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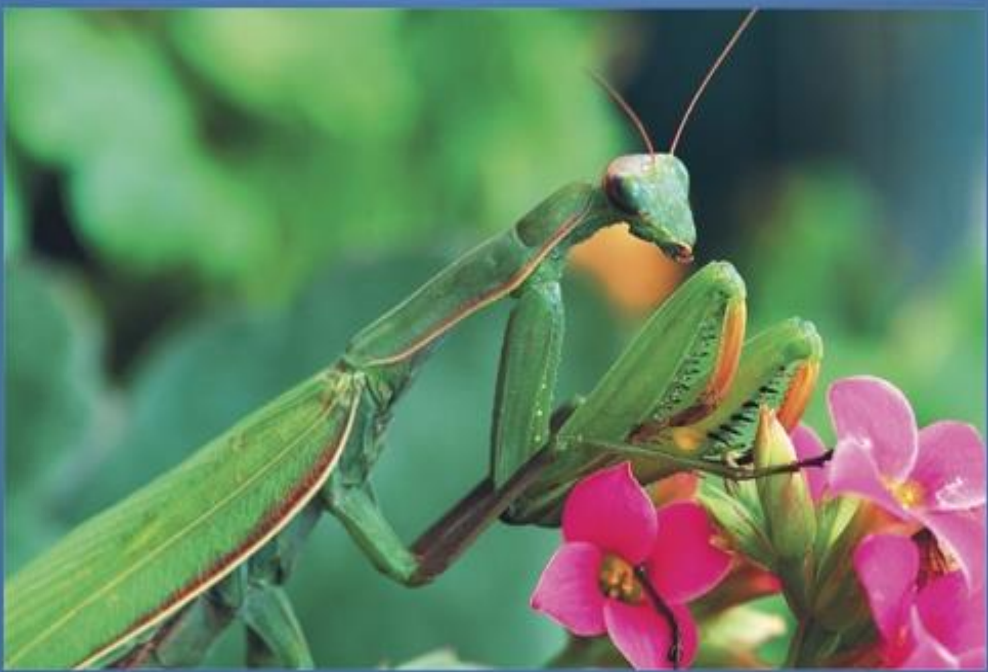

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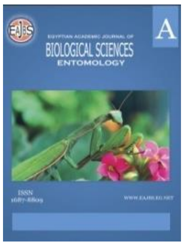

Vector Competence of Five Ixodid Tick Species in Egypt for *Borrelia*

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ABSTRACT

Ticks are vectors of several disease agents affecting human and animal health. The ability of ticks to acquire, maintain, and transmit disease agents are factor determining the competence of ticks as vectors of etiologic agents. The present study investigates the ability of five ixodid tick species, *Hyalomma dromedarii*, *H. excavatum*, *H. impeltatum*, *H. analoticum* and *Rhipicephalus annulatus* to acquire, transstadially and transovarially maintain and transmit *Borrelia sp.* isolated from their population in nature to a laboratory animal. Although the five ixodids successfully acquired the borrelial infection by feeding on infected New Zealand rabbits, only four of the ixodids showed the ability to transstadially transfer *Borrelia* from the Larval-nymphal stage to the adult male, and female. Also, only *R. annulatus*, *H. dromedarii*, *H. impeltatum* succeed in transovarially transferring *Borrelia* from adult females to their progeny. *Rhipicephalus annulatus* was the most efficient species in acquiring borrelial infection in the laboratory (69.17%) and in transstadially transferring their natural *Borrelia sp.* from the infected nymphs to the emerged adult males and females with infection rates of 60 and 80%, respectively All infected females *R. annulatus* transovarially transmitted their natural *Borrelia* species to the highest percentage of the unfed larval progeny in each of F1 and F2. This was followed by *H. dromedarii* and *H. impeltatum* with 54.58% and 39.17% acquisition infection rates, and transstadially transfer *Borrelia* to males and females (50, 70%, and 30, 50%), respectively. Infected females of both species transmit *Borrelia* to the F1 and F2 (100,90%), respectively. No transovarial transmission was observed in *H. impeltatum* and *H. analoticum*. In each tick species, the transmission of *Borrelia* to uninfected rabbits increased by increasing the number of feeding infected stages per rabbit. Generally, female ticks were more efficient than males and adults than immatures in transmitting borrelial infection.

