The compact of temperature on *Hyalomma dromedarii* larval ticks that feed on *Testudograeca* turtles

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Abstract: Ticks are obligate parasites that feed on the blood of many animals. Haylommaticks are parasites which attach to turtles and other organisms. This study was conducted to determine the probability of *Hyalommad romedarii* ticks parasitism on the turtles bred at homes as is a common hobby in Jeddah, Saudi Arabia. *H. dromedarii* ticks are widely spread in the desert areas in North Africa, Sudan and the Arabian Peninsula and are the major vectors of *Borrelia burgdorferi* bacteria that cause Lyme disease. In some areas, reptiles are considered the mainhosts of the genus Haylomma during the juvenile stages which makes this relationship between host and parasite an exciting one. In order to shed more light on the complex relationship between host and parasite, we infected *Testudograeca* turtles with *Hyalomma dromedarii* ticks. The goal was to study of the effects of temperature on feeding duration, i.e. the number of days needed for reaching repletion, the number of ticks that succeed in feeding to repletion, and the feeding efficiency that resulted in repleting *H. dromedarii* larval tick mass. Experiments were conducted on the turtles through exposing them to three levels of temperature (25°C, 35°C and 40°C). Larvae feeding at 25°C temperature took twice the time taken by larvae feeding at of 40°Ctemperature and approximately the two-thirds of the time that it takes at 3° C. It is posited that temperature during feeding period plays a role through its effects on the circulatory system of the host and functionality of ticks' salivary proteins. Study results demonstrated that temperature is a factor that impacts on the feeding of *H. dromedarii* and similar species of ticks. Further research is needed to clarify the exact mechanisms behind the effects of temperature on ticks feeding and the role of local climate and microclimate in a more detailed way.

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1. Introduction

The *H. dromedarii*miteis the most studied mite species in Saudi Arabia but no study has been conducted on parasitism of juvenile or adult phases when infecting turtles. Keeping turtles at home as a common hobby amongst family members has been noted recently. Research has reported that turtles belong to the hosts parasitized by rigid ticks that are known for their ability to transfer a number of pathogens and motivated the present study. "Acariasis" is the medical term used to describe infections by mites and ticks.

Mites and ticks are the most common ectoparasites that prevalence on reptiles. They can attach to lizards, snakes, turtles, tortoises and terrapins. There are seven species of ticks and more than 250 species of mites that can parasitize reptiles including chiggers (Pollock *et al.,* 2015(.Both mites and ticks feed on the blood of reptiles. In the case of severe infection, they may cause anemia. Ticks may also transfer a variety of diseases and perhaps viruses, parasites and protozoa that live in the blood stream. In some continents such as Africa, ticks may present a real danger in the case of appropriate environmental conditions. They may transmit diseases such as heart water, which is devastating to other animals such as farm livestock and wild animals. They may also transfer diseases that affect humans such as Lyme disease, disease causing spirochete. In general, signs of infection by ticks are usually very clear for they can be seen with the naked eye, especially when they are filled with blood. Infection by ticks may result in the abnormal shedding of skin in reptiles, idiomatically known as dysecdy is, or localized reactions that appear in the place of their attachment to the host. If left untreated, they may cause a variety of morbid symptoms in severe cases and suffocation as observed in some reptiles when ticks close the respiratory passages. Infection by mites may cause various morbid symptoms. Scales and crusts may fall or cracks in the skin may occur with slight hemorrhage. Sores may also be observed in lizards, especially in *iguana schameleon*. Infection may also cause depression or loss of appetite to the infected animals and such animals may be seen rubbing their bodies by the floor the cages they are kept in during the experiments in the laboratory or in the houses of amateur breeders. Infected animals may also immerse their bodies in water for long periods. Severe cases may lead to levels of anemia that cause death. Diagnosis by the naked eye is possible and ticks can be seen under scales or gills in snakes while in the lizards it is common to see them close to the vent or gills. In turtles, ticks often stick to the vent or the soft skin under the shell at the front or back parts of the legs (Burridge, 2005).

Mites are much smaller than ticks and are harder to distinguish. They may be seen as small black or red spots on animals, often around eyecaps or under the scales. They may also be seen floating on the water or on the researcher after carrying the infected reptile.

Ixodid ticks generally appear on three hosts through their life cycle. During the larval stage they feed on two distinct host species while during the nymphal and adult stages, they feed on many types of mammals (Carpenter *et al.,* 1996).

