borrelia persica	1321	1321	97%	0.0	99.18%
Borrelia persica strain H1042 flagellin (flaB) gene, partial cds	Borrelia persica	1317	1317	97%	0.0	99.18%
Borrelia persica isolate HB4 flagellin (flaB) gene, partial cds	Borrelia persica	1312	1312	96%	0.0	99.18%
Borrelia persica isolate TG52 flagellin (flaB) gene, partial cds	Borrelia persica	1310	1310	96%	0.0	99.18%
Borrelia persica clone Bg233n flagellin (flaB) gene, partial cds	Borrelia persica	1306	1306	94%	0.0	100.00%

C2: BLAST of *Borrelia turcica fla B* against reference strains sequences in the gene bank. Sequences producing significant alignment. (Bacteria, coverage and identity are marked with red).

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident
Borrelia turcica flaB gene for flagellin, partial cds, strain: IST4	Borrelia turcica	418	418	100%	2e-112	98.72%
Borrelia turcica isolate HAE90 flagellin B gene, partial cds	Borrelia turcica	412	412	100%	8e-111	98.30%
Borrelia turcica IST7 chromosome, complete genome	Borrelia turcica IST7	412	412	100%	8e-111	98.30%
Borrelia turcica flaB gene for flagellin, partial cds, strain: IST7	Borrelia turcica IST7	412	412	100%	8e-111	98.30%
Borrelia turcica IST7 flagellin gene, partial cds	Borrelia turcica IST7	412	412	100%	8e-111	98.30%
Borrelia turcica isolate SW236-12 flagellin gene, partial cds	Borrelia turcica	412	412	100%	8e-111	98.30%
Borrelia turcica strain 33NM1854-LC flagellin gene, partial cds	Borrelia turcica	412	412	100%	8e-111	98.30%
Borrelia turcica flaB gene for flagellin, partial cds, strain: IST2	Borrelia turcica	412	412	100%	8e-111	98.30%
Borrelia turcica flaB gene for flagellin, partial cds, strain: ISTF2	Borrelia turcica	412	412	100%	8e-111	98.30%

C3: BLAST of *Borrelia lonestari fla B* against reference strains sequences in the gene bank. Sequences producing significant alignment.

Borrelia lonestari isolate MO2002-V2 flagellin (fla) gene, complete cds	409	409	100%	1e-110	95%	AY850064.1
Borrelia lonestari flagellin gene, complete cds	409	409	100%	1e-110	95%	AY166716.1
Borrelia lonestari isolate 022072 flagellin gene, partial cds	409	409	100%	1e-110	95%	AY237713.1
Borrelia lonestari isolate 021937 flagellin gene, partial cds	409	409	100%	1e-110	95%	AY237711.1
Borrelia lonestari isolate 021935 flagellin gene, partial cds	409	409	100%	1e-110	95%	AY237710.1
Borrelia lonestari isolate 021888 flagellin gene, partial cds	409	409	100%	1e-110	95%	AY237709.1
Borrelia lonestari isolate 021853 flagellin gene, partial cds	409	409	100%	1e-110	95%	AY237708.1
Borrelia lonestari isolate 021477 flagellin gene, partial cds	409	409	100%	1e-110	95%	AY237704.1
Borrelia lonestari isolate 020772 flagellin gene, partial cds	409	409	100%	1e-110	95%	AY237700.1
Borrelia lonestari isolate 020589 flagellin gene, partial cds	409	409	100%	1e-110	95%	AY237698.1
Borrelia lonestari isolate 020119 flagellin gene, partial cds	409	409	100%	1e-110	95%	AY237697.1
Borrelia lonestari isolate 012522 flagellin gene, partial cds	409	409	100%	1e-110	95%	AY237694.1
Borrelia lonestari isolate 011323 flagellin gene, partial cds	409	409	100%	1e-110	95%	AY237687.1
Borrelia lonestari isolate 011292 flagellin gene, partial cds	409	409	100%	1e-110	95%	AY237686.1
Borrelia lonestari isolate 011235 flagellin gene, partial cds	409	409	100%	1e-110	95%	AY237685.1
Borrelia lonestari isolate 011166 flagellin gene, partial cds	409	409	100%	1e-110	95%	AY237684.1

C4: Multiple alignment of *Borrelia lonstari with reference sequence and B. Persica sequence.*