INTRODUCTION

Various species of ixodid ticks harbor and transmit spirochetes of the genus *Borrelia* (Order: Spirochaetales, Family: Borreliaceae) that are pathogenic for human, domestic, and wild animals (Burgdorfer *et al.*, 1989; Margos *et al.*, 2018; Trevisan *et al.*,

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2021). Ixodid ticks were recognized as major vectors of bovine borreliosis in cattle, sheep, deer, horses, and other large animals, and Lyme disease or borreliosis that commonly found in humans and animals such as deer, mice, and rodents (Spielman *et al.,* 1985; Cutler *et al.,* 2017). *Borrelia theileri*, the etiologic agent of bovine borreliosis has been demonstrated in the blood of cattle and livestock in Europe, North America, and Asia (Uilenberg *et al.,* 1988; Khan *et al.*, 2023). It is prevalent in Africa, India, Indonesia, South America, and Australia, and is associated with ixodid ticks (McCoy *et al.*, 2014). *Borrelia theileri* was detected in four *Rhipicephalus* Species *R. microplus*, *R. annulatus*, and *R. decoloratus R. evertsi* in South Africa, Nigeria, Brazil, and Mexico (Smith *et al.*, 1978; McCoy *et al.*, 2014). Certain spirochetes of the 11 spp. of *Borrelia Burgdorferi* senso lato (s.L) complex are the etiologic agents of Lyme disease or borreliosis of human, domestic, and wild animals (Grubhoffer, 2005; Rudenko *et al.*, 2011; Eisen, 2020). *Borrelia burgdorferi* Senso lato species were detected or isolated from more than 18 ixodid tick spp. belonging to five genera including *Ixodes*, *Dermacentor*, *Haemaphysalis*, *Amblyomma*, and *Rhipicephalus* (Lane *et al.*, 1991). Different *Ixodes spp.* have been identified as the primary or principal vectors transmitting *B. Burgdorferi* of Lyme disease in the United States, Europe, North Africa, Asia, and Australia (Takada *et al.*, 1994; Singh and Girschick, 2003; Eisen, 2020). In contrast, other tick genera were considered secondary vectors that may have the potential to transmit *B. burgdorferi* but are not efficient vectors of Lyme disease spirochetes (Teltow *et al.*, 1991; Eisen, 2020).

In epidemiological studies, the demonstration of vector competence of an arthropod for the etiologic agent of a disease provides important evidence to incriminate the arthropod as an effective vector of the disease (Spielman *et al.,* 1985; Barker and Reisen, 2019; Eisen, 2020). The parameters defining vector competence of ticks for pathogens such as acquisition, maintenance, and transmission to the vertebrate host have been studied by several authors (Peisman and Sinsky, 1988; Lane *et al.,* 1994; Rollend *et al.,* 2013; Lynn *et al.,* 2022). However, the role of ixodid ticks as vectors of borrelial diseases has been scarcely investigated in Egypt and was mainly concerned with Lyme disease (Hammouda *et al.*, 1995, El-helw *et al.*, 2014 and Samir *et al.,* 2015).

Hyalomma dromedarii, *H. excavatum*, *H. impeltatum*, *H. analoticum*, and *Rhipicephalus annulatus* are widely distributed in Egypt infesting cattle, camels, sheep, and other livestock and transmitting various diseases to them including babesiosis, theileriosis, and rickettsiosis (Shoukry *et al.*, 1993; Adham *et al.*, 2009; Barghash *et al.*, 2016; Abdullah *et al.*, 2016). The five ixodids were found to be infected with *Borrelia* in different localities of Egypt (Adham *et al.*, 2010; Abdelbaset *et al.*, 2022) which may represent a probable source of pathogenicity and disease to livestock and other domestic animals in Egypt. In the present study, the abilities of the aforementioned ixodid tick spp. to acquire, transstadially, and transovarially maintain and transmit *Borrelia* isolated from their population in nature to a laboratory animal are determined and compared.