Hyalomma dromedary i ticks are prevalence through desert areas such as Sudan, Egypt and the Arabian Peninsula. In juvenile stages such as larval and nymphal stages, they feed on lizards, birds and rodents and other small mammals. In their adult stage, they feed on a large variety of mammals (Castro and Wright, 2007).Reptiles in general and *Testudo graeca* desert turtles are among the major hosts of the juvenile stages of rigid ticks such as *H. dromedarii, Hyalomma aegyptium* and *I. spacificus* and the percentage of juveniles in some habitats exceeds 90% (Casher *et al.,*2002). The relationship between host and parasite in these species was studied in detail and *S. occidentalis* was found refractory to the infection that causes Lyme diseases which cause spirochete and *Borrelia burgdorferi* (Lane and Quistad 1998). Juvenile ticks carry Borrelia bacteria which parasitize reptiles. Infected reptiles can be cleared of infection through complement-mediated innate immunity (Kuo *et al.,* 2000). Therefore, the role of reptiles in providing a suitable ecology for Lyme disease has seen more frequent study. Some studies have shown that the removal of some species of reptiles such as *S. occidentalis* from oak forests communities can have a dramatic and complex impact on the prevalence of bacteria (*Swei et al*., 2011).

Studying the minor details of *Ixodes* feeding mechanisms and host response may help explain the inherent complexities in the nature of the relationship between host and parasite. The present study differs with respect to the immune responses of *Borrelia* on the reptiles as a host compared to small mammals and birds. This is attributed to the physiologyof thermoregulation for reptiles where Poikliothermic organisms are classified as ectothermis which means that the temperature of their bodies varies during the day because they derive most of their heat from external sources (Pough *et al.,* 2004, To ews and Barker, 2004).

Ticks that feed on lizards gain experience in daily fluctuations in temperature. If found in environments with low temperatures, they may regulate their body buffered heat at such low temperature environments through the warm blood in the fur and plumage of the host. Because ticks are ectothermic like lizards, their physiological processes such as feeding, digestion and excretion are affected by temperature in turtles, which leads to decreased pharyngeal pumping and decreased salivary ejection in *Hyalomma ageyptium*nymphs. This decrease in feeding was observed in recent studies conducted on *Sceloporus undulates, Ixodes pacific s*and *Ixodess capularis* lizards (Rulison *et al.,*2014).

The effects of the behavior of the host on thermoregulation and repeated infestations of larvae for feeding duration in warm temperatures (25.8 - 36.6°C), and in cold temperatures (24.9 – 28.4°C) has been investigated in many previous studies. It was found that ticks fed more quickly in warm temperatures and repeated infestations. Low temperatures result in a n increase in feeding duration and decreased ticks feeding success. For example, decrease of temperature in the ears of the sheep leads to increased feeding duration while acclimatization with low temperatures leads to increased engorgement weight (Norval, 1978). Such effects may be more pronounced in ectothermic hosts like *S. occidentalis* as low temperature can ha ve a negative impact on feeding such as when ticks obtain less blood or even become incapable of feeding to repletion (Pollock *et al.,* 2015).

Feeding on low temperature hostsextends the feeding duration and this became evident during testing the effects of temperature on the success, efficiency and duration, *H. dromedarii*l arvae were fed on *Testudo graeca*. Feeding success is defined as the proportion of ticks that feed successfully to repletion after attaching to host while efficiency of feeding is defined as the rate growth of tick body mass when fed to repletion and the rate of heavy ticks which possess high efficiency due to containing a large quantity of blood from the host. Feeding duration is defined as the average time in days to reach repletion after infesting the host. We studied the hypothesis that low temperatures inhibit the rate of sensitive processes included in feeding ticks on turtles (*T. graeca*) in laboratory in addition to *H. dromedarii* larvae by incubating them at low, intermediate and high temperatures, noting the time required for success of feeding and its efficiency. If the hypothesis is supported, low temperature should increase the feeding duration, increasing the period of success of feeding and lowering efficiency.

2. Material and Methods

Animal collection and maintenances

Adult *H. dromedarii* ticks were selectively collected from camels from a farm in a suburb of Jeddah, Saudi Arabia. Replete females were separated and put in plastic 2 ml. vials and lids were tightly closed by using a mesh in preparation to keeping them in incubators until ovi position stage. The vials contained a mixture of Parisian plaster and activated charcoal to prevent desiccating and retard mold growth. Temperature of incubators was 32°C and humidity was maintained at 85% in a (14- 8) hours light / dark photoperiod. Larvae were kept in laboratories for about four weeks post emergence before placement on lizards to ensure readiness to feed. Turtles were examined to make sure that each group was subject to treatment in at the appropriate standards. Each turtle was placed in a beaker and 50 larvae were put on each one. Beakers were closed by covers secured with rubber bands to prevent the escape of ticks from the beaker. Infestation period were 48 hours during which beakers were sprayed with distilled water to prevent desiccation of the larvae. Beakers were also put beside 60 watt lamps for 12 hours each day. At the end of the infestation period, turtles were separately placed in mesh cages with dimensions of 20/10/10 raised on vials filled with about 4 cm of water so that when they would scatter, they would fall in the vials and float to be collected daily by the examiner according to Pollock et al. (2015). The sides of the vials were wrapped with fluon to prevent escape. Viles were put in 8:14 night / day environmental chambers. It should be that the temperature of turtles ranged between 25°C to 35°C and 40°C similar to the treatment groups. It was found that the temperatures of lizards resting under objects on the ground under (Pollock *et al.,* 2015). Water and leaves of lettuce were provided every day to ensure that animals fed to satisfaction. Replete and unfed ticks were collected daily by collecting the ticksthat dislodged in water for experiment daysuntil collecting all the ticks. Repletelarvae were kept in 120 ml vials for each turtle. At the end of the experiment, the replete arval ticks were quantified and weighed by using a microbalance to the nearest 0.0001g.

Data analysis

Feeding duration was known by estimating the number of days needed by the larva to feed to repletion and to calculate feeding duration. The equation had been applied according to of Pollock et al. (2015). Feeding success was known by calculating the rate of the mass of the larvae that were able to feed to repletion after attaching to the host. It was calculated as the number of larvae fed to repletion / the total number of attaching larvae multiplied by 100. Feeding efficiency was known by dividing the total mass of all repletes by the total number of replete larval ticks' mass to obtain the rate of replete tick mass as according to Pollock et al. (2015). All comparisons, feeding duration, success and efficiency across temperatures were analyzed with different temperatures through using Paired-Samples Test comparative. Analysis was conducted by using Spssversion 21, all probability.

3. Result

The number of larvae attached to their hosts ranged between 25-48 but by calculating the rate, they reached 29 larvae. The larvae were incubated at a temperature of 40°C, fed to repletion and were then dropped from their hosts and replaced by larvae that were incubated at a temperature of 35°C.At 25°C, larvae took longer to reach repletion and then fell from their hosts (Table 1). Feeding duration rate was 7.9-9.6 days when the larvae were incubated at temperatures 40°C, 35°Cand 25°C respectively. Feeding duration was observed as significantly higher at 25°C temperature compared to that of larvae incubated at temperatures of 35°C and 40°C. Feeding duration at temperatures 35°C and 40°C show any significant difference (Table 1). Feeding success and feeding efficiency was not affected by temperature (Tables 1,2) (Figure 1).

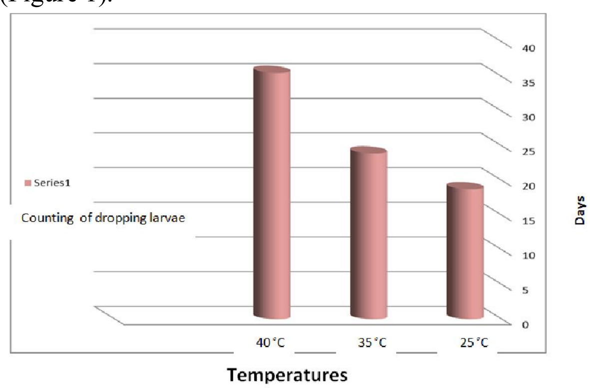

Fig. 1:The number of dropping larvae in the duration of the experiment at three different temperatures (Larvae fed significantly faster at 40°C and 35° C compared to 25°C)

Table 1: Cumulative number of replete larval ticks a t25° C,35° C and 40° C. Values are shown as means ± 1 SEM.

		Mean	N	±Std. Deviation	±Std. Error Mean	sig
Pair 1	numb1	18.7500	28	9.21201	1.74091	
	days	14.5000	28	8.22598	1.55456	0.0
Pair 2	numb2	23.9286	28	10.73564	2.02885	
	days	14.5000	28	8.22598	1.55456	0.0
Pair 3	numb3	35.5357	28	11.79605	2.22924	
	days	14.5000	28	8.22598	1.55456	0.0

numb1:number of dropping ticks at 25°C
numb1:number of dropping ticks at 35°C
numb1:number of dropping ticks at 40°C

Table 2: Feeding success: The mean number of replete larval ticks fed to replete on the total number of larvae feeders recorded for each temperature.Values are shown as means ± 1 SEM.

		Mean	N	Std. Deviation	ectoparasites Std. Error Mean	sig
Pair 1	succ1	.5720	5	.01304	.00583	0.329
	succ2	.6060	5	.00894	.00400	
Pair 2	succ1	.5720	5	.01304	.00583	0.229
	succ3	.9620	5	.02280	.01020	
Pair 3	succ2	.6060	5	.00894	.00400	0.322
	succ3	.9620	5	.02280	.01020	

succ1: Feeding success at 25°C; succ 2: Feeding success at 35°C succ3: Feeding success at 40°C

Table 3: mean body masses (feeding efficiencies) for replete larval ticks fed at 40, 35, and 25 C. table represent the range of body mass recorded for each temperature. Values are shown as means ± 1 SEM.

		Mean	N	±Std. Deviation	±Std. Error Mean	sig
Pair 1	eff1	.7560	5	.00894	.00400	0.685
	eff2	.8740	5	.00894	.00400	
Pair 2	eff1	.7560	5	.00894	.00400	0.278
	eff3	.4540	5	.48475	.21679	
Pair 3	eff2	.8740	5	.00894	.00400	0.867
	eff3	.4540	5	.48475	.21679	

eff1: feeding efficiency at 25°C eff2: feeding efficiency at 35°Ceff3: feeding efficiency at 40°C

4. Discussion

Ticks belong to a class of arthropods that parasitize turtles. The danger of ticks on man, his cattle and his pets at home lies in their ability to transfer diseases as they have the ability to transfer a number of diseases to humans such as lapsing fever and western equine encephalitis virus. Some studies reported that certain types of disease-causing ticks were transported while importing infected turtles (Simmons and, Burridge, 2000). It has been known for many years that the import of turtles from the areas infested by ticks to other areas causes spread of the diseases carrying their pathogens (Burridge, 2001).

Eight species of ticks have been at least discovered to be transported to Florida with turtles (Burridge *et al.,* 2000a). These ticks were observed to have the ability to infect humans, dogs and other mammals with diseases such as Q fever Studies have also reported the role of rigid ticks in the transfer of many haemogregarines infections by turtles (Burridge *et al.,* 2000a, Tavassoli *et al.,* 2007)

Feeding duration, efficiency and success are all important factors for the growth of tick populations. If the juveniles are capable of feeding successfully to repletion and drop off and molt, this is likely to result in allowing molting of larger numbers of adult ticks whose development depends on them reaching adult stage during the feeding period due to their ability to feed successfully. To reach this, ticks depend on several factors like host species and their immune function with respect to speed and type of immune reaction in addition to tick species and the ir life stage and temperature (Aponaskevich and Oliver, 2014). With rabbits, for example, there is no relationship between environment temperature and feeding duration of *Haylomma dromedarii* ticks (Hagras and Khalil, 1988). But there are such differences when observing that feeding duration of (Dermacentoran dersoni) ticks in cattle varies depending on the temperature and cattle strains (Lysyk, 2008).It is clear that there are several factors that affect ticks' ability to get the necessary blood meal, but most studies have focused on endothermic species like mammals. The studies that have been conducted on endothermic species are limited. The studies conducted on *I. pacificus* and *S. occidentalis* showed the effects of host sex, reproductive state, and the percentage of hematocr it in the host's blood on the feeding duration (Pollock *et al.,* 2012; Pittman *et al.,* 2013). The effects of temperature on tick feeding has been conducted only in the study by Pollock et al. (2015).This study showed the impact of temperature on the feeding duration but not on feeding efficiency and success in ectothermic poikilothermic species. Despite the lack of significant effect of temperature on feeding efficiency, the variation in replete larval mass was the highest at the temperature of 40°C. This variation may contribute to correlation between the number of replete larvae and their masses. The rate of replete larvae mass decreases with the increase of the larvae dropping off host lizards. In previous studies, the effects of tick density on tick feeding and feeding efficiency was investigated and mixed results were obtained. However, there was some support for the idea that increasing tick density leads to increased feeding deficiency (Sutherst *et al.,* 1973). Other studies also reported that the increase in the density of ticks leads to increase in feeding efficiency (Davidar *et al.,* 1989, Ogden *et al.,* 2002). Pollock *et* *al.* (2015) found the that the duration needed by *I. pacifiacus* larva to feed to repletion on *S. occidentalis*lizards increases at low temperatures, which agrees with what we found in this study, the feeding period taking longer at 25°C. Similar results were observed in a similar parasitic relationship with *S. undulatus* and *I. scapularis* in the United States where the larvae fed faster to repletion with warmer temperatures (Rulison*et al.,* 2014).

