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Parasites of equine piroplasmosis and its potential tick vectors in Israel

A literature review

A ló piroplazmózisát okozó paraziták és potenciális kullancs vektorai Izraelben

Irodalmi áttekintés

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Abstract:

Equine piroplasmosis (EP) is an infectious tick-borne disease with a significant economic impact on the equine industry. The two causative agents of EP are *Babesia caballi* and *Theileria equi*. This thesis highlighted first the clinical presentation of the disease, which is similar to either parasite and is related to intravascular hemolysis and associated systemic illness and can manifest in different forms: peracute, acute, subacute, and chronic. Although most infected horses survive, the infection can be fatal, and most horses remain asymptomatic and inapparent carriers. EP is an endemic disease in many countries worldwide, including Israel. Therefore, this thesis focused mainly on EP and its potential tick vectors in Israel, located at the crossroads of three continents. Ticks are the vectors of *B. caballi* and *T. equi* and their definitive hosts. Thus, to understand the disease's pathogenesis and epidemiology, it is essential to understand the relationship between the parasites and the vector ticks, as well as highlight the tick species involved in the transmission of the disease. Thus, we emphasized here the etiology and pathogenesis of EP, the biology and systematics of ticks and we also listed and described the main species of ticks that are considered potential vectors of EP in Israel.

Összefoglalás:

A ló piroplazmózisa (EP) kullancsok által terjesztett fertőző betegség, amely jelentős gazdasági hatással van a lótenyésztésre. Az EP két kórokozója a *Babesia caballi* és a *Theileria equi*. Ez a dolgozat először a betegség klinikai megjelenését emeli ki, amely mindkét parazita esetében hasonló; intravaszkuláris hemolízissel és az ehhez kapcsolódó szisztémás betegséggel jár.Különböző formákban jelentkezhet: perakut, akut, szubakut és krónikus. Bár a legtöbb fertőzött ló túléli a fertőzést, tünetmentesen és tünetmentes hordozóként marad, a fertőzés végzetes is lehet. Az EP endémiás betegség a világ számos országában, így Izraelben is. Ez a dolgozat elsősorban a ló piroplazmózisával foglalkozott Izraelben, amely három kontinens kereszteződésében található. A kullancsok nemcsak a *B. caballi* és a *T. equi* vektorai, hanem végleges gazdái is. Így a betegség patogenezisének és epidemiológiájának megértéséhez fontos megérteni a paraziták és a vektor kullancsok közötti kapcsolatot, valamint rávilágítani arra, hogy mely kullancsfajok vesznek részt a betegség átvitelében. Ezért itt az EP etiológiáját és patogenezisét, a kullancsok biológiáját és szisztematikáját emeltük ki, valamint felsoroltuk és leírtuk a kullancsok fő fajait, amelyeket Izraelben az EP potenciális vektorainak tartanak.

Table of content

1 Introduction

Equine piroplasmosis (EP) is a tick-borne protozoal disease of equids (horses, mules, donkeys, and zebras) caused by one of the two erythrocytic apicomplexan protozoan parasites, ***Babesia caballi,*** and ***Theileria equi*** [1–4].

EP, also known as **equine Babesiosis**, **equine theileriosis** (concerning *T. equi*), and **biliary fever**, is a disease with non-specific clinical signs that may be seen as per-acute, acute, subacute, or chronic infections [3, 4]. Most infected horses remain asymptomatic and usually become inapparent carriers of the disease. The disease is characterized by fever, sometimes of an intermittent nature, anemia, icterus, hepato-, and splenomegaly. In addition, bilirubinuria and hemoglobinuria may be present, usually during the later stages of the disease [5]. Sudden death is not commonly seen in cases of EP, and the fatality rate of the disease is reported to be as low as 5%-10% but can reach up to 50% in case of outbreaks [6].

Equine piroplasmosis has a significant economic impact worldwide, primarily because of the limitation in the international movement of horses between endemic and non-endemic countries for trade and participation in sports events [7, 8]. The disease is endemic in many parts of the world, mainly in tropical, subtropical, and temperate areas, including Israel [9, 10]. The spread and distribution of the disease globally depend on the presence of the tick vectors responsible for transmitting the pathogens to the equines [1]. Therefore, identifying the specific tick species that are potential vectors of EP is a key factor in controlling the disease. Recently Glen A. Scoles and Massaro W. Ueti [8] listed 33 tick species implicated as competent vectors for *B. caballi* and *T. equi*, all of which belong to the family Ixodidae (hard ticks).

Ticks, specifically hard ticks, are blood-feeding ectoparasites that occur worldwide and are a significant group of arthropod vectors. They are characterized by the diversity of pathogens they can transmit, by their impact on human and animal health, and by their socioeconomic implications.

Since ticks play a major role in the transmission of EP and are required for the completion of the parasite's life cycle, understanding the biology of the ticks is essential for a better understanding of the disease.

Due to Israel's geographical location and climate, the country is endemic to many tick-borne diseases affecting humans and other animals, including pets and livestock. EP is one of those endemic diseases, and several studies have described it as highly prevalent in the country [9, 11, 12]. In addition, Israel provides a habitat for various ixodid tick species that have been reported to infest horses.

In this review, we first go through the general features and the epidemiology of Equine Piroplasmosis. Secondly, we discuss the etiology and pathogenesis of the causative agents with a detailed description of their reproduction and life cycle. After that, we describe the biology and systematics of hard ticks. And finally, we list and represent the potential tick vectors of EP in Israel.

2 General features of Equine Piroplasmosis

2.1 CLINICAL FINDINGS

The clinical signs of EP are often not specific, and the disease can manifest in different forms: peracute, acute, subacute, and chronic. Similar clinical signs may be seen in horses infected with either *B. caballi* or *T. equi* [3]. The disease is characterized by intravascular hemolytic anemia due to the erythrocytes' damage caused by the parasite replication [7]. Although clinical signs might appear similar following infection with both parasites, clinical signs tend to be milder in *B. caballi* infection and more severe in cases of *T. equi*. Still, symptoms and severity can vary significantly from one region to another [3, 7]. Even though most infected horses survive, there are 10-50% fatality rates of documented cases [6].

Most infected horses (especially with *B. caballi*) remain asymptomatic and can become inapparent carriers. Inapparent carrier is the most common clinical presentation of the disease in horses infected with either one of the parasites [4]. Horses infected with *T. equi* will most likely remain lifelong carriers of these parasites and serve as a source of infection to the vector ticks that will transmit the pathogen to another horse [10]. Horses infected with *B. caballi* are believed to be inapparent carriers for a shorter period than those infected with *T. equi*, and that is because they can undergo self-clearance of the parasite [2].

2.1.1 Peracute Equine Piroplasmosis

The peracute form of the disease is rare. It can be seen primarily in neonatal foals following in-utero infection and in adult horses that have just moved to an area with many infected ticks [3]. This form of the disease is characterized by severe and sudden onset of signs. In foals, clinical signs can be seen at birth or sometimes 2 to 3 days after delivery. Symptoms are often non-specific, such as decreased suckling, fatigue, weakness, fever, lethargy, anemia, severe icterus, and malaise. Hemoglobinuria and petechiae might also be seen [3, 4].

2.1.2 Acute Equine Piroplasmosis

Acute piroplasmosis is the most common form of the disease. It is characterized by non-specific signs such as a high fever that usually exceeds 40°C, decreased appetite and malaise, dehydration, tachypnea and tachycardia, congested mucous membranes, anemia, limb edema, and in severe cases, hemoglobinuria/bilirubinuria, icterus, and even death [3, 6]. Signs of colic, constipation, diarrhea and catarrhal enteritis may also be involved at the terminal stage of the disease [3]. Other less common clinical symptoms include secondary pneumonia, pulmonary edema, laminitis, and signs of central nervous system disease, including ataxia, myalgia, and seizures [2].

2.1.3 Subacute Equine Piroplasmosis

Horses with subacute EP show signs similar to the acute form but are often accompanied by intermittent fever and weight loss. Infected horses show various degrees of anorexia, malaise, normocytic normochromic anemia, limb edema, tachycardia, and tachypnea. Mucous membranes vary from pale pink to pink, or pale yellow to bright yellow, with petechiae and ecchymoses. In addition, horses may show signs of mild colic due to slightly depressed bowel movements. Horses with acute EP usually have an enlarged spleen (splenomegaly), which can often be palpated rectally [3, 5, 6].

2.1.4 Chronic Equine Piroplasmosis

Chronic cases of EP often present non-specific clinical signs of chronic inflammation or infection. Including poor body condition, reduced exercise tolerance, poor performance, mild anorexia, weight loss, and lethargy. Anemia may be minimal. Clinicopathological abnormalities may include reduced red blood cell count, platelet count, and hemoglobin concentration [2–5].

2.2 PATHOLOGICAL FINDINGS

Gross pathological findings vary depending on the form and severity of the disease. For example, the gross examination might show evidence of anemia, edema, splenomegaly, and different degrees of icterus. Other findings include subcutaneous and subserosal edema, pulmonary edema, epicardial and endocardial hemorrhages, hydropericardium and hydrothorax, hepatomegaly, enlarged pale to red-brown kidneys, enlargement of lymph nodes, and ascites [3–5].

3 Epidemiology of Equine Piroplasmosis

3.1 GLOBAL DISTRIBUTION

Equine Piroplasmosis is endemic in many parts of the world, mainly in tropic, subtropic, and temperate areas, and poses a significant economic impact globally. EP is a tick-transmitted disease of equids, and its presence requires a competent vector. Furthermore, the infected horse may remain a life-long carrier of the disease and act as the infection source of the vector ticks [6]. EP is present worldwide in most countries, and according to several publications, it is estimated that 90% of the total horse population of the world inhabits EP-endemic areas [3–5, 7]. Therefore, there is a constant danger of introducing pathogens to non-endemic areas.

It is important to emphasize that the worldwide distribution of EP is directly related to the distribution of its competent arthropod vectors since tick-borne transmission is required for the completion of the life cycle of the parasite. Therefore, the spread of the disease cannot occur without competent tick vectors [8]. According to the World Organisation for Animal Health (OIE: Office International des Epizooties) **(http://www.oie.int/),** the parasites occur in **Europe**, **Central, and Eastern Asia**, **Africa**, **Cuba**, **South and Central America**, and certain parts of the **southern United States of America**. *Theileria equi* has also been reported from Australia (but apparently never established itself in this region) and is now believed to have a wider general distribution than *B. caballi*. (**AETIOLOGY (woah.org)**).

3.2 THE PRESENCE OF EP IN ISRAEL

Israel and its surrounding countries are considered endemic to EP. The geographical location of Israel and the fact that it is located at the junction of three different continents (Europe, Asia, and Africa), the country's climate ranges from Mediterranean to highly arid and provides a habitat for a variety of ixodid tick species. Therefore, Israel is endemic to many arthropod-borne diseases, including Equine Piroplasmosis [11]. Several tick species, mainly of the genus ***Hyalomma,*** considered a possible vector of EP, have been reported to infest horses in Israel. A recent study was published by Dr. Sharon Tirosh-Levy [13] showing the equine tick infestation of the horse population in Israel. The species and the total number of collected ticks were found to be different depending on the location of the farm and the season [13]. The results of this study are detailed later in this literature review (see: 6.1 Potential tick vectors in Israel).

Babesia caballi and *T. equi*, the two causative agents of EP, are endemic in Israel. And therefore, several papers were published about the genome and the prevalence of these parasites [11, 14–18]. The seroprevalence of *B. caballi* is **69.6%,** its molecular prevalence is **9.7%** [14], and that of *T. equi* is **51%** (seroprevalence) and **26%** (molecular prevalence) [16]. Due to those high numbers, the disease is considered endemic in the country. To the recent day, every year few serologically positive cases are reported by the Israeli Veterinary Services and Animal Health.

4 Etiology and Pathogenesis of Equine Piroplasmosis

4.1 CLASSIFICATION OF THE ORDER PIROPLASMIDA

The genera *Babesia* and *Theileria* belong to the family **Babesiidae** and **Theileriidae**. Both families belong to the Phylum **Apicomplexa**. Apicomplexa is a eukaryotic phylum of obligate intracellular parasites with more than 6000spp. The phylum Apicomplexa is divided into 2 classes: **Aconoidasida** and **Conoidasida.** The class Aconoidasida is further divided into 2 orders: **Haemosporida** and **Piroplasmida**, and the course Conoidasida is divided into 2 subclasses: **Coccidiasina** and **Gregarinasina.** The order Piroplasmida contains three genera: ***Babesia***, ***Theileria***, and ***Cytauxzoon*** [19] (Figure 1). *Babesia caballi* is a typical species of *Babesia*, but it is not the case for *T. equi*. The phylogenetic position of *T. equi* has been controversial, and the organism has been renamed several

times. There is still uncertainty about the appropriate taxonomic classification for *T. equi*. Studies using small-subunit ribosomal ribonucleic acid (rRNA) gene analysis suggest that *T. equi* belongs to a distinct phylogenetic group positioned between *B. bovis* and *Theileria spp* [3, 20].

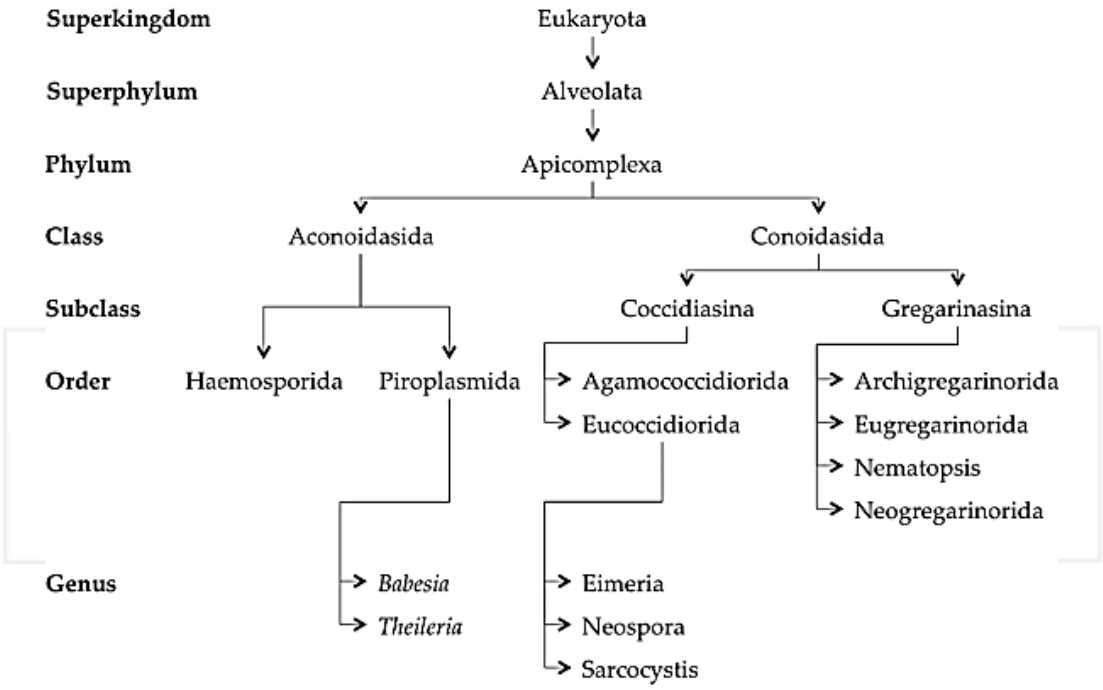

Figure 1. Classification of phylum Apicomplexa. Taxonomic categories are shown in bold (left) (taken from [19])

4.2 GENERAL MORPHOLOGY OF THE ORDER PIROPLASMIDA

The order was named Piroplasmida after its pear-shaped (pyriform) intra-erythrocytic stages. And since they are members of the phylum Apicomplexa, all Piroplasms share several morphological characteristics (as seen in Figure 2) [21]. As the name Apicomplexans states, all Piroplasms contain an apical complex that consists of cytoskeleton structures and membrane-bound organelles. Some of these structures might be missing depending on the order or the family [22]. The apical complex can include a **conoid (**not present in *Babesia* nor *Theileria* spp**.)** composed of counter-clockwise spiral microtubules that form a cone-shaped structure, one or more **polar rings**, **rhoptries, dense granules, spherical bodies,** numerous **micronemes** and **subpellicular microtubules** that extend from the polar ring towards the posterior pole [23].

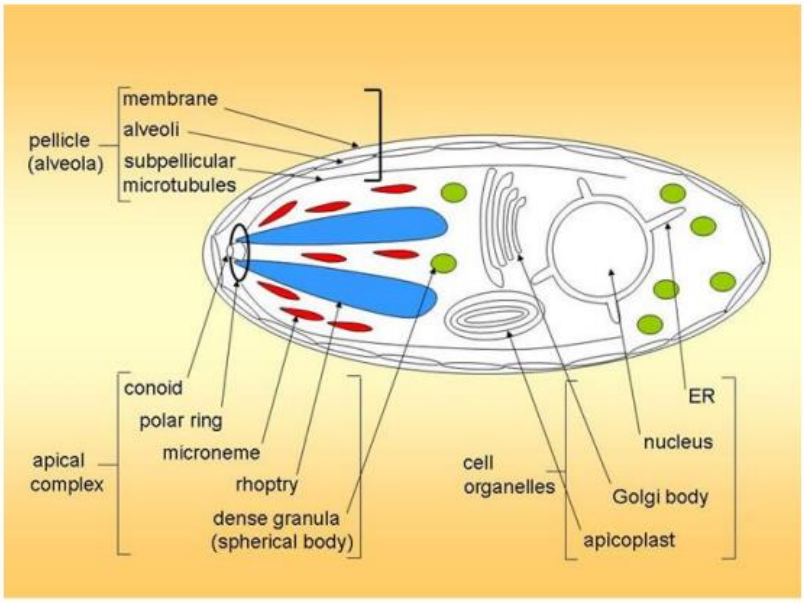

Figure 2. Morphology of an Apicomplexan. (taken from [23]).

4.3 REPRODUCTION AND LIFE CYCLE

Piroplasms include mainly two genera (*Babesia* and *Theileria*). Depending on the type of the organism, the reproduction and the life cycle of piroplasms can be different. Since these parasites are tiny, at first, only asexual reproduction within the vector ticks' salivary glands and the blood cells of vertebrates could be identified [24]. Later through the years, the development of the parasite (*T. equi*) in the gut of the vector ticks was studied by light microscopy [25].

In general, the life cycle of piroplasms (Figures 3 and 4) consists of the following [21]:

4.3.1 **Schizogony and Merogony** – asexual multiplication in the vertebrate host blood cells.

All parasites belonging to the group Piroplasmida reproduce asexually inside the blood cells of their host. **SPOROZOITES** are transmitted to the host following the tick bite through saliva secretion. The sporozoites target different blood cells according to the piroplasmida species, And the infection of the host starts after the invasion of the sporozoites [21]. *Theileria equi* (and all *Theileria* spp.) are characterized by **SCHIZOGONY**, an intra-leukocytic (monocytes and lymphocytes) asexual reproduction before red blood cell invasion. Schizogony starts after the invasion of the sporozoite into the leukocytes and the production of **MEROZOITES** [21]. The invasion of sporozoites

into the leukocytes is a complicated process that requires the involvement of the tick's saliva and several changes in the metabolism of the invading sporozoites [21].

This process includes several successive stages and is completed within about 3 minutes:

(1) Recognition of the target cell and the attachment to the cell membrane;
(2) Junction formation of the parasite with the cell membrane;
(3) Internalization (endocytosis) of the parasite fully into the host cell; (4-5)
(4) + (5) Separation and breakdown of the enclosing membrane;
(6) The formation of a microtubule network [21].

Once inside the host cell, sporozoites change into a multinucleate schizont (the first microschizont, which will develop into macrosichizont). Then the schizont-infected cells will circulate in the bloodstream. Finally, the end of the intra-leukocytic schizogony is marked by the production of uninucleated merozoites, which will be released into the bloodstream, where they invade erythrocytes [21].

Both *Babesia* and *Theileria* spp. go through **MEROGONY,** asexual division occur in the red blood cell and starts with sporozoite (*Babesia* spp.) or with merozoite (*Theileria* spp.) [21]. In *Babesia*, sporozoites are injected into the host's bloodstream during feeding an infected tick, which will lead to the initiation of merogony within the host red blood cells; merogony results in the propagation of pyriform merozoites and may lead to long-lasting persistence [26]. Proteins secreted by the APICAL COMPLEX mediate the invasion of the RBC; unlike *Theileria*, *Babesia's* orientation of the apical end establishes the junction between the parasite and the host cell membrane [21, 26]. Intracellularly sporozoites will develop into trophozoites, and trophozoites further divide into merozoites [26]. Later, merozoites are released from ruptured cells and invade other healthy erythrocytes [21].

Merogony of piroplasms is an asynchronous process; therefore, trophozoites and merozoites occur in the bloodstream simultaneously [21]. Merozoites are characterized by a piriform shape, forming pairs or tetrads, and the size of merozoites varies according to the piroplasm spp. As well as the vertebrate host species [21].

4.3.2 **Gamogony** – sexual reproduction in the gut of the tick vector, later followed by the invasion of kinetes into the internal tissues of the ticks.

It is generally assumed that the sexual reproduction of *Theileria* is identical to that of *Babesia*; the first sexual stages of piroplasms appear in the host red blood cells and are

referred to as **GAMETOCYTES** [21, 24]. Unlike merozoites, gametocytes do not grow and reproduce. They are believed to be larger than merozoites and unusually shaped compared to the asexual stages [24]. Following a blood meal from the infected host, gametocytes of both *Babesia* and *Theileria* develop into **GAMETES** in the lumen of the tick gut, and this process is called **GAMOGONY** [21]. Gametocyte metamorphosis is asynchronous, and the process is accompanied by microtubular reorganization, which will result in the formation of gametes [25]. Gametes of both parasites are haploid and have a unique appearance characterized by a tail, arms, and arrowhead [21].

Fertilization of piroplasms is induced by the fusion of two gametes of different types and may occur at the early stages of gamete formation [26]. Gamete fertilization results in the formation of a **ZYGOTE** [21]. The zygote of piroplasmida, often referred to as **KINETE** or OOKINETE, is a motile stage that will penetrate the peritrophic matrix for further development [21, 26]. Matrix penetration begins immediately after zygote formation and is accomplished by enzymes released from the arrowhead of the zygote. Since the matrix forms a strong barrier, the penetration process is an active process where the arrowhead will open the way for the zygote body [21, 24, 26]. Once penetrated, the zygote enters the ecto-peritrophic space and immediately invades gut epithelial cells, where the invagination membrane will disappear, and the zygote is found loose in the cytoplasm [21].

In most apicomplexans, the zygote forms a surrounding wall resulting in the oocyst, which will undergo a meiotic division [27]. In contrast, the zygote of *Babesia* does not develop an oocyst. Instead, it undergoes meiotic division inside the gut cells, producing unicellular kinetes (haploid stages result from the meiotic division of a diploid zygote)[26]. Kinetes are released from the gut epithelial cells into the hemolymph, where they are disseminated throughout the whole tick body and invade internal tissues [21].

4.3.3 **Sporogony** – asexual reproduction in the tick vector resulting in the generation of multiple infective Sporozoites.

The sporogony of *Babesia* sensu stricto species tends to differ from that of *Theileria*. The kinetes of Babesia species are believed to be involved in two cycles of asexual multiplication [24, 28]. Among all apicomplexan parasites, *Babesia* sensu stricto species possess a unique feature: transovarial transmission, where the parasite invades the vector tick's ovarian cells and transmits it to the tick larvae [24]. *Babesia* kinetes undergo the

first asexual cycle, where the kinetes invade different tissues throughout the tick and go through the second asexual multiplication producing secondary kinetes [21, 29]. Finally, secondary kinetes will invade the salivary gland, where sporogony occurs [24].

The *Theileria* sensu stricto species and *Theileria equi* kinetes are believed to migrate directly to the salivary glands [30, 31]. The kinetes of both *Babesia* and *Theileria* further develop in the tick salivary gland to produce **Sporozoites**, an invasive stage of the parasites [21]. Sporozoites are the responsible form for the transmission of the parasite from the tick to the vertebrate host. Sporogony starts after the invasion of the kinetes to the salivary glands [29]. After the invasion, kinetes rapidly enlarged and transformed into **Sporont.** This polymorphous single-membrane syncytium will later evolve into a **sporoblast,** a multinucleated and relatively undifferentiated three-dimensional branching meshwork[21]. To ensure the parasite's survival through the tick's constructional changes, the invading kinetes arrest in the form of a sporoblast.[26, 32]. Once the tick attaches to a host, the maturation of the sporoblast begins, and the infective **sporozoites** will be released from the syncytium continually into the host's bloodstream during the tick's feeding. [26].

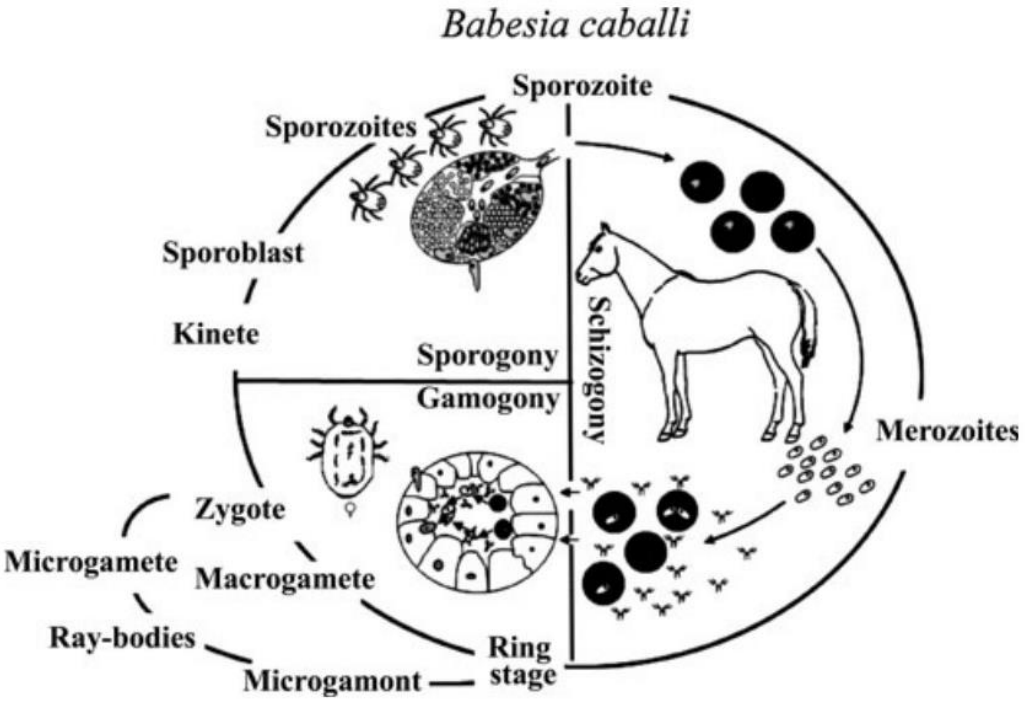

Figure 3. Life cycle of *Babesia caballi* (taken from[2].)

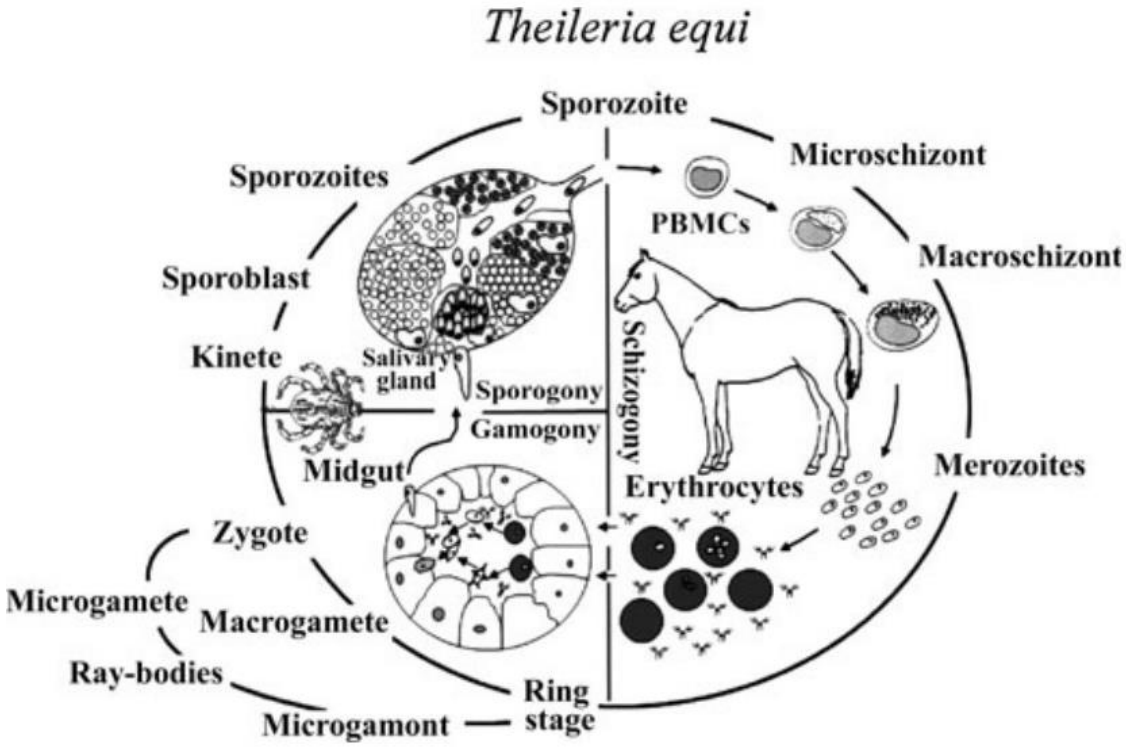

Figure 4. Life cycle of *Theileria equi* (taken from[2])

5 Biology and Systematics of Ticks

Ticks are blood-sucking arthropods that transmit infectious bacterial, viral, and protozoan agents. Ticks, specifically hard ticks (Ixodidae), are the definitive host for EP pathogens [8]. In addition, *Babesia caballi* and *T. equi* must undergo sexual-stage development within the tick to complete their life cycle. Therefore, ticks play a critical role in the disease's epidemiology and pathogenesis [33].

Understanding the relationship between the vector tick and the epidemiology of the disease plays a crucial role in the control of Equine Piroplasmosis, and knowing which tick species are involved in the transmission of the disease is the beginning of understanding such a relationship [8].

Basic knowledge of ticks' biology and systematics is also essential to better understand the disease.

5.1 CLASSIFICATION OF TICKS

Ticks belong to the phylum **Arthropoda**, further divided into the subphylum **Chelicerata**, and arranged into the class **Arachnida**, which is further divided into the subclasses of the **Acari** [34]. Mites and ticks belong to this group, characterized by an interior body part called Gnathostome and a posterior section, the Idiosoma [35]. Ticks are represented by the order **Ixodida**, which is either obligatory or only temporary blood-sucking ectoparasites. Ixodida comprises 3 families: **Ixodidae**, **Argasidae**, and **Nuttalliellidae** [35]. Ixodidae, or hard ticks, are the dominant tick species and consist of approximately 700 species, mainly of veterinary and medical importance [8]. Ixodidae is arranged into two major groups, **Prostriata** and **Metastriata,** composed of 5 subfamilies and 13 genera [35]. Argasidae or soft ticks contain approximately 170 species assigned to 5 subfamilies and 2 genera; ***Ornithodoros*** comprises about 100 species, and Argas includes 56 species [35]. The last family, Nuttalliellidae, is an unusual monotypic family that consists of only one species, ***Nuttalliella namaqua*** [35].

Taxonomy of ticks		
Phylum: Arthropoda		
Subphylum: Amandibulata		
Class: Arachnida		
Subclass: Acari		
Parasitiformes		
Metastigmata (Ixodida)		
	Ixodidae	*Amblyomma, Anocentor, Boophilus, Dermacentor, Haemaphysalis, Hyalomma, Ixodes, Rhipicephalus*
	Argasidae	*Argas, Ornithodorus, Otobius*
	Nuttalliellidae	*Nuttalliella*

Figure 5. Taxonomy of ticks (taken from [36]).

5.2 MORPHOLOGY OF TICKS

Ticks consist of two parts, the **capitulum** (gnathosoma) and the **body** (Idiosoma) (see Figure 6) [37]. No distinct head can be seen, and the mouth parts are found on the capitulum. Mouth parts include **Two 4-segmented palps**, each of which has several chemosensory sensillae located in the distal fourth segment. **Pair of 2-segmented tubular chelicerae** that extend from the base of the capitulum and are located medially to the palps. Two moveable and highly sharp **cutting digits** are situated at the extremities of the

chelicerae shafts. The digits are located laterally and are used to cut the skin during feeding [37]. A medially positioned, large **hypostome** with ventral, backward-pointing teeth (denticles) on its external surface is used as a food canal and a holdfast organ.

The basal portion of the capitulum, the basis capituli, is attached to the tick's body by a flexible membrane [37]. The body is posterior to the capitulum and is attached to the legs. Larval ticks have 6 legs, while nymphs and adults have 8 legs. The jointed 6-segmented legs are used for locomotion. A **Haller's organ** located on the tarsus of the first leg detects temperature, odor, and chemicals. The genital pore is located at the anterior part of the body, while the spiracles and the anus are situated at the posterior part. The genital aperture is closed, cannot be seen in larvae and nymphs, and is open only in adults [37]. In ixodid ticks, **scutum,** a hardened shield, cover the dorsal anterior portion of the body of females and immature ticks. In males, the scutum covers the entire body[37].

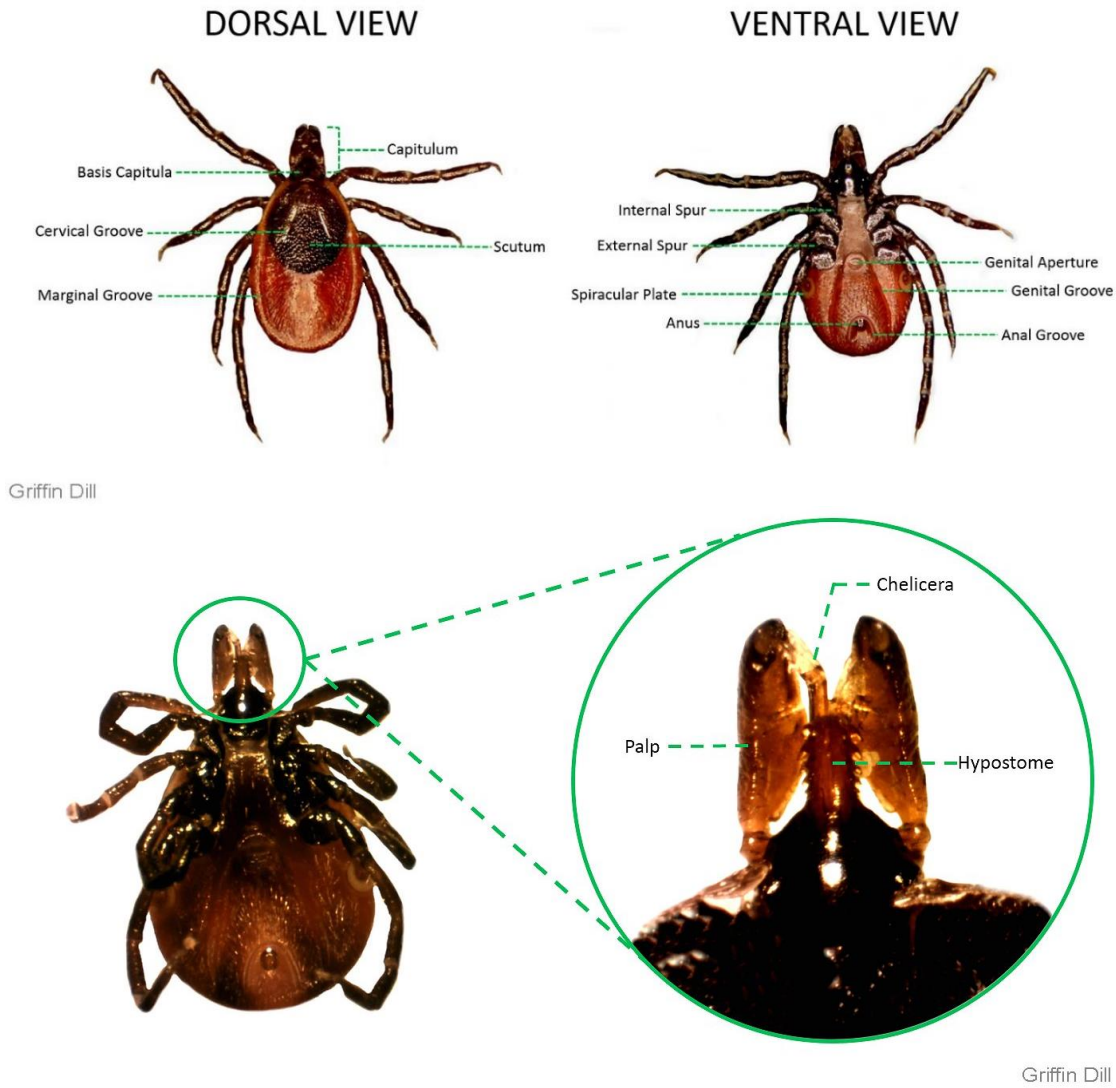

Figure 6. The morphology of deer tick (*Ixodes scapularis*).
(https://extension.umaine.edu/ticks/tick-biology/ download: 09/11/2022)

5.3 REPRODUCTION AND LIFE CYCLE

The life cycle of all hard ticks consists of 4 developmental stages, the **egg** and 3 active stages: **larva**, **nymph**, and **adult** [38]. Ixodidae ticks have only one nymphal instar, which differs from soft ticks (Argasidae) with several nymphal instars [35]. Each active stage feeds blood only once during its life, although females removed from their hosts could reattach to another host and continue their meal [38]. Hard ticks differ from other ticks because they need a more extensive blood meal; each stage requires several days. Ixodids females feed a large meal of blood and increase up to 100 times in size, then they lay one big mass of eggs and die. An obligatory factor that determines the female's ability to complete feeding is mating [35, 38]. The average number of eggs a female tick can lay is a few thousand; this number varies depending on the tick species and engorgement [38].

Most ixodid ticks are characterized by a 3-host life cycle (see Figure 7), where each active stage (larva, nymph, and adult) is involved with a different host [39]. Usually, larvae and nymphs of mammal-feeding ixodids engorge small-sized animals, while adults feed on larger animals. In a 3-host cycle, the larvae attach to a host and feed for around 3-7 days; upon completion of feeding, the larvae are going to drop off from the host and molt to a nymphal stage in the environment. After that, the nymph will attach to a host (different or same host) and feed for 4-8 days; the nymph drops off from the host and molts to the adult stage. Adults then will find their final host, where the female is going to mate, feed for a period of 7-12 days, and upon completion, they drop off the host, lay a big mass of eggs, and die. Males ixodids do not gorge on blood like the females and the immatures. Instead, they remain on the host for several weeks or months, feeding intermittently on small blood meals [35, 38].

Several ixodid ticks evolved and no longer required multiple hosts; those ticks are characterized by a 2- or 1- host life cycle [38]. In the case of the 2-host life cycle, the larvae do not drop off from the host. Instead, they ecdyse on the host, and the resulting nymph reattaches to the same host and completes feeding; the rest of the cycle is similar to the 3-host cycle [35].In the case of the 1-host life cycle, larvae, nymphs, and adults feed on the same host, and only the females will drop off once they are ready to lay the eggs [38].

The reproductive activity of ixodid ticks is regulated by several strategies [38]. For ticks in the Prostriata group, gametogenesis begins after the nymph molt and continues or is completed when the adult emerges. Consequently, females may mate before or during the feeding [38]. For the Metastriate ticks, adults hatch immature sexually, and gametogenesis is initiated during blood feeding. Therefore, mating can occur only when the female feeds on a host and not before [38]. Copulation and insemination in metastriate ticks induce several changes in the recipient female, including the engorgement of the blood meal, synthesis, and secretion of vitellogenin, incorporation of vitellogenin into the oocytes, and the enlargement of the oocyte. After mating, engorgement, and drop-off from the host, oviposition will occur, and the mated female will lay thousands of eggs at once and die after; on the other hand, males feed and mate multiple times [38, 39].

Several sex pheromones regulate the mating of ticks that the female secretes; the pheromones will attract fed males, identify the female as a suitable partner and guide the male to the genital pore of the female [39].

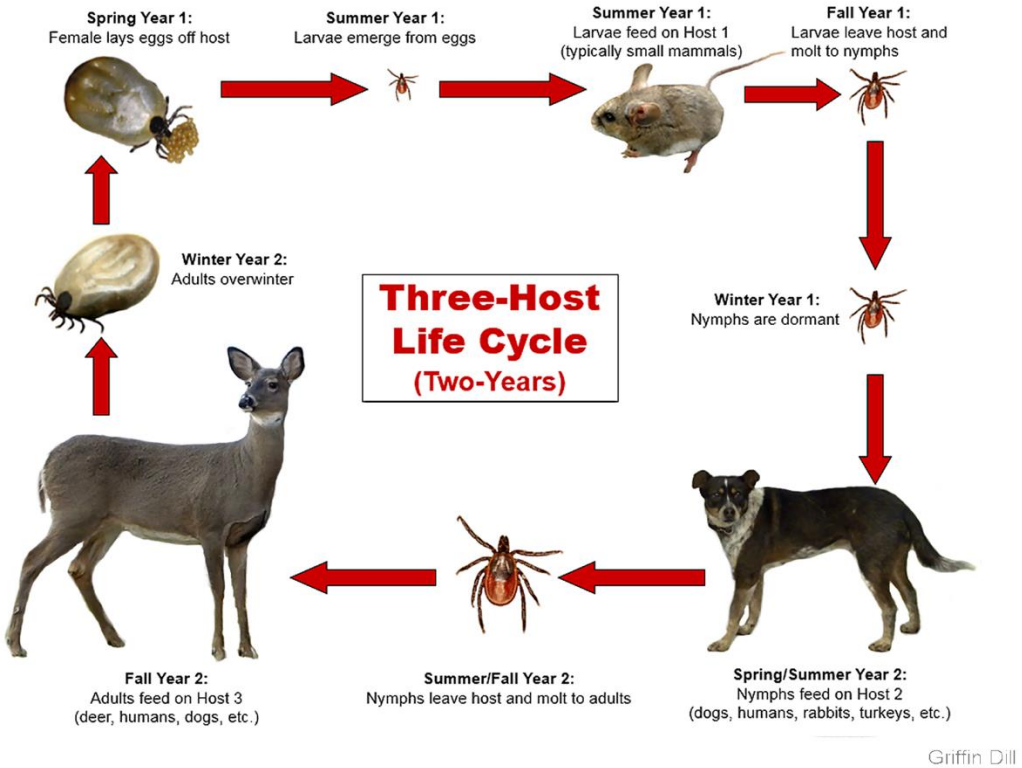

Figure 7. The life cycle of a 3-host ixodid tick
(https://extension.umaine.edu/ticks/tick-biology/ Download: 09/11/2022)

5.4 HOST - VECTOR - PATHOGEN INTERACTION

A key feature to understanding the pathogen prevalence in questing ticks and vertebrates is understanding the interactions and the relations of the Host – Vector – Pathogen system. **Vector competence**, which is the capability of the vector to transmit a pathogen either mechanically or biologically, is only one component of the overall **vectorial capacity**, which is the efficiency of vector-borne disease transmission, among many other factors [8].

To qualify a tick as a vector of a specific pathogen, 3 criteria must be confirmed [40]:

(1) The ability of a naive tick to attach to an infected animal, feed on its blood, and become infected.

(2) The ability of the infected tick to pass the pathogen transstadially to the next stage of the life cycle, or the F1 generation.

(3) The ability of the adult tick to transmit the pathogen to a new non-infected host during blood feeding.

In other words, to become a vector, the tick must feed on an infected host, uptake the pathogen during the blood meal, keep the pathogen through one or more developmental stages, and transmit the pathogen to a naïve host while feeding again.

To understand the transmission dynamics of tick-borne pathogens, it is essential to know how the tick's life cycle interacts with the parasite's life cycle. The interaction between the life cycle of the tick and the parasite will result in the mode of transmission of the disease. Therefore, the mode of transmission of tick-borne pathogens, which in our case is *B. caballi* and *T. equi*, depends mainly on the interaction between the life cycle of the vector tick and the life cycle of the parasite [8].

The agents of Equine Piroplasmosis are transmitted through 3 distinct models [8]:

(1) **Intrastadial transmission** means that the same tick stage will take the pathogen from an infected host and transmit it to a different host.
(2) **Interstadial or transstadial transmission**: where one stage of the tick (larvae) takes the pathogen, and the following stage (nymph) transmits it to the host.
(3) **Transovarial transmission**, in which the female tick gets infected with the pathogen and passes it to her offspring, who will transmit it to the host.

Many other factors, such as the climate and distribution, the behavior of the tick, the behavior of the host, the tick abundance, intrinsic physiological characteristics of the vector, etc., play a crucial role in the vectorial capacity of a tick. Therefore, for a tick to be an effective vector, it must be vector competent and have a combination of several factors that will lead to a vector capacity [8, 41].

Tick-host interactions are complex, dynamic encounters among several defense mechanisms by the host and countermeasures to those defenses that will result in successful blood feeding and infectious agent transmission. Based on the number of tick species, the diversity of potential host species, and the natural environment, an assortment of tick-host interactions occurs [42]. Currently, there are 907 valid species of hard ticks that feed on a wide variety of mammals, birds, and reptiles [43]. Ixodid ticks stay attached to the host for extended periods; larvae and nymph each feed for up to 8 days, and adults feed for 12 days and sometimes longer [37]. Different tick species have developed an independent adaptation for blood feeding, reflected in their salivary glands' gene composition [44]. Ticks must counteract their hosts' pain and itch responses, immune defenses, hemostasis, and wound healing to maintain a continuous attachment where they can feed for days[45].

6 Potential tick vectors of Equine Piroplasmosis in Israel

In a recent study by Glen A Scoles and Massaro Ueti, a list of 33 ixodid species in 6 different genera have been implicated as competent vectors for *B. caballi*, *T. equi*, or both [8]. Ticks listed in the study include ticks of the genera

(1) ***Amblyomma***
(2) ***Dermacentor***
(3) ***Haemaphysalis***
(4) ***Hyalomma***
(5) ***Ixodes***
(6) ***Rhipicephalus***.

The ticks listed here are ticks with a strong host association with equines or an association with an outbreak of EP.

Since this review focuses on EP in Israel, the following chapter lists the tick species found to infest horses in Israel. The list is taken from a study done by Doctor Sharon Tirosh-levy [13]. And have been published through the Parasites & Vectors journal (Parasites & Vectors | Home page (biomedcentral.com)). This study investigated the species distribution, epidemiology, and seasonal dynamics of ticks infesting horses in Israel (see Figure 8). For the analysis, 3267 ticks were collected from 396 different horses in 24 farms across the country. The authors described five major tick species found to infest horses in Israel: ***Hyalomma excavatum***, ***Hyalomma marginatum***, ***Rhipicephalus turannicus***, ***Rhipicephalus annulatus***, and ***Haemaphysalis parva*** [13].

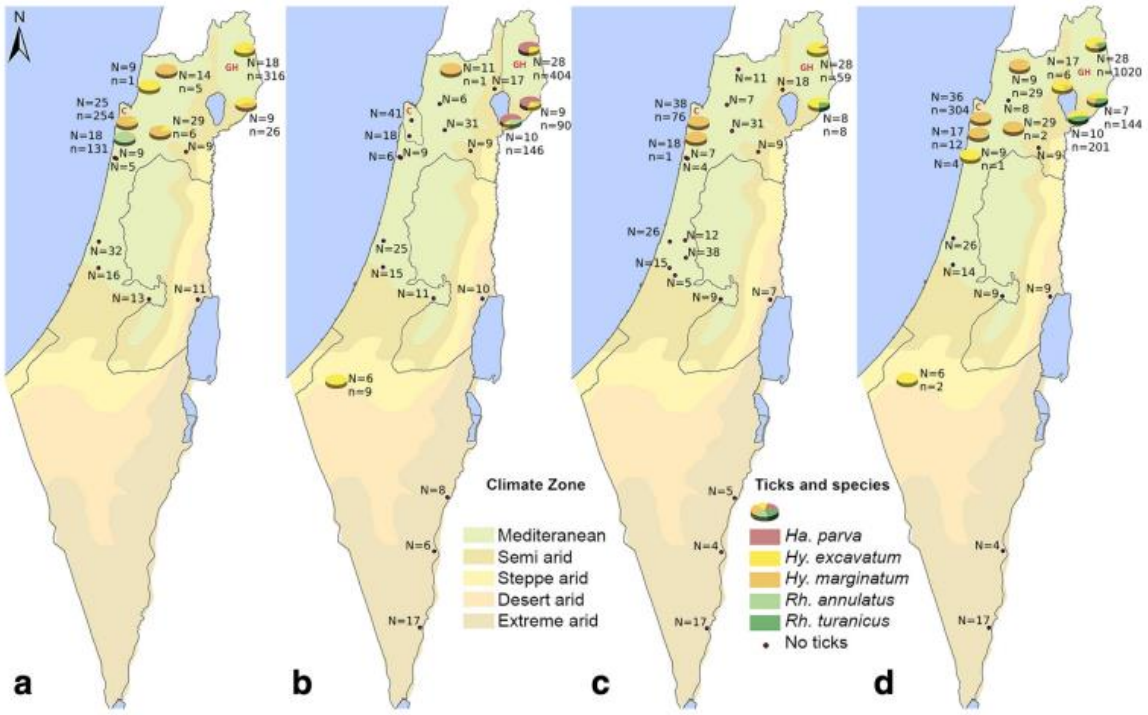

Figure 8: Geographical and seasonal distribution of equine ticks in Israel. Tick species distribution in each farm is depicted in a pie chart. **a** Summer (July – August 2014). **b** Autumn (November – December 2014). **c** Winter (February – March 2015). **d** Spring (May – June 2015). N = number of horses scanned, n = number of ticks collected, C = Carmel mountain ridge, CG = Golan Heights. (taken from [13]

6.1 HYALOMMA EXCAVATUM (KOCH, 1844)

Until recently, this species was considered a subspecies of *Hyalomma anatolicum* [34].

Hyalomma excavatum is a two-host or 3-host exophilic tick. Adult ticks prefer mainly cattle, but they frequently infect sheep, goats, and horses. The immature stages prefer rodents, leporids, and insectivores as their hosts [46]. When fed on calves, the whole life

cycle takes 113-161 days. Female ticks engorge in 8-12 days. Preoviposition takes 14-22 days, oviposition 28-38 days, and larval hatching takes 48 days [46].

The prevalence, population density, and distribution of *Hy. excavatum* depends on the availability of hosts for immature stages. Commonly coexists and interbreed with large populations of *Hy. anatolicum* in semi-desert and steppe areas [47]. In Israel, larvae are found during the summer, and adults infest animals between April and November [48]. The distribution of *Hy. excavatum* covers the whole Mediterranean region, including Israel, Jordan, Syria, and Lebanon, as well as Iraq, Iran, Kazakhstan, Turkmenistan, Uzbekistan, Afghanistan, Turkey, Cyprus, Greece, Egypt, Sudan, Libya, Morocco, Somalia, Algeria, and Albania [46, 47]. In Israel, *Hy. excavatum* was found to be the most prevalent species in the Golan Heights, peaking in the spring and summer [13].

