

# INFESTATION MODALITIES OF *HYALOMMA AEGYPTIUM* (ACARI, OXYDIDAE) ON THE SPUR-THIGHED TORTOISE *TESTUDO GRAECA* IN SEMI-ARID AREAS OF ALGERIA

H. AOURAGH<sup>1</sup>, R. CHAIBI<sup>2</sup>, A. SI BACHIR<sup>3\*</sup>

<sup>1</sup> Department of Nature and Life Sciences, Faculty of Exact, Nature and Life Sciences, University Mohamed Khider, Biskra, Algeria

<sup>2</sup> Laboratory of Biological and Agronomic Sciences (LSBA), Faculty of Exact, Nature and Life Sciences, University Amar Telidji, Laghouat, Algeria

<sup>3</sup> Department of Ecology and Environment, Faculty of Nature and Life Sciences, University Mostefa Benboulaïd, Batna 2, Algeria

\* Corresponding author: a.sibachir@univ-batna2.dz

*HYALOMMA AEGYPTIUM*  
TICK  
*TESTUDO GRAECA*  
SPUR-THIGHED TORTOISE  
TICK INFESTATION  
SEMI-ARID REGION  
ALGERIA

**ABSTRACT.** – In semi-arid areas of the Saharan Atlas region in Algeria, 33 tortoises (*Testudo graeca*) were examined for ticks in 2017-2018. All ticks found were adults and they were identified as *Hyalomma aegyptium*. For the total sample studied, the infestation prevalence was 63.6 %. The mean infestation intensity was 4.04 and the parasite abundance was 2.57. The infestation prevalence, the mean infestation intensity and the parasite abundance in female tortoises were higher than in males, but this variation was statistically insignificant. The number of ticks was positively and significantly correlated with tortoises' body measurements: body weight, straight carapace length, carapace height, mid-body carapace width and plastron length. The parasitism by female and male ticks separately was not statistically significant. A total of 40 % of ticks were found attached to the hind limbs, 37.7 % to the forelimbs, 10.6 % to the neck, 11.7% to the tail, and no individuals were detected on the carapace. Statistically, the forelimbs and the neck were the preferred attachment areas for *H. aegyptium*.

## INTRODUCTION

The Spur-thighed tortoise (*Testudo graeca* Linnaeus, 1758) has a large geographic distribution under a very wide range of climatic and environmental conditions, across the Mediterranean region, to Turkey and Iran (Fritz & Havaš 2007, Anadón *et al.* 2012). In North Africa, it exists from Morocco to Libya; populations were probably introduced to several Mediterranean islands (Corti *et al.* 2011). It can colonize different habitats as wastelands, forested areas, sandy terrains, open steppes with bushy vegetation, gardens, and cultivated fields. It lives from sea level to about 2090 m of altitude and from 116 to 1093 mm of annual precipitation (Anadón *et al.* 2012). In Algeria, the Spur-thighed tortoise (*T. graeca*) has a wide distribution area across the littoral regions to the Saharan Atlas (Tiar *et al.* 2019).

According to IUCN (2002), *T. graeca* is classified as vulnerable. In Algeria, it is classified as a threatened and protected wildlife species. Natural populations suffer from a big reduction in populations due to collection, sale, pesticide and insecticide, deaths and fires. The animal suffers from great degradation and alteration in its natural habitat. Otherwise, parasites may reduce the tortoise population densities by having an impact on the host's fecundity and survivability (Hudson *et al.* 1998, Albon *et al.* 2002).

Tick infections are characterized, in some cases, by complicated ecological and epidemiological relation-

ships between the parasite and the host. This relation is regulated by many factors as host density, host age, host sex, and parasite density, which may determine the spatial and temporal dynamics of both the parasite and the host. Rosá and Pugliese (2007) have proved that the transmission and the persistence of the ticks are controlled by the dynamics of the tick population and by their host densities. Considering the role of parasites in affecting population dynamics, on the other hand, tortoises are becoming very popular as pets and they can spread parasitic diseases to humans. Thus, studies related to the parasitic diseases in the Spur-thighed Tortoise have gained attention in recent years.

In Algeria and North Africa, *T. graeca* is not largely studied. Few authors have worked on its tick parasitic-fauna but mainly in the northern regions (Benyacoub *et al.* 2007, Rouag *et al.* 2008, 2017, Gharbi *et al.* 2015; Tiar *et al.* 2016, Segura *et al.* 2019). To our knowledge, no study was focused on the parasitic-fauna of Spur-thighed tortoise in semi-arid areas.

Therefore, the aim of this study was to identify the tick species that parasitize the Spur-thighed tortoise in the Saharan Atlas region (semi-arid area of Algeria) and to investigate the infestation modalities by analyzing the impact of tick sex; tortoise sex; tortoise body size, body weight and body region on the variations of tick parasite parameters in the studied tortoise populations.

## MATERIALS AND METHODS

**Study site and sampling method:** Tortoises were collected in May 2017 and 2018, from Tibikaouine area (35°22'36"N; 6°26'27"E), in Batna province (north of the Saharan Atlas, Algeria), which is located at the semi-arid climate stage. Sampling was conducted while walking and tortoises were captured by hand, from a rural territory, characterized by low vegetation cover density with some vegetable crops. They were placed in a dark box to remain tranquil and transported to the laboratory for age and sex identification and to undergo different body measurements.

Finally, captured tortoises were transported and released back into their natural habitat, in the same locality where they were captured. All experimentations were made in respect of ethical considerations, with care to the animals, and according to the national legislation texts concerning threatened and protected wild animal species in Algeria. This study was also approved by the local authorities (Forest Conservation Office of Batna).

**Biometric and morphometric parameters measured on tortoises:** A total of 33 tortoises were examined (20 males, 10 females and 3 juveniles). Captured individuals were sexed using the morphological characters of the plastron and the tail (Ben Kaddour *et al.* 2005). This was easy for specimens with a carapace size greater than 10 cm; those with a carapace of less than 10 cm were classified as juveniles. The tortoise age was estimated through the counting of scute rings on the carapace (Wilson *et al.* 2003, Rodriguez-Caro *et al.* 2015).

Total body weight in g (WT) was measured by a digital balance (precision  $\pm 1$  g). Measurements were taken in centimeters with a caliper (precision  $\pm 0.1$  cm) for: straight carapace length (CL: maximal anteroposterior carapace length), carapace height (H: maximal height of the carapace), mid-body carapace width (CW: body width in the middle of the abdominal scute), plastron length (PL: along midline from gulars to anal scutes).

Tortoises were macroscopically examined for the parasitological study. Ticks were still attached to tortoises during transport. In the laboratory, ticks were collected to study the different parasite forms of tortoises. All ticks were collected, counted and placed in ethanol 70 % for later identification. Stage and sex were recorded, using standard phenotypic keys (Meddour-Bouderda & Meddour 2006). The attachment site on the tortoise body was also recorded: carapace, plastron, forelimb, hind limb, neck and tail.

**Parasitological indicators and statistical analysis:** According to Bush *et al.* (1997), three parasitological indicators were calculated: infestation prevalence (%) = (number of infested tortoise  $\times$  100) / total number of examined tortoises; mean infestation intensity = number of parasites / number of infested

tortoises; parasite abundance = number of parasites / total number of examined tortoises.

Most data had a non-normal distribution; they were analyzed by U Mann-Whitney test to assess the statistic variation of the infestation by tick sex and tortoise body regions. The variation of infestation by tortoise sex was studied using Chi square test. Differences considered statistically significant when  $P \leq 0.05$ . Spearman's correlations were used to test the relationships between the ticks' number and tortoise body measurements (CL, H, CW, PL, and WT), also between the total number of ticks on each tortoise and their number in each attachment area of the tortoise body. Correlations were considered significant at  $P \leq 0.05$ . All analysis was carried out with IBM SPSS Statics for Windows, version 25.0. Armonk, NY: IBM Corp.

## RESULTS

### *Overall parasitism and its variation by host sex*

All ticks collected on *Testudo graeca* were identified as *Hyalomma aegyptium* (Linnaeus, 1758) (Acari: Ixodida, Ixodidae) (Fig. 1). A total of 85 ticks (24 females, 61 males) were collected and no immature ticks were found. Among the 33 examined tortoises, 21 were infested by ticks. The number of ticks per host ranged from 1 to 13. In relation to the total population, the infestation prevalence was 63.6 %; it ranged from 0 % in juveniles to 39.4 % in males. The mean infestation intensity was 4.04 and the parasite abundance was 2.57 (Table I).

Among the 10 examined females, 8 were infested by a total of 50 ticks; the tick number by host ranged from 1 to 13. For the 20 examined males, 13 were infested by 35 ticks; the tick number by host ranged between 1 and 8. Infestation prevalence in females (80 %) was higher than in males (65 %). The mean infestation intensity in females was 6.25 and only 2.7 in males. The parasite abundance was higher in females (5.0) than in males (1.75) (Table I). The parasitism variation regarding the tortoise sex was not statistically significant ( $\chi^2=0.17$ ; ddl = 1;  $P = 0.67$ ).

### *Parasitism variation by the host's body size and weight*

The plastron length (PL) of *T. graeca* ranged from 3 to 18.8 cm (mean =  $11.08 \pm 0.5$  cm), the straight carapace length (CL) from 3.7 to 18.5 cm (mean =  $13.5 \pm$

Table I. – Variation of tick infestation of *Testudo graeca* in the Algerian semi-arid region according to tortoise sex.

	Range (individuals)	Infestation prevalence (%)	Mean infestation intensity	Parasite abundance
Males (n = 20)	1–8	39.4	2.70	1.75
Females (n = 10)	1–13	24.2	6.25	5.00
Juveniles (n = 3)	–	–	–	–
Total (n = 33)	1–13	63.6	4.04	2.57



Fig. 1. – The tick *Hyalomma aegyptium* collected on Spur-thighed tortoise (*Testudo graeca*) in the semi-arid region of Algeria. **A:** Male, dorsal view; **B:** Male, ventral view; **C:** Female, dorsal view; **D:** Female, ventral view; magnification x20.

0.5 cm), the mid-body carapace width (CW) between 3.3 and 13.0 cm (mean =  $9.9 \pm 0.3$  cm) and the carapace height (H) ranged between 2.0 and 10.8 cm (mean =  $6.8 \pm 0.2$  cm). Within all classes of straight carapace length, only two classes were infested (Fig. 2A): the ]10-15] and ]15-20] cm classes. All parasitological indicators (infestation prevalence, mean infestation intensity and parasite abundance) were higher in the ]15-20] cm class for both males and females (Table II).

Both the total number of collected ticks and separately the number of males and females were positively and sig-

nificantly correlated with all the tortoise body sizes (PL, CL, CW and H) (Table III).

*Testudo graeca* weight (WT) ranged from 12 g to 640 g (mean =  $296.1 \pm 24.2$  g). Within the four weight classes in males, only three were infested (Fig. 2B): in the ]150-300] g class, the infestation prevalence was 50 %, the mean infestation intensity was 1.5 and the parasite abundance was 0.75. In the ]300-450] g class, the infestation prevalence was 88.8 %, the mean infestation intensity was 2.62 and the parasite abundance was 2.33. In the ]450-600] g class, the infestation prevalence was 100 %,

Table II. – Infestation prevalence, mean infestation intensity and abundance of *Hyalomma aegyptium* ticks according to the infested straight carapace length classes of *Testudo graeca*.

Carapace length classes (cm)	Tortoise males			Tortoise females		
	Infestation prevalence (%)	Mean infestation intensity	Parasite abundance	Infestation prevalence (%)	Mean infestation intensity	Parasite abundance
]10-15]	57.14	2.62	1.50	66.6	4.00	2.66
]15-20]	83.30	2.80	2.33	85.7	7.00	6.00

Table III. – Relationship between ticks' numbers and tortoise sizes (R and P-value from Spearman's correlations).