MATERIALS AND METHODS

Tick Collection:

Ticks were collected from camels and sheep, in the Berkash camel market, Kerdasa and Abu-Rawash, Giza governorate, Egypt. Adult ticks on each host were carefully picked up with fine forceps and placed in separate vials. In the laboratory, ticks were identified according to Hoogstraal 1956 and Walker *et al.*, 2003, sorted by species, sex, uninfected, and infected with *Borrelia*, and counted.

Borrelia Detection:

Smears of adults and nymph’s hemolymph (HL) and larval squashes of ticks were examined for *Borrelia* using Fontana stain (Conn *et al.,* 1960) and direct immunofluorescence technique (Piesman *et al.,* 1986) and examined microscopically by light and epifluorescence microscopy.

Tick Colonies and Maintenance of *Borrelia-infected* tick Colonies in the Laboratory:

Uninfected and infected laboratory colonies for each of the collected 5 ixodid species were successfully maintained in the laboratory. Uninfected F1 adults produced from field-collected ticks showing no spirochetes in HL samples were used as the parents (P1) to start the uninfected laboratory colonies. To infect the New Zealand white rabbits, twenty naturally infected females of each tick species were fed on each rabbit. Seven days to one month later, a Fontana blood film from the rabbit or an indirect immunofluorescent test (Lane and Manweiler, 1988) for antibodies in the serum of the laboratory animals were examined to confirm spirochetemia. Laboratory-infected ticks were obtained by feeding ticks on spirochetemic rabbits that had been infected by *Borrelia* from the corresponding naturally infected tick species. Rabbits infected with *Borrelia* from each tick species were kept in separate cages from each other and from the uninfected rabbits.

In tick species that demonstrated transovarial and transstadial transfer of spirochetes, the infected laboratory tick colonies were established by rearing the infected progeny of the infected field-collected females as described by Gaber *et al.* (1984). All the tick species were held at 28±1°C, 75%RH 16 hr light/day.

Acquisition of *Borrelia* by Ticks:

Eighty laboratory-reared uninfected larvae, males, and females of each tick species were fed on rabbits infected in the laboratory by *Borrelia* from the corresponding naturally infected tick species. The larval squashes or adult gut contents of 10 ticks from each species were examined 30 – 60 minutes (immediately) post-feeding to ensure that the spirochetes had been acquired with the blood meal. Subsequently, the HL of 10 adults or larval squash of each tick species were examined for *Borrelia* every two days for 14 days to ensure the persistence and spread of the infection inside the tick body.

Transstadial Transfer of *Borrelia* in Ticks:

A hundred uninfected larvae from each tick species were infected in the laboratory by feeding on *Borrelia*-infected rabbits, as previously described. Following molting, the resulting nymphs were fed on uninfected rabbits. Subsequently, the emerged adult males and females of each tick species (30 ticks) were fed on uninfected rabbits (10 adult ticks/ rabbit), and the rabbit blood was examined 7–30 days after feeding for each tick species. The HL of each transstadially resulting adult tick was individually examined for *Borrelia* immediately post-feeding on the uninfected rabbit. Three replicates of each experiment were used.

Transovarial Transmission of *Borrelia*:

Ten engorged infected field-collected, and laboratory-reared infected females of each tick species (Parents, P1) were observed for oviposition and hatching. Ten unfed F1 larvae from each female were examined individually for *Borrelia*. The remaining larvae and subsequent nymphal stages were fed on uninfected rabbits until the adult stage was reached. Ten F1 females that were found to be positive for the spirochetal infection were allowed to feed on uninfected rabbits in the presence of males and followed up till oviposition and larval hatching as was mentioned in P1. Ten F2 larvae resulting from each F1 female were examined for spirochetes. According to Hoogstraal (1985) definition, the percent of infected females that pass the spirochetes to their progeny (the transovarial infection rate) and the percent of infected unfed larval progeny derived from an infected female (filial infection rate were determined in each of the two successive generations of the infected ticks. The experiment was replicated three times for each tick species.

Infectivity of Ticks to Host:

For each of *H. dromedarii*, *H. excavatum,* and *Rhipicephalus annulatus* unfed F1 larvae and adults (male and female) derived from infected field collected parents were fed on uninfected rabbits. Different numbers of ticks of each life stage were allowed to feed to repletion on each rabbit (groups of 10, 50, 100 larvae/rabbit or 1, 10, 20 males, females/ rabbit). Seven to thirty days after tick feeding, blood samples from each rabbit were examined weekly for *Borrelia* to determine if these rabbits had acquired infection. The experiment was replicated three times for each life stage of each tick species.