6.2 HYALOMMA MARGINATUM (KOCH, 1844)

Hyalomma marginatum is a non-nidicolous, two-host tick with one generation per year in nature [46]. This tick has been reported to be involved with several veterinary and public health pathogens. It is most known as the primary vector of the zoonotic disease, **Crimean-Congo hemorrhagic fever** (CCHF) [49], which has been reported in Israel and can be transmitted from ticks to horses [50]. In addition, *Hy. marginatum* has been said to be a vector tick for *T. equi*, one of the causative agents of EP [16, 51]. Under laboratory conditions, the life cycle of *Hy. marginatum* seems to depend on the host's species, where the shortest time required to complete one cycle varies, ranging from 73 to 97 days [46]. Adult *Hy. marginatum* feed on various mammals, mainly wild and domestic ungulates, particularly bovines. The larvae and the nymphs are specific to small mammals, particularly leporids, insectivores, and ground-dwelling birds [49, 52].

All stages are rarely seen during winter and are mainly active during the spring and summer. Adults begin to appear in March and will reach their peak activity in May. Larvae are primarily active through June and July, while nymphs first appear in July [13, 46, 47]. Since those ticks are non-nidicolous, their host-seeking behavior relies on an active locomotory hunting strategy. Whenever a chemical, mechanical or sensorial stimulus indicates the presence of a suitable host, the tick will run across the ground to attack [46].

Hyalomma marginatum has a Palaearctic distribution showing ecological plasticity [34]. It is adapted to several biogeographical regions supporting an extensive range of

conditions, from the humid Mediterranean climates to the steppe arid environments. In Israel, *Hy. marginatum* is the most prevalent species in the Carmel Mountain ridge, a limestone mountain ridge located near the Mediterranean Sea. It peaks at 546 meters above sea level and features a Mediterranean climate and vegetation [13, 53]. The tick has been reported in many countries, including the countries of the Mediterranean region, southern Europe, northern Africa, and some parts of Asia [46].

6.3 RHIPICEPHALUS TURANICUS (POMERANTZEV, 1940)

Rhipicephalus turanicus is still under discussion recently due to the morphological, biological, and molecular variability of ticks identified as *R. turanicus* in different countries [46]. For this reason, many literature records are currently pending speculative confirmation [34].

Rhipicephalus turanicus is a Palaearctic 3-host tick that lives in lowland desert, semi-desert, steppe, and open woodlands. In Israel, the tick has been found only in the Golan Heights [13, 54]. This species feed on mammals, birds, and lizards. Human infestation has been also reported [54].

Adults of *R. turanicus* have been found questing during the spring and summer months. In Israel, it is found to peak during the spring [13]. However, since this species is still under discussion, many distribution records are currently speculative, and therefore the precise geographical distribution of the tick remains unknown [46]. And its role as a vector of human and animal pathogens detected in ticks designated as *R. turanicus* needs to be confirmed.

6.4 RHIPICEPHALUS ANNULATUS (SAY, 1821)

Before 2001, most records listed *Rhipicephalus annulatus* as a species of the genus *Boophilus* under the name *Boophilus annulatus*. However, in 2001 Beati and Keirans published a molecular study of this species that led to *Boophilus* becoming a subgenus of the genus *Rhipicephalus* [34, 55].

Rhipicephalus annulatus is a one-host tick with all stages feeding on the same animal. Under favorable conditions, the life cycle can take less than 2 months, and two generations can occur yearly [46]. Due to this fast life cycle, a massive infestation of animals can

occur, resulting in substantial economic losses [56]. The primary host of *R. annulatus* is cattle, but sheep, goats, and wild ungulates are occasionally infested. It has been reported to infest other hosts, such as dogs, horses, donkeys, and even humans but infestations on such hosts seem to be maintained exclusively by cattle [56].

Rhipicephalus annulatus is well known as a vector tick for **Babesiosis,** mostly known as a vector for *B. bovis* and *B. bigemina*, in which it transmitted those parasites to cattle. It is also known as a competent vector of bovine anaplasmosis. *Rhipicephalus annulatus* prefer a Mediterranean and savanna vegetation habitat, but it can also be found in humid steppe areas with hot and dry seasons [57]. In Israel, *R. annulatus* was found on two farms, one located in the Golan Heights and the other in Carmel Mountain ridge, and horses of both farms were used for herding cattle[13]. This tick is widely distributed, and reports from Africa, Southeast Asia, the middle east, Europe, and the US confirms the presence of this tick in those countries.[46, 47]

6.5 HAEMAPHYSALIS PARVA (NEUMANN, 1897)

Haemaphysalis parva was designated initially as *Dermacentor parvus*; later, it was recognized as a member of the *Haemaphysalis* and found identical to *H. otophila*. Therefore, *D. parvus* and *H. otophila* are synonyms of *H. parva* [34].

Haemaphysalis parva is a three-host exophilic tick. Adult ticks feed on medium to large-sized wild and domestic animals, and immatures feed mainly on small to medium-sized animals, including wild mammals, birds, and reptiles [34]. Under laboratory conditions, the entire life cycle of *H. parva* is completed in 50-80 days. Larvae feed for 2-7 days and moult to nymphs in 15-28 days. Nymphs feed for 2-8 days and moult to adults in 17-26 days. Female tick feed for 3-14 days and lay up to 3000 eggs [52]. Experimentally, *H. parva* can transmit *B. ovis*, and it has been reported to be associated with ovine babesiosis cases in Russia [52, 54].

Haemaphysalis parva is a Mediterranean species, and it prefers various types of habitats. It cannot survive in steppe climates with low humidity and is mainly observed around places with grazing livestock [46, 58]. The tick is characterized by autumn-winter-spring activity and can be found on hosts from August to May, with a peak in October and November. In Israel, the tick has been found on horses at the Golan Heights only, and the peak activity of the tick was in autum[13].

7 Discussion

Equine Piroplasmosis and its vector ticks have been a field of interest for veterinarians for many years, and till today there is still some missing information that needs further investigation.

The fact that the taxonomy of *T. equi* has changed a few times over the years and till today still not fully clear should raise a few questions regarding the old data on the disease [20]. Also, we should consider that several studies regarding the molecular characteristics of *B. caballi* and *T. equi* that were conducted in the past used serological and molecular methods that are not considered the most accurate nowadays[11].

The epidemiology and the distribution of EP are the main factors in the control of the disease. Several studies were conducted regarding the prevalence of *B. caballi* and *T. equi* in Israel [9, 12, 14, 59]. The diagnosis of EP is usually made by the detection of the agent either microscopically or by PCR, and most of the time, the diagnosis is performed on a jugular vein blood sample. A recent study done by Isabel Ribeiro [60] shows that 20% (5/25) of horses evaluated for piroplasm by PCR were positive for splenic blood samples but negative in jugular vein blood samples. In addition, several studies report a higher seroprevalence than PCR prevalence for *T. equi*, suggesting that the serological methods' sensitivity is higher than the sensitivity of the molecular methods [61–63].

8 Conclusion

EP is endemic in many countries worldwide, including Israel, and the distribution of the disease is directly related to the distribution of its tick vector, which can vary depending on several factors.

The causative agents of EP were found to be significantly more prevalent in the northern parts of Israel than the central or southern parts of the country, which defined northern Israel as a hyperendemic region for EP. Furthermore, and in accordance, the potential tick vectors of *T. equi* were also found to be more prevalent in the northern parts of the country, which proves the direct relation between the epidemiology of the disease and the distribution of the tick vector.

Further investigations can be performed regarding the prevalence of EP in Israel since knowing the exact tick species that are the potential factor of EP is essential for studying EP prevalence.

Understanding the interaction between the tick vector and the pathogen is essential in controlling the disease's epidemiology. And therefore, a better understanding of this relationship is required by the veterinarians and the equine community.

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