	Total ticks		Female ticks		Male ticks	
	R	P	R	P	R	P
Plastron length	0.55	0.001	0.40	0.021	0.54	0.001
Straight carapace length	0.50	0.005	0.42	0.019	0.50	0.005
Mid-body carapace width	0.44	0.013	0.40	0.026	0.43	0.016
Carapace height	0.54	0.000	0.56	0.001	0.60	0.000

Table IV. – Individuals number, infestation prevalence, mean infestation intensity and abundance of *Hyalomma aegyptium* ticks according to the *Testudo graeca* sex.

	Individuals number		Infestation prevalence (%)		Mean infestation intensity		Parasite abundance	
	♂	♀	♂	♀	♂	♀	♂	♀
Tortoise sex								
Female ticks	1-3	1-4	18.18	18.18	1.66	2.33	0.30	0.40
Male ticks	1-5	1-11	36.30	21.20	2.08	5.14	0.75	1.09

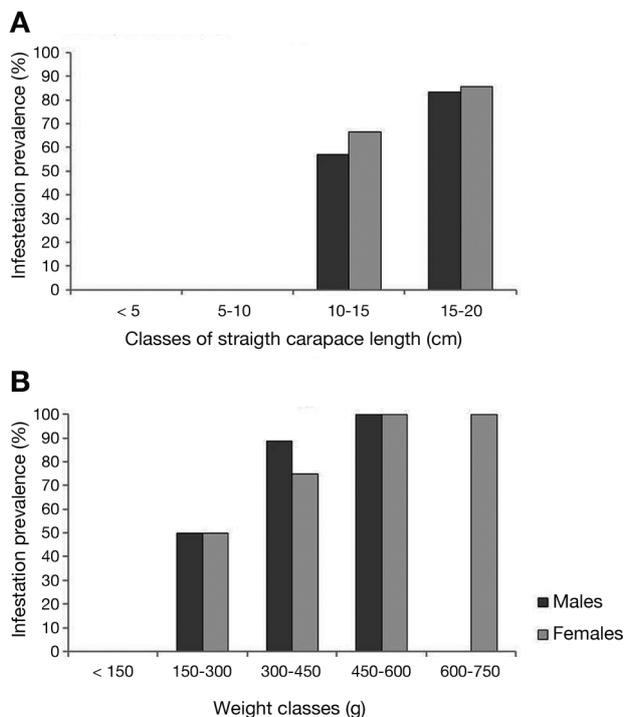


Fig. 2. – Infestation prevalence of the tick *Hyalomma aegyptium* according to the classes of straight carapace length (A) and weight classes (B) of Spur-thighed tortoise (*Testudo graeca*) in the semi-arid region of Algeria.

the mean infestation intensity was 8.0 and the parasite abundance is 8.0.

In females, the four weight classes were infested (Fig. 2B): [150-300], [300-450], [450-600] and [600-750] g with an infestation prevalence of 50 %, 75 %, 100 % and 100 %, respectively. The mean infestation intensity was 1, 4.33, 7.66 and 13, respectively. The parasite abundance was 0.5, 3.25, 7.66 and 13, respectively. The ticks' total number and the number of male and female ticks separately were positively and significantly corre-

lated with the body weight of tortoises (total ticks:  $R = 0.80$ ,  $P < 0.001$ ; female ticks:  $R = 0.73$ ,  $P < 0.001$ ; male ticks:  $R = 0.77$ ,  $P < 0.001$ ).

#### Relationship between infestation and ticks' sex

For the total of 85 collected ticks, 24 were females, 10 (41.6 %) were collected from 6 males and 14 (58.3 %) were collected from 6 female tortoises (Table IV). The variation in parasitism by female ticks was not statistically significant ( $U = 64.00$ ;  $P = 0.07$  Mann-Whitney test). For the

total collected ticks, 61 were males. Among the male ticks, 25 (41 %) were collected from 12 male tortoises and 36 (59 %) from 7 females (Table IV). The variation in parasitism by male ticks was not statistically significant ( $U = 65.5$ ;  $P = 0.11$  Mann-Whitney test).