In the case of tick species (*H. impeltatum* and *H. anatolicum*) that did not demonstrate transovarial and transstadial transfer of *Borrelia*, this experiment on infectivity of the tick to host was only performed on unfed infected field-collected adult males and females.

Statistical Analysis:

Fisher's exact test was used to compare the proportion of infection rates in different tick species and life stages with the aid of SPSS Program version 27.

RESULTS

I.Tick Prevalence and Infection Rate:

A total of 3969 ticks were collected from 481 hosts (259 camel and 222 sheep) from different animal markets in Giza (Table 1). *Hyalomma dromedarii* were the most prevalent (44.54%), followed by *H. excavatum* (23.3%), *Rhipicephalus annulatus* (21.34%), *H. impeltatum* (7%) and *H. analoticum* (3,8%). Collected ticks were examined for borrelial infection, and all tick species were detected to be naturally infected with *Borrelia*. *R. annulatus* had the higher infection rate (50%), followed by *H. dromedarii* (47.78%), *H. excavatum* (27.14%), *H. analoticum* (20%), and *H. impeltatum* (11.71%). Infection rates of females mostly exceed males, except for *H. impeltatum* where the infection rate of males and females is almost the same.

Table 1: The numbers of collected tick species, their hosts, and percent infection with *Borrelia* of the collected ticks.

Tick Species	Number of collected ticks			% infection			Host
	Male	Female	Total	Male	Female	Total	
H. dromedarii	787	981	1768	38.78	54.098	47.78	Camel and Sheep
H. excavatum	433	492	925	21.66	31.25	27.14	Camel and Sheep
H. impeltatum	154	124	278	11.76	11.66	11.71	Camel and Sheep
H. analoticum	116	35	151	10	30	20	Camel
R. annulatus	391	456	847	22	64	50	Sheep

II. Acquisition of *Borrelia* Infection in Ticks:

The uninfected larvae and adult males and females of each of the five studied ixodid tick species acquired the spirochete infection by feeding on New Zealand white rabbits infected with *Borrelia* isolated from the natural population of the corresponding tick species. Spirochetes were detected in larval squashes and gut contents of adults immediately (30-60 min.) and in HL smears of ticks examined every two days during 14 days of observation after feeding, of immatures or adults.

Larvae and adults of *Rhipicephalus annulatus* acquired the highest total infection rate ($P<0.05$) with *Borrelia* species isolated from its natural population (69.17%). This is followed by *Hyalomma dromedarii* (54.17%) then *H. excavatum, H. anatolicum,* and *H. impeltatum* acquiring total infection rates of 39.17, 35.83 and 29.17%, respectively, with the corresponding natural *Borrelia* species for each tick species (Table 2).

Females of the aforementioned tick species acquired the spirochete (77.50, 61. 25, 43.75, 45, and 37.50%, respectively) at higher percentages (P<0.05) than their males. Also, larvae of each tick species acquired its natural *Borrelia* sp. at higher rates (71.25, 58.75, 40, 37.50 and 25%, respectively), than males except for *H. excavatum* and *H. impeltatum* (p>0.05).

Table 2: The ability of different stages of five ixodid ticks to acquire *Borrelia sp.* from laboratory-infected rabbits. Infection rate: (No. infected/No. examined) %

Tick species	Infection rate			Total %
	Larva	Male	Female	
H. dromedarii	58.75 (50-70)	43.74 (40-50)	61.25 (50-70)	54.58%
H. excavatum	40 (30-50)	33.75 (30-40)	43.75 (40-50)	39.17%
H. impeltatum	25 (20-30)	25 (20-30)	37.50 (30-50)	29.17%
H. analoticum	37.5 (30-50)	25 (20-40)	45 (40-50)	35.83%
R. annulatus	71.25 (60-80)	58.75 (50-70)	77.50 (70-90)	69.17%

A total of 80 ticks were examined for 14 days post-feeding at 2-day intervals for 10 ticks of each life stage.

III. Transstadial Transfer of *Borrelia* in Ticks:

Four of the studied ixodid tick species which had acquired their natural *Borrelia* species in the laboratory during the larval-nymphal stages transferred the spirochete from the positively infected nymphs to their adults which were fed on uninfected rabbits (Table 3). *Rhipicephalus annutalus* showed the highest frequencies of the transstadial transfer (Tst) of their natural *Borrelia* sp. from the infected nymphs to the emerged adult males and females with infection rates of 60 and 80%, respectively. This was followed by Tst of the corresponding natural *Borrelia* sp. from infected nymphs to adult males and females of *H. dromedarii* (50 and 70%), *H. excavatum* (30 and 50%), *H. anatolicum* (0 and 10 %). Except for *H. anatolicum*, all transstadially infected adult males and females transmitted infection to clean rabbits (10 ticks/rabbit) while feeding upon them (Table 3). *Rhipicephalus annulatus* was the most efficient in this respect showing the highest infection rates (P<0.05) of rabbits fed upon by Tst infected males and females (66.67 and 100%); followed by *H. dromedarii* (33.33 and 66.67), and *H. excavatum* (33.3 and 33.33%).

Table 3. Transstadial transfer (Tst) of *Borrelia sp.* to adults of 5 ixodid ticks fed as larvae on laboratory rabbits infected with *Borrelia sp.* isolated from each tick natural population and transmission of *Borrelia sp.* to uninfected rabbits.

Tick species	Life stage				
	Larva	Male		Female	
	% infection	% infection	% transmission	% infection	% transmission
H. dromedarii	60	50	33.3	70	66.6
H. excavatum	50	30	33.3	50	33.3
H. impeltatum	40	0	0	0	0
H. analoticum	50	0	0	10	0
R. annulatus	80	60	66.6	80	100

VI. Transovarial Transmission of *Borrelia* in Ticks:

Examination of the unfed F1 larval progeny of field-collected (Fc) and laboratory-reared *Borrelia*–infected parent females (P1) showed that 90, 90, 100% and 70, 80, 100 of the parent female *H. dromedari*, *H. excavatum* and *Rhipicephalus annulatus*, respectively, transovarially (Tov) transmitted *Borrelia* infection to their F1 larval progeny (Table 4). In both cases, respectively, the filial infection rate was the highest (P<0.05) in F1 larvae of *Rhipicephalus annulatus* (27 and 18%) followed by *H. dromedarii* (18 and 11%) and *H. excavatum* (17 and 13%). Generally, the percentages of the Tov transmission and filial infection rate of the natural *Borrelia* were higher (P<0.05) in field-collected P1 females and their F1 larvae than in laboratory-reared females of each tick species. In the second generation, *Borrelia* Tov transmission in F1 adult females and the filial infection rates of their unfed larval progeny (F2) were higher (P<0.05) than in the first generation (P1 females and F1 larvae) in each tick species (Table 4). The frequencies of the Tov transmission were 100% in the three tick species in the field-collected females and 90, 90, and 100% in laboratory-reared F1 females. Also, the filial infection rates of F2 larval progeny were increased (P<0.05) to 28, 31, 44% in field-collected and to 17, 18, 28% in laboratory-reared *H. dromedarii, H. excavatum,* and *Rhipicephalus annulatus,* respectively. No Tov transmission of borrelial infection to F1 and F2 larval progeny was observed in both infected field-collected and laboratory-reared *H. impeltatum* and *H. anatolicum*.

Table 4: Percentage of transovarial transmission (TOV) and filial infection rates during two successive generations of field-collected (FC) and laboratory-reared (Lab) females of 5 ixodid ticks infected with *Borrelia sp.* from the natural population of each corresponding tick species.

Tick species	FC infected female				Lab. Infected female			
	Infection rate (%)							
	F1		F2		F1		F2	
	%TOV	% Filial	%TOV	% Filial	%TOV	% Filial	%TOV	% Filial
H. dromedarii	90	18 (0-30)	100	28 (20-40)	70	11 (0-30)	90	17 (0-30)
H. excavatum	90	17 (0-30)	100	31 (20-50)	80	13 (0-30)	90	18 (0-30)
H. impeltatum	0	0	0	0	0	0	0	0
H. analoticum	0	0	0	0	0	0	0	0
R. annulatus	100	27 (10-40)	100	44 (30-60)	100	18 (10-30)	100	28 (10-40)

V. Transmission of *Borrelia* from ticks to Rabbits:

The transmission frequencies of *Borrelia* from infected ticks to uninfected New Zealand white rabbits varied (P<0.05) in the five studied ixodid tick sp., their life stages, and the number of ticks (infected with their natural *Borrelia* sp.) fed on each rabbit (**Table 5**). A hundred percent of the tested rabbits (3) were infected with *Borrelia* from *Rhipicephalus* when exposed individually to the feeding of 10, 20, and 100 infected females and males or immature larvae, respectively. This was followed by *H. dromedarii* (100, 66.67 and 66.67%), *H. excavatum* (66.67, 33.33 and 33.33%) and *H. impeltatum* and *H. anatolicum* (33.33, 33.33 and 0 %) infectivity to rabbits individually fed upon by 20, 20 and 100 females, males and larva, respectively (Table 5).

In each tick species, transmission frequencies in each life stage decreased (P<0.05) by decreasing the number of feeding infected tick stages per rabbit than the aforementioned frequencies (Table 5). Generally, female ticks were more efficient than males, and adults than larvae in transmitting their borrelial infection (P<0.05), where lower numbers of these life stages of ticks were required to produce similar or higher levels of transmission to rabbits fed upon by the other stages of each tick species (Table 5). One adult female or male *Rhipicephalus* was enough to transmit the borrelial infection to 66.67 and 33.33% of the exposed rabbits. This was followed by *H. dromedarii* where one female or 10 males per rabbit transmitted borrelial infection to 33.33 of the exposed rabbits. Ten males or females *H. excavatum* and *H. impeltatum* were required to infect 33.33% of the rabbits fed upon.

Table 5: Percent Transmission of *Borrelia sp.* to rabbits by different stages and numbers of infected ixodid tick species.

No. of infected ticks/rabbit	*H. dromedarii*			*H. excavatum*			*H. impeltatum*			*H. analoticum*			*R. annulatus*		
	L	Male	Female	L	Male	Female	L	Male	Female	L	Male	Female	L	Male	Female
1	-	0	33.3	-	0	0	-	0	0	-	0	0	-	33.3	66.6
10	0	33.3	66.6	0	33.3	33.3	-	33.33	33.3	-	0	0	33.3	66.6	100
20	-	66.6	100	-	33.3	66.6	-	33.3	33.3	-	33.3	33.3	-	100	100
50	33.3	-	-	33.3	-	-	-	-	-	-	-	-	66.6	-	-
100	66.6	-	-	33.3	-	-	-	-	-	-	-	-	100	-	-

DISCUSSION

Adult and immatures of *Hyalomma dromedarii*, *H. excavatum*, *H. impeltatum*, *H. anatolicum,* and *Rhipicephalus annulatus* collected from camels and sheep in Giza governorate Egypt, varied in their ability to acquire, transstadially and transovarially maintain and to transmit their natural *Borrelia* species to laboratory mammalian host (rabbit).

Acquisition of *Borrelia*:

The ability of the five studied ixodid tick species to be infected with *Borrelia* species isolated from natural populations of the corresponding ticks was consistently demonstrated in the larval squashes and HL of adults in the laboratory, which has given one of the convincing evidence of the identity of a vector tick species (Spielman *et al.*, 1985). Frequencies of laboratory acquisition of *Borrelia* in adults of the five ixodid tick species ran in parallel with their borrelial infection rates in the field-collected adults as was calculated in the present study. In both cases, respectively, *Rhipicephalus* annulatus showed the highest percentages of infection (69.17 and 50%) followed by *H. dromedarii* (54.58 and 47.78%), *H. excavatum* (39.17 and 27.14%), *H. anatolicum* (35.83 and 20%) and *H. impeltatum* (29.17 and 11.71%) where percentages of *Borrelia* laboratory acquisition and field infection rates of female mostly exceeded males in each tick species. The observed higher percentages of borrelial infection in the laboratory than in nature might be attributed to the relatively optimum conditions of survival offered to the tick and pathogen in the laboratory versus the challenging and varied conditions in nature as environmental temperature, humidity, vertebrate hosts kinds, availability etc. which have been found to affect different physiological processes and interrelationships in both organisms (Schwan *et al.*, 1995; Shanbaky *et al.,* 2009; Wallace *et al.,* 2019).

