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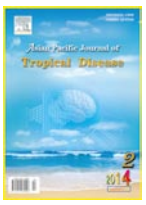

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Survey on morphometric characteristic of different developmental stages of *Dermacentor marginatus* under laboratory conditions

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ABSTRACT

Objective: To study the morphometric characteristics and biology of different developmental stages of *Dermacentor marginatus* (*D. marginatus*) under laboratory conditions.
Methods: *D. marginatus* ticks were collected from sheep in Shahmirzad and suburb. The identification of *D. marginatus* was carried out by means of stereoscope and light microscope according to available systematic keys. Nourished female ticks weight and their length of body, capitulum and mouth parts were measured. After laying eggs and breeding, the weight of all developmental larva stages and the length of mouth parts were measured and recorded carefully.
Results: The mean of egg dimension was 566 μm×436 μm. The length of unfed larva body, hypostome and capitulum were (690±10) μm, (75±5) μm and (172±7) μm, respectively. The weight of egg was calculated 0.05 mg and the weight of unfed larva, nymph and female were 0.02 mg, 0.14 mg and 4.66 mg, respectively; whereas the weight of replete larva, nymph and female were recorded 0.5 mg, 11 mg and 380 mg, respectively. Moreover, the length of unfed nymph, hypostome and capitulum were recorded (1300±50) μm, (135±5) μm and (280±10) μm, respectively. The longest length and width in replete female were observed to be 12.6 mm×8.4 mm.
Conclusions: The current investigation presents new information on biology of *D. marginatus* under standard laboratory conditions. Besides, investigation on ticks under laboratory conditions increases our knowledge regarding their biology and potential risks.

1. Introduction

Ixodidae family is considered as an obligatory ectoparasite of humans and animals that they have a wide variety of hosts and it occurs broadly in the world. Hard ticks are considered important both in medical and veterinary field due to being able to transfer different causative agents of diseases, particularly *Dermacentor* species which are among the most medically important of all ticks[1-3].

Dermacentor marginatