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TICK INFESTATION OF THE MEDITERRANEAN SPUR-THIGHED TORTOISES (*TESTUDO GRAECA*, LINNAEUS, 1758) FROM WESTERN REGIONS OF ALGERIA

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ABSTRACT

The Spur-thighed tortoise has a large geographic distribution and can be infested by several tick species, mainly *Hyalomma aegyptium*. This tick has recently been confirmed as a natural carrier of numerous pathogenic, including zoonotic agents. The purpose of this study was to estimate the level of hard tick infestation in Mediterranean tortoises living in some regions of the west of Algeria (Oran, Mostaganem, and Tiaret) during the spring and summer of the year 2019. Among 39 turtles collected from the different study regions, 22 tested positive, which represents an infestation rate of 56.41 %. A total of 224 adult ticks and one nymph were collected, which can display a tick infestation intensity of 10.23 %. All of these ticks belong to a single species, which is *Hyalomma aegyptium*. In terms of abundance, this study showed 5.78 ticks/tortoise. Regarding sex, we have collected 198 male and 26 female ticks with a sex ratio of 7.62. The preferred attachment sites of *Hyalomma aegyptium* ticks were the inguinal, the limbs, and to a lesser degree, the neck. A few rare cases have been collected from the carapace. Further studies are needed to assess the impact of the intensity of *H. aegyptium* infestation on the tortoise's health status and to assess the potential transmission of zoonotic pathogens.

Key words: *Hyalomma aegyptium;* prevalence; *Testudo graeca;* west of Algeria

INTRODUCTION

The Mediterranean spur-thighed tortoise *T. graeca* is one of the five species of Mediterranean tortoises belonging to the family *Testudinidae* and the genus *Testudo,* native from North Africa [8, 17]. Its distribution extends from high-quality areas of northern and southern Morocco, which are considered key areas [18], to Algeria and Tunisia, which would be medium and sub-optimal areas, respectively [1, 28]. Its habitats include forests, wooded areas and even deserts [9]. These turtles can easily be identified by the presence of conical-shaped spurs on each

thigh (although some may appear a bit flattened due to attrition), from which it receives its common name.

Ticks are obligate blood-feeding ectoparasites of wild and domestic terrestrial or semi-aquatic vertebrates in almost all regions of the world. They are currently considered the second most common vector of human infectious diseases in the world next to mosquito [22].

The majority of ticks of the genus *Hyalomma* are difficult to identify [25] but the tortoise tick *Hyalomma aegyptium* (Linnaeus, 1758) is easily recognizable thanks to its two equal spurs well separated from coxa I in both sexes [32]. *H. aegyptium* is a hard tick with a typical three-host life cycle [4]. The main hosts for adults are Palearctic tortoises of the genus *Testudo* [19].

Also, rare cases of adult-stage ticks were reported in other hosts, like hedgehogs and hares [11]. They have an extremely long feeding period compared to most of the other tick species [12]. Nevertheless, larvae and nymphs of *H. aegyptium* are less host-specific and feed on various vertebrates such as tortoises, lizards, birds, small mammals, and even humans [14, 23].

Recent studies have shown that *H. aegyptium* can be an important vector of various zoonotic agents: *Theileria* spp. [27], *Rickettsia* spp. [5], *Ehrlichia* spp., *Coxiella* spp., *Anaplasma* spp. [23], Crimean-Congo hemorrhagic fever virus [12, 29], *Hemolivia mauritanica* [10] and *Hepatozoon kisrae* [21].

The fact that nymphs and adults of *H. aegyptium* are found sporadically in humans, could play a role in the zoonotic transmission of pathogens and therefore, pose a potential risk to human health [17, 36]. This situation is exacerbated by the international illegal trade of *T. graeca* originating from their native areas to other countries, particularly Europe [16].

Due to the great importance of this species of tick and given the absence of their data in western Algeria, the present study aimed to evaluate the tick infestation of spurred-thigh tortoises and to calculate some parasitological indicators (mean intensity infestation and abundance) in this region.

MATERIALS AND METHODS

Study areas

The present study was carried out during the spring and summer seasons of the year 2019. The turtles that were the subject of this study were spotted in several sites belonging to three districts; Tiaret (19 tortoises), Oran (17 tortoises), and Mostaganem (3 tortoises) in the west of Algeria. These turtles lived in the surrounding forests (Fig. 1).

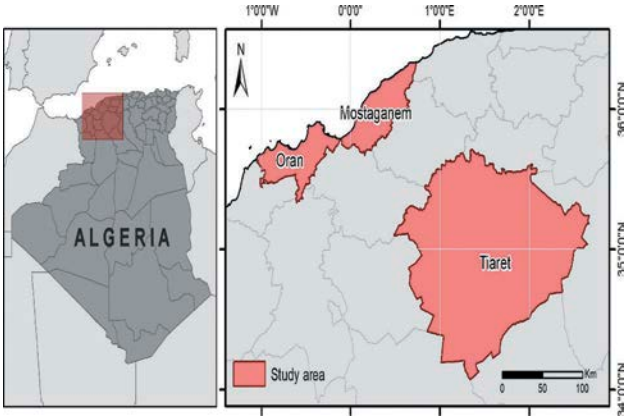

Fig. 1. Map of Algeria representing study areas

Field study and sample collection

A total of 39 spurred-thigh tortoises (*T. graeca*) were studied. The tortoises were captured by hand and their body surfaces were thoroughly examined for the presence of different stages of Ixodid ticks. The ticks were collected mainly in the perianal region, the hind limbs, and sometimes the neck, the forelimbs, and the carapace. All the ticks were collected in labelled flasks containing 70 % ethanol.

Ethical considerations

Handling of tortoises: for the majority of turtles, tick sampling was done on site and the turtles were released directly into their living areas. For others, the sample was taken in the laboratory and afterward they were also returned to their places of residence.

Tick identification

The collected ticks were sexed, counted, and identified morphologically to species level using appropriate morphological keys [37].

Data on infestation prevalence (%; number of infested tortoises/numbers of examined tortoises × 100), mean infestation intensity (number of ticks/number of infested tortoises), and tick abundance (number of ticks/number of examined tortoises) were calculated [28].

RESULTS AND DISCUSSION

Among 39 turtles collected from the different study regions, 22 tested positive, which represents an infestation rate of 56.41 %. All of these ticks belong to a single species, which is *Hyalomma aegyptium*. No other species have been identified (Fig. 1).

A total of 224 adult ticks and one nymph were collected, which can display a tick infestation intensity of 10.23 %. In terms of abundance, this study showed 5.78 ticks per tortoise, with a minimum of 1 and a maximum of 41.

Regarding sex, we have collected 198 male and 26 female ticks with a sex ratio of 7.62. These results are summarized in Table 1.

Table 1. Parasitological indicators of tick infestation in Mediterranean spur-thighed tortoises examined

Prevalence (%)	Infestation intensity	Sex ratio	Abundance
56.41 (22/39)	10.23 (225/22)	7.62 (198/26)	5.78 (225/39)

Our study showed a prevalence of 56.41 %. A close result was reported in Batna (northeastern Algeria) by Aouragh et al. [2] and in Tunisia by Gharbi et al. [9], with 63.6 % and 66.19 % prevalence, respectively.

On the other hand, Široký et al. [31] reported a lower infestation prevalence in the arid area of the Souss Valley in Southern Morocco (20.69 %). However, Segura et al. [28] displayed an overall prevalence of 92.5 %, ranging from 95 % in adult tortoises to 62 % in juveniles in the Maaamora forest of Morocco. Also, Najjar et al. [20] reported a global infestation prevalence of 91 %, varying from 82 % to 100 % by capture location.

In this survey, all the ticks collected belong to a single species which is *Hyalomma aegyptium* (Fig. 1). This result is in accordance with all studies carried out in the Saharan Atlas or the northeast of Algeria [2, 5, 35], and several studies in different areas in the world, like Tunisia [6, 9, 20], Iran [29, 34]), and Malta [33]. Indeed, this tick has long been classified as specific to turtles [11, 24].

However, among a subsample of collected ticks, 96.5 % were identified as *H. aegyptium* and the remaining 4.4 % of ticks were identified as *H. marginatum, Hyalomma excavatum*, and *Hyalomma scupense* in spur-thighed tortoises from Morocco [28].

T. graeca is also a natural host of *Rhipicephalus sanguineus* (Latreille, 1806) (Ixodida, Ixodidae) and *Haemaphysalis sulcata* (Canestrini and Fanzago, 1878) (Ixodida, Ixodidae) in itsnatural habitat in Turkey and Balkan countries [32].

During the present study, we have collected 224 adults and one nymph. Our result corroborates with that of Tiar et al. [35] who reported that nymphs did not exceed 2 % of the overall number of immature ticks collected. In addition, Robbins et al. [26] assume that nymphs are more common in late summer and fall.

On the contrary, several studies reported the total absence of immature ticks, in Algeria [2], in Tunisia [9, 20], and in Morroco [15].

Compared to larvae and nymphs, adults *H. aegyptium* are more host-specific, dominantly feeding on *Testudo* tortoises, while *T. graeca* is one of the preferred tortoise hosts [30, 32].

Although larvae and nymphs of *H. aegyptium* tend to also attach themselves to tortoises, they are less specific and have been reported to feed on other animals such as lizards, birds, small mammals, and even humans [3, 7, 12, 23, 36].

Regarding the sex of the ticks, we found the highest percentage to be male *H. aegyptium*. This result is compatible with the previous studies [2, 32]. The higher percentage of males may be due to the fact that male ticks remain longer in tortoise hosts. Females with an average feeding period of 25 days leave after they are fully engorged, whereas males remain on their hosts [30].

The preferred attachment sites of *Hyalomma aegyptium* ticks were the inguinal, the hindlimbs, the forelimbs, and to a lesser degree, the neck. A few rare cases have been collected from the carapace (Fig. 3).

Similar trends in attachment site preferences were observed by Tiar et al. [35]. The authors suggest that this location provides ticks with a relatively stable micro-habitat (in terms of temperature and humidity), protection against crushing or dislodging during the retraction of limbs, and rich vascularization in the anal area with thin skin [24].

These sites have been cited by several studies in different parts of the world. Brianti et al. [6] have reported that the hind limbs were the preferred site for *H. aegyptium* on its host. Aouragh et al. [2] found ticks in areas of soft skin that are protected by the carapace which allows ticks an easy attachment and feeding with a good protection.

Also, Gharbi et al. [9] reported that the predominant attachment site was the limbs (hind more than fore).

Interestingly, we have identified in some tortoises that the ticks have adhered to the carapace, which is not a typ-

Fig. 2. *Hyalomma aegyptium*, male dorsal view (A), male ventral view (B), female dorsal view (C), female ventral view (D).

Fig. 3. Some tick attachment sites: on the inguineal region (A); on carapace (B); hindlimbs (C); and on forelimbs (D).

ical location of tick infestation in tortoises. Similar results related to this location were observed by G h a r b i et al. [9] who reported the attachment of a female tick on the seams of the shell of a female tortoise.

An Infestation was observed on the lesions of the break carapace of a turtle (*Testudo Graeca Ibera*) in Southwest Iran [13].

CONCLUSIONS

The present study demonstrated the presence of *H. aegyptium* on tortoises from western Algerian regions with an infestation rate of 56.41 %, a tick infestation intensity of 10.23 %, an abundance of 5.78 ticks per tortoise, and a sex ratio of 7.62. The preferred attachment sites of *Hyalomma aegyptium* ticks were the inguinal region and the limbs. The findings should encourage new research (next step) by focusing on the main pathogens transmitted to these tortoises which can be potential agents of zoonoses and to evaluate the impact of the intensity of *H. aegyptium* infestation on the tortoise's health status in the study areas.

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