#### Relationship between ticks' infestation and tortoises' body regions

A total of 36.3 % of the studied tortoises (12) had ticks on their forelimbs; 39.40 % (13) on hind limbs; 27.27 % (9) on the tail and 21.21 % (7) on the neck (Table V). No ticks were found on the carapace or plastron. The variation in tick infestation according to tortoises' body regions was statistically significant with neck ( $U = 58.0$ ;  $P = 0.012$  Mann-Whitney test); with forelimbs ( $U = 59.5$ ;  $P = 0.04$  Mann-Whitney test) and it was not significant with hind limbs ( $U = 79.0$ ;  $P = 0.30$  Mann-Whitney test) and tail ( $U = 82.5$ ;  $P = 0.33$  Mann-Whitney test) (Table V). Spearman's correlation shows a significant positive correlation between the total number of attached ticks and the tick numbers on all body regions' attachment (Table VI).

## DISCUSSION

Despite the sufficient number of tortoises examined in this study, *Hyalomma aegyptium* was the only tick found. Studies have clearly demonstrated the potential of this tick to infect humans and different animals such as eastern hedgehogs, cows, buffaloes, lizards and hares (Hoogstraal 1956, Bursali *et al.* 2010, Gazyağci *et al.* 2010, Rafique *et al.* 2015). It should be noted that *H. aegyptium* is still the dominant tick that infests natural populations of *T. graeca* in Northern Africa, the Middle East (Široký *et al.* 2009), Balkan countries (Široký *et al.* 2006), Iran (Javanbakht

Table V. – Number of ticks, infestation prevalence, mean infestation intensity and parasite abundance of *Hyalomma aegyptium* ticks according to the *Testudo graeca* body regions.

Body region	Number of ticks	Percentage of ticks (%)	Infestation prevalence (%)	Mean infestation intensity	Parasite abundance
Hind limbs	1-5	40.0	39.40	2.61	1.03
Forelimbs	1-6	37.7	36.30	2.66	0.96
Tail	1-2	11.7	27.27	1.11	0.30
Neck	1-2	10.6	21.21	1.28	0.27
Carapace	–	–	–	–	–
Plastron	–	–	–	–	–

Table VI. –Relationship between the total number of the attached ticks and the tick numbers on tortoise body regions (R and P-value are from Spearman's correlations).

Parameters	R	P
Total number of ticks attaching / number of ticks on neck	0.60	0.000
Total number of ticks attaching / number of ticks on forelimbs	0.87	0.000
Total number of ticks attaching / number of ticks on hind limbs	0.87	0.000
Total number of ticks attaching / number of ticks on tail	0.38	0.027

et al. 2015), Malta (Loporto et al. 2017) and Turkey (Kireççi et al. 2013). In Morocco, Segura et al. (2019) has identified in a population of *T. graeca*: *H. aegyptium*, *H. marginatum* (Koch, 1844), *H. excavatum* (Koch, 1844) and *H. scupense* (Schulze, 1919). *T. graeca* is also a natural host of *Rhipicephalus sanguineus* (Latreille, 1806) (Ixodida, Ixodidae) and *Haemaphysalis sulcata* (Canestrini & Fanzago, 1878) (Ixodida, Ixodidae) in its natural habitat in Turkey and Balkan countries (Široký et al. 2006, 2009). This can be accidental without biological or epidemiological effects (Široký et al. 2006). However, to our knowledge, the Spur-thighed tortoise studied in Algeria is infested exclusively by *H. aegyptium* (Široký et al. 2009, Harris et al. 2013; Tiar et al. 2016) and no one has reported the presence of this ectoparasite on other hosts. Sadly this tick species has been found to be a vector of various human and animal pathogens in Algeria, such as *Rickettsia aeschlimannii* infection (Bitam et al. 2009), Crimean-Congo hemorrhagic fever (CCHFV) (Kautman et al. 2016) and *Hemolivia mauritanica* infection (Tiar et al. 2010).

In accordance with previous studies, our results show that the infestation prevalence varies widely on *T. graeca*, especially according to the studied regions. Široký et al. (2009) have counted 11 infected tortoises from a sample of 16 in north-eastern Algeria (68.75 %), which is close to the global value obtained in our study. However, Tiar et al. (2016) have reported various levels of infestation prevalence in different areas in Algeria (from 9 to 100 %). The highest prevalence found may be explained by the collection of ticks at adult and immature stages. In Tunisia, Gharbi et al. (2015) have reported an infestation prevalence of 66.19 %.