In Rulison *et al.* (2014) and the current study, the impact of temperature on the feeding duration of *Ixodid* ticks clearly manifested. However, the impact of feeding duration on the life cycle of ticks in general has not been directly demonstrated. It is very possible that the decline in feeding duration of larval ticks may increase their survival for their subsequent life stages. For example, *Hyalomma* larvae hatch in the middle of the winter and start their quest for a host in early spring and this extends until the first half of winter in the outskirts of Jeddah which are the areas of our study. However, it has been observed that the other species, *Ixodespacificus*, only hatch in late summer on in cold countries and then begin their quest for the host at the beginning of spring which represents the peak larval densities period at the end of April and early May (Padgett and Lane, 2001; Eisen *et al.,* 2002) in conjunction with the peak breeding of *S. occidentalis* species, the primary larval host (Bromwich and Schall, 1986; Casher *et al.,* 2002). Replete larvae drop off and turn into nymphs during mid summer while the larvae that failed to realize their life cycle and encounter an appropriate host for feeding fail to molt and thus fail to survive (Padgett and Lane 2001).This variation in the duration of ticks activity and breeding during different seasons in the outskirts of Jeddah in the west region of Saudi Arabia in the desert of the Arabian Peninsula is the reason behind this abundance in number and active breeding, which nearly persists over the entire year due to the warm climate temperature necessary for completion of their life cycle and breeding several generations in shorter periods. Hence, due to the fact that larval ticks feed to repletion faster at high temperatures, the development duration for engorged larvae to transform into nymphs is shorter at high temperatures (Ogden *et al.,* 2002). Molting is expected to increase in the nymphal stage and therefore the chances of survival in hot countries such as Saudi Arabia increase resulting in their effectiveness as a potential infection factor in most seasons of the year. However, determining the mechanism of how temperature governs decline in feeding duratrion of Ixodid ticks remains unclear. But there is one potential way to increase tick feeding duration with low temperature while under the effects of parasite behavior and host circulating system. Low-temperature environments have shown a decrease in the feeding activity of the ticks and coordination (Clark, 1995), which in turn requires a longer duration for the tick to obtain sufficient blood meal in addition to the fact that temperature affects the ticks feeding while in endothermic species. The decrease in temperature decreases cardiac output, decreases the heart beatrate, and decreases the volume of blood circulation (Pollock *et al.,* 2015). Each of these reasons leads to a slowdown in the flow of blood of feeding ticks resulting in a significant increase of the duration required for feeding to repletion (satiety).

There is another way that may affect the feeding duration which is through changing tick protein expression and activity. Depending on its attachment to the host, the tick produces salivary cocktail proteins including anti-coagulants, bandages platelet, anti-platelets and vasodilators that maintain blood flow to the feeding ticks, and immunomodulators which balance and counteract the host inflammatory and immune responses(Steen *et al.,* 2006).Due to the fact that these salivary components are proteinaceous and have temperatures that represent the peak at which they operate efficiently, salivary proteins show a drop in activity at low temperatures. Therefore, the temperature of 22°C showed decline in salivary proteins functions which may lead to increased feeding period due to reduced ability to maintain necessary and adequate blood flow to feed ticks, which was supposed by Pollock *et al.* (2015).

Some anticoagulants in ticks, for example, are identified as slow-binding competitive inhibitors at low temperatures (Waxman *et al.,* 1990; Limo *et al.,* 1991) similar to the effects of the proteins known as hemostatic proteins. It has been observed that ticks can modify the immunomodulatory of salivary proteins at low temperatures. As a result of that, the tick extends the duration of feeding due to the difficulty in obtaining adequate blood meal while combating the host immune response in the ectothermic host reptiles. Thus, their immune cells and proteins may also be affected by heat. In fact, it has been observed that immune functions are affected by decrease in temperature in preserving the survival of several ectothermic species (Mondal and Rai, 2001and Merchant *et al.,* 2003)

Generally, we believe that it is unlikely to have effects related to temperature on the immune functions of the host that play a significant role in observing the decrease in feeding duration of the tick at 25°C temperature. Regardless of temperature, ticks feed successfully and similar to engorgement mass, variations in the success and efficiency of feeding among temperature groups are expected for temperatures which have effects on the role of immune functions.

In a study by Gable and Oliver (1992), lack of resistance after multiple tick infections on the host by the same species of ticks was indicated. Ticks feed on lizards more efficiently than they feed on mice and guinea pigs.

Future research should be concerned with the probability of the existence of a potential relationship between temperature, host competence and ticks transfer to *Borrelia* bacteria.

Some research reports that 10% of ticks around the world were found to be parasitic on turtles and were recognized by their ability to cause significant damage (Burridge, 2001). To identify the role of ticks in spreading diseases, it is necessary to recognize similarities and differences in the biological and physiological aspects between all involved species in the spread of diseases and parasites. The importance of such studies originates from ticks' economic and medical impact on human communities and domesticated animals in their surroundings. It has been found that Haylomma ticks are the most widespread parasites in warm environments, desert areas and semi-desert areas, and in general, low and mid high are as and the countries characterized by long dry seasons in central and southwest Asia, Africa, and else where.It has been found that under the subgenus Haylomma, there are 15 genera that have national veterinary and public health importance Tavassoli *et al.,* 2007). Some studies showed frequent documented infections by ticks, especially *ornithodoro sparkeri* and as an ecto-parasite on turtles in addition to the fact that ticks transfer mycoplasms that are involved in spreading vine pleuropneumonia disease in Africa. This has been seen in earlier studies and was not included under natural conditions. In studies conducted in southern Europe, it was found that the *H. aegyptium* tick belongs to the parasites whose main hosts are turtles, lizards, dogs, horses, hedgehogs, hamsters and birds. It was also found in Italy on partridge birds and in Egypt on quails, pigeons and songbirds (In Pakistan, it was found on oxen and cattle. In Turkey, a study reported *H. aegyptius* ticks'ability to transfer *Theileria thirici* to sheep (Tavassoli*et al.,* 2007).

Crimean-Congo hemorrhagic fever virus (CCHFV) is mainly transferred to humans after being bitten by *Hyalomma*ticks (Tavassoli *et al.,* 2007). In Iran, ticks play role in transmission of (50) infectious diseases from animals to humans. Since there are a lot of people who like to keep turtles in their homes, they have to observe periodic veterinary checkups and pay attention to their cleanliness. The danger of infection by diseases transmitted by ticks still exists for people with weak immune systems such as the elderly and children less than ten years. It is necessary to take some precautions such as keeping them clean and keeping them away from kitchens and food processing areas to reduce their danger in addition to the necessity of performing veterinary examinations before bringing animals home.

In conclusion, the direct relationship between temperature and the duration of feeding in this species (*H. dromedarii*) that parasitizes poikilothermic ectothermic (*Testudo graeca*) turtles on the blood circulatory system of the host or the functional role of ticks salivary proteins although the survival of this hypothesis is still subject to experimental evaluation according to Pollock *et al.* (2015). Salivary proteins were isolated and characterized in Ixodid ticks (Francischetti *et al.,* 2010).Future research should focus on the effects of temperature for it may have a role on the kinetics of these proteins. Due to evidence in this study, I hypothesized the role of the host immune function on ticks feeding. Future studies have to seriously explore the existence of potential effects of temperature on the ectothermic host immune response on ticks feeding.

Finally, our results presented here do not suppose existence of the effect of temperature on the transfer of *B. burgdorferi* and Pollock *et al.*(2015) report a study that was conducted on *S. occidentalis* showing that it is an incompetent host (Levin *et al.,* 1996). Nevertheless, ectothermic species act as hosts of *Borrelia* species, *B. lusitaniaee* and *Padarcis* species (Richter and Matnschka 2006 and Pollock *et al.,* 2015). Temperature could possibly have an effect on the role of transmission dynamics.

Future research should be concerned with the probability of the existence of a relationship between temperature, host competence and ticks' transfer of bacteria, viruses, protozoa and other pathogens.

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