Transstadial Maintenance of *Borrelia* in Tick Stages:

Rhipicephalus annulatus showed the highest frequency and efficiency (%Tst) and efficiency (infectivity) of transstadial transfer of its borrelial infection from nymphs to the resulted adult males and females (60 and 80%) which in their turn succeeded to transmit the spirochete, respectively to 66.6 and 100% of the uninfected rabbits fed upon. *Rhipicephalus annulatus* was followed by *H. dromedarii*, *H. excavatum* with higher percentages in the females. Transstadial transmission and efficiency of infectivity to rabbits have reached zero in *H. impeltatum* but only infectivity in *H. analoticum* was zero. Several previous studies have reported the transstadial transfer of *Borrelia* in ixodids (Lane and Burgdorfer, 1987; Derdakova *et al.,* 2004; Kalmar *et al.,* 2015, Lynn *et al.,* 2022) and Argasid (Gaber *et al.,* 1984; Lane and Manweiler, 1988), suggesting a vectorial capacity.

Transovarial Maintenance of *Borrelia* in Tick Generations:

The infected field collected and laboratory-reared *Rhipicephalus annulatus*, *Hyalomma dromedarii* and *H. excavatum* showed transovarial transmission (Tov) of their natural *Borrelia* species to the eggs and hatched unfed larval progeny during the two subsequent generations. *Rhipicephalus annulatus* was the most efficient species where all infected females transovarially transmitted their natural *Borrelia* species to the highest percentage of the unfed larval progeny in each of F1 and F2 compared to the two other tick species. Generally, percentages of Tov and filial infection rates of larval progeny were higher in field-collected than laboratory-reared females and in F2 than in F1 progeny in each tick species. This may suggest a higher borrelial load in the field-collected females and dissemination of *Borrelia* in their ovary tissue. Rollend *et al.,* 2013**,** reported that one field-collected female *Ixodes scapularis* transmitted the borrelial infection to 100% of offspring. The Filial infection prevalence (FIP) was 100%, and the transstadial survivorship of *Borrelia* spirochetes was also 100% for subsequent nymphs and adults. Tissue smears from this infected female revealed a disseminated heavy infection, with spirochetes observed in multiple tissues including the central ganglion, Malpighian tubules, midgut, ovaries, and salivary glands.

In the present study, the higher percentages of the female transovarial transmission of *Borrelia* and infection rates of their larval progeny in the second generation more than the first generation conform to findings in both Ixodid (Bellet-Edimo *et al.,* 2005) and Argsids (Gaber *et al.,* 1984) where this was attributed to the higher number of spirochete invaded ovaries of the mother ticks in the second generation of *Ixodes ricinus* by *Borrelia burgdorferi* and in the second gonadotropic cycle of *Ornithodoros erraticus* by *B. crocidurae*, respectively.

Transmission of Borrelial Infection to Rabbits:

The efficiency of *Borrelia* transmission to the New Zealand white rabbits varied in the different tick sp. examined, their life stages, and the number of infected ticks fed on each rabbit. *Rhipicephalus annulatus* showed the highest *Borrelia* infectivity rates in the rabbits fed upon where one infected adult female and male transmitted *Borrelia* to 66.67 and 33.33% of the rabbits, followed by *H. dromedarii*, and *H..excavatum* where one female or 10 male/rabbit and 10 female or male/rabbit, respectively, transmitted *Borrelia* to one-third of rabbits. Also, 100% of the rabbits fed upon were infected using the least number of infected life stages of a *Rhipicephalus* annulatus (10, 20 and 100 female, male and immatures) in comparison to other examined tick species. This was followed by *H. dromedarii* and *H. excavatum* using a higher number of each tick to obtain similar or lower percent transmission as compared to *R. annulatus*. On the other hand, *H. impeltatum* and *H. analoticum* were the least efficient in transmitting *Borrelia*, where only adult males and female ticks transmitted *Borrelia* to one-third of the rabbits using 10 and 20 adult *H. impeltatum* and *H. anatolicum*, respectively per rabbit.