Otherwise, our results indicate that the tortoise sex had

no role in the tick infestation as similarly to what was indicated by Gharbi et al. (2015) and Tiar et al. (2016). On the other hand, males of *T. graeca* in Morocco, those of *T. graeca nikolskii* in Russia and males of *Homopus s. signatus* (Gmelin, 1789) in South Africa have a higher parasitism by ticks than females (Robbins et al. 1998, Loehr et al. 2006, Segura et al. 2019). The tick infestation on tortoises can be influenced by habitats of each sex; for example, females of *T. graeca* have larger home ranges than males (Rouag et al. 2017). In addition, in some periods, males can spend nearly twice as much time walking than females (Lagarde et al. 2002); consequently, this makes them more prone

to ticks. Also, the relatively large carapace openings of males, compared to females, may explain why males have more ticks than females (Loehr et al. 2006). Salvador et al. (1996) have indicated that an elevation of testosterone levels in plasma makes animals more infested by ticks.

Our results showed that large adult tortoises were infested, meanwhile juveniles were not parasitized. This corresponds to the results of Široký et al. (2009) and Tiar et al. (2016). In fact, larger animals provide larger external surfaces for ticks (Široký et al. 2009, Esser et al. 2016). In addition, larger animals move further, they may visit diverse habitats; this help to elucidate why they acquire more ticks than smaller animals (Diaz-Paniagua et al. 1995, Loehr 2002, Loehr et al. 2006, Esser et al. 2016).

The individual number of both males and females of *H. aegyptium* did not differ between tortoises. It can be explained by mating habits of the sexes, males stay fixed to tortoises waiting for females for a long time until their removal or death (Široký et al. 2011). Females normally engorged detach quickly from the tortoises to produce and lay eggs and die. On the other hand, virgins can stay attached to the host for a long time waiting for a male. Otherwise, virgins remain attached until the host immunological responses may finally result in their elimination or death (Kaufman 2007). Gharbi et al. (2015) in Tunisia and Tiar et al. (2016) in Algeria have indicated that *T. graeca* is significantly more infested by male than female ticks, possibly the previous studies coincided with the detachment phase of the females to lay eggs.

In our study, ticks were found in areas of soft skin that are protected by the carapace; we did not find any ticks on the carapace or plastron. This allows ticks an easy attachment and feeding with a good protection. It should be

noted that the preference for attachment sites varies with tick species and sometimes with host species. For example, adults of *Amblyomma sylvaticum* (de Geer, 1778) were found attached to the carapace of its host, they are able to produce histolytic secretions that can dissolve the carapace and create pits for attachment (Pearcy & Beyer 2013). The segregation of tick attachment areas on reptiles may be explained as: (i) a result of interspecific relations; (ii) adaptations to increase mating and feeding success and (iii) protection from physical disturbance (Fielden & Rechav 1994).

The forelimbs and the neck were the preferred attachment areas for *H. aegyptium* in our study. Brianti *et al.* (2010) had found that 89.8 % of the infested tortoises had ticks on their hind limbs, 21 % on forelimbs, 18.6 % on the tail and pre-anal area, and 4.8 % on the head. They reported that the hind limbs were the preferred site for *H. aegyptium* on its host. Plausibly, the ticks may attach to the neck and the forelimbs because they are the first regions encountered when the tortoise moves. Pearcy & Beyer (2013) have said that the anterior preference may be due to the high density of the ticks. Similarly, *Bothriocroton hydrosauri* (Denny, 1843), *Amblyomma limbatum* (Neumann, 1899) and *H. aegyptium* attach more often to the front of their hosts at high densities than at low densities (Petney & Al-Yaman 1985). In our study, a strong positive correlation was found between the density of ticks attaching and their number on the neck, hind limbs and forelimbs. The relatively large carapace openings may help explain why ticks are at high density in these body regions.

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