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             1       10        20        30        40        50        60        70        80        90        100       110       120       130
             |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
  B.persica  TGGAATGCAACCTGCAAAAATTAATACACCAACTTCATTAACTGGGTCACAAGCTTCATGGACATTAAGAGTACATGTGGGTGCAAATCAAGATGAAGCAATTGCTATTAATATTTATGCAGCTAATGTT
B.lonestari  TGGAATGCAACCTGCAAAAATTAATACACCAGCATCACTAACTGGAGCACAAGCTTCATGGACATTGAGAGTTCAGGTAGGTGCAAATCAGGATGAAGCAATTGCTGTTAATATTTTCTCAACTAATGTT
 A27FlaFull  TGGAATGCAACCTGCAAAAATTAACACACCAGCATCACTAACTGGAGCACAAGCTTCATGGACATTAAGAGTTCAAGTGGGTGCAAATCAGGATGAAGCAATTGCAGTTAATATTTTTGCAACTAATGTT
1.3kFlaFull  TGGAATGCAACCTGCAAAAATTAATACACCAGCATCACTAACTGGAGCACAAGCTTCATGGACATTAAGAGTTCAAGTGGGTGCAAATCAGGATGAAGCAATTGCAGTTAATATTTTTGCAACTAATGTT
  Consensus  TGGAATGCAACCTGCAAAAATTAAtACACCAgCaTCAcTAACTGGagCACAAGCTTCATGGACATTaAGAGTtCA.GTgGGTGCAAATCAgGATGAAGCAATTGCtgTTAATATTTttgCAaCTAATGTT

             131     140       150       160       170       180       190       200       210       220       230       240       250253
             |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+--|
  B.persica  GCAAATCTTTTTTCAGGTGAGGGTGCTCAACAAGCAACTCAAAATCAGGAAGGAGTACAACCAGCAG---------CAGCTCCAATTCAAGGTGGAATTAATTCTCCAGTTAATGTTACAACT
B.lonestari  GCAAATCTTTTTGGTGGAGAAGGTGTTCAA---GCGGCTCCAGCTCAAGAGGGTGCACAACAGGAAGGAGTTCAACCAGCTCCAGCTCAAGGTGGGATTAGCTCTCCAATTAATGTTACAACT
 A27FlaFull  GCAAATCTTTTTGGTGGAGAAGGTGTTCAA---GCAGCTCCAGCTCAAGAGGGTGCACAACAGGAAGGAGTTCAACCAGCTCTAGCTCAAGGTGGAGTTAGCTCTCCTATTAATGTTACAACT
1.3kFlaFull  GCAAATCTTTTTGGTGGAGAAGGTGTTCAA---GCAGCTCCAGCTCAAGAGGGTGCACAACAGGAAGGAGTTCAACCAGCTCTAGCTCAAGGTGGAGTTAGCTCTCCTATTAATGTTACAACT
  Consensus  GCAAATCTTTTTggtGGaGAaGGTGtTCAA...GCagCTCcAgcTCAaGAgGGtGcACAACagGaAGgagttcaacCAGCTCcAgcTCAAGGTGGaaTTAgcTCTCCaaTTAATGTTACAACT
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التوصيف الجزيئي لبكتيريا البوريليا كمسبب للحمى الانتكاسية المنقولة بالقراد في القراد الصلب والناعم في فلسطين

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الملخص

المقدمة: الحمى الانتكاسية المنقولة بالقراد (TBRF) تُسببها بشكل أساسي عدة أنواع من بكتيريا البوريليا (*Borrelia*) التي تنتقل عبر لدغات القراد. تتميز هذه الحمى بشكل رئيسي بتكرار ظهور علامات وأعراض غير محددة، بما في ذلك الحمى، الصداع، آلام العضلات (myalgia)، آلام المفاصل (arthralgia)، وأحياناً تؤدي لظهور بعض المضاعفات العصبية. تنتقل أنواع بكتيريا البوريليا *Borrelia* في الغالب عن طريق القراد الصلب مثل قراد ريبيسيفالوس *Rhipicephalus*، والقراد الناعم مثل أورنيثودوروس *Ornithodoros*، الذي يُعرف بأنه الناقل الرئيسي لبكتيريا البيرسيكا *B. persica* في فلسطين. من الملاحَظ أن البيانات الجزيئية الجينية المتعلقة بأنواع بكتيريا البوريليا *Borrelia* والحيوانات التي تحتضنها في فلسطين نادرة جدًا، مع ملاحظة أن بوريليا البيرسيكا *B. persica* تمت دراسته مسبقاً في فلسطين.