In each tick species, transmission frequencies in each life stage increased by increasing the number of feeding infected stages per rabbit. Generally, female ticks were more efficient than males and adults than immatures in transmitting borrelial infection as lower numbers of these life stages are required to produce similar or higher levels of infectivity to the rabbit fed upon by each species. The present results agree with those of previous investigators of other ixodids (Piesman & Sinsky, 1988) and argasid ticks

(Shanbaky and Helmy 2000) Those authors found that the efficiency of transmitting *Borrelia* in ticks had increased by increasing the number of infected ticks per host animal, apparently the feeding of a larger number of infected ticks inoculates the host animal with a bigger number of spirochetes than the feeding of a smaller number. Lane *et al.*, (1994) found that the plasma antibody titer of *B. burgdorferi* increased from 1.128 to 1.256 in mice exposed to feeding by one and two infected nymphs, respectively in *I. pacicicus*. Balashov (1972) suggested that spirochetes within the tick body need to reach a threshold level to be able to infect vertebrates.

In conclusion, differences in frequency and efficiency levels of the parameters used to assess vector competence of the 5 tested ixodid ticks for their natural *Borrelia*(e) species pointed to differences in the capacities of the ticks as vectors and their *Borrelia* (e) species as agents of borreliosis. The high percentages of the frequency and efficiency of *Rhipicephalus annulatus* followed by *H. dromedarii* and *H. excavatum* in the acquisition, and maintenance of transmission of their natural *Borrelia* (e) proved the capacities of these tick species to serve as reservoirs and vectors of *Borrelia* (Hoogstraal 1985, Eisen *et al.*, 2020) in Egypt. Also, the high levels of parameters such as Tst and Tov transfer of *Borrelia* in the tick life stages and generations, respectively, and transmission to the vertebrate host might reflect tick specificity for its natural *Borrelia* species (Hoogstraal, 1985 and Shanbaky and Helmy 2000). On the other hand, *H. anatolicum* and *H. impeltatum* life stages showed the ability to acquire *Borrelia* isolated from their corresponding natural populations but generally failed to transstadially and transovarially maintain the infection till adult stage and next generations and only infected field-collected adult ticks transmitted their *Borrelia* with the lowest percent to rabbits fed upon in laboratory. These findings suggested the incompatibility as vectors or refractoriness of the latter two tick spp. for the acquired *Borrelia* in nature. However, further investigations are required to identify the species of *Borrelia* (e) naturally infecting each of the five studied ixodid ticks before proceeding to other ecological and physiological studies on the epidemiological importance of these tick sp. and their *Borrelia* (e). Using the PCR technique, Hassan *et al.* (2017) identified *Borrelia sp.* in *Rhipicephalus annulatus* collected from cows in different localities in Egypt as *B. theileri*. Adham *et al.* (2010) identified *Borrelia* infecting the present ixodid tick species in addition to the argasid *Ornithodoros savignyii* collected from the Giza governorate as being *B. burgdorferi* senso lato. However, Barghash *et al.* (2016) using the PCR technique reported the absence of *Borrelia* burgdorferi in *H. dromedarii* and other *Hyalomma sp.* (H. *ruficepes*, *H. trucatum*, and *H. analoticum excavatum* and *H. impeltatum*) collected on camels in Matrouh governorate. Furthermore, only ixodid ticks were found to be principal vectors of *B. burgdorferi* since its identification as the etiologic agent of LD (Spielman *et al.*, 1985, Grubhoffer *et al.*, 2005). Other ticks and arthropod spp. as blood-sucking insects that accidentally ingested *B. burgdorferi* contaminated blood meals were refractory (Piesman and Sinsky, 1988, Grubhoffer *et al.*, 2005) or failed to transmit the spirochete to clean laboratory animals (Magnarelli *et al.*, 1987; Magnarelli & Anderson, 1988). However, mechanical transmission was not completely excluded (Piesman, 1989).

Declarations:

Ethical Approval: This research paper was approved by the research ethics committee from the Faculty of Science, Ain Shams University (ASU-SCI/ENTO/2024/1/3).

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