أهداف الدراسة: هدفت هذه الدراسة إلى دراسة التوصيف الجزيئي لأنواع البوريليا *Borrelia* في القراد الصلب والناعم الذي يتطفل على بعض الحيوانات، حيث تم جمع هذا القراد من حيوانات مختلفة منتشرة في بعض المناطق الفلسطينية.

المنهجية: تم جمع عينات القراد من حيوانات مختلفة تقيم في مناطق متعددة في فلسطين، وتم تحديد جميع عينات القراد على مستوى الجنس والنوع اعتماداً على معايير تصنيفية محددة مسبقاً. تم استخراج الحمض النووي (DNA) باستخدام إجراءات مخبرية خاصة. تم فحص وجود الحمض النووي لبكتيريا البوريليا *Borrelia* بواسطة تفاعل البلمرة المتسلسل (PCR) مستهدفًا جين flagellin (*fla B*). وتم تأكيد جميع العينات الإيجابية بواسطة تفاعل البلمرة المتسلسل لجين *16 sRNA*. تم تحميل منتجات PCR على الترحيل الكهربائي عبر الأجاروز للكشف عن الاشارة التشخيصية عند حجم 250 زوجًا قاعديًا لجين *fla B* و523 زوجًا قاعديًا لجين *16 sRNA*. تمت دراسة التسلسل الجيني للعينات الإيجابية وتحليلها لتحديد نوع البوريليا باستخدام البنك الجيني BLAST. وتم إجراء التحليل الإحصائي للنتائج باستخدام برنامج IBM SPSS v27.0.

النتائج: من بين جميع عينات القراد والتي بلغت 851 عينة، تم تحديد 117 عينة (14%) من عائلة القراد الناعم (*Argasidae*). و734 عينة (86%) على أنها من عائلة القراد الصلب (*Ixodidae*) ، بشكل عام، كان 76% من القراد الصلب ينتمي إلى جنس ريبيسيفالوس *Rhipicephalus* (92% من نوع سانجوينوس *R. sanguineus* و5.5% من نوع تورانيكوس *R. turanicus*)، و12% ينتمي إلى قراد من مجموعة هيميفيساليس *Haemaphysalis* (80% من نوع بارفا *H. parva* و20% من نوع أدليري *H. adleri*)، و11% ينتمي إلى مجموعة قراد هيالوما *Hyalomma* (84% من نوع دروميداري *H. dromedarii*، و6% من نوع إمبيلتيتم *H. impeltatum*، و5% من نوع ايجيبتيوم *H. aegyptium*). أما جميع عينات القراد الناعم (117 عينة) فتم تحديدها على أنها من نوع أورنيثودوروس ثولوزاني *Ornithodoros tholozani*. من بين 734 عينة قراد صلب، تم الكشف عن الحمض النووي لبكتيريا البوريليا في 2% منها (يعادل 13 عينة) باستخدام تقنية PCR. وكشفت تحليلات التسلسل عن وجود بوريليا بيرسيكا *B. persica* في 77% (10 عينات) من العينات الإيجابية، بينما كانت 15% (عينة 2) بوريليا تورسيكا *B. turcicae*، و8% (عينة 1) بوريليا لونستاري *B. lonestari*. من جهة أخرى، تم العثور على 9% من القراد الناعم (17 عينة) تحتوي على بوريليا بيرسيكا *B. persica*.

الاستنتاجات: تُبرز نتائجنا وجود بوريليا البيرسيكا *Borrelia persica* بين القراد الصلب، رغم وجودها المعتاد بين القراد الناعم، مما قد يشير إلى اكتساب عرضي أو تداخل بيئي. كما لوحظ النمط الجيني لـبوريليا بيرسيكا *B. persica* أيضًا في القراد الناعم. ومن الجدير بالذكر أن قراد من نوع ريبيسيفالوس سانجوينيوس *R. sanguineus* يُعد الناقل الرئيسي لأنواع البوريليا بين القراد الصلب في فلسطين، في حين أن قراد اورنيثودوروس ثولوزاني *O. tholozani* هو الناقل الرئيسي بين القراد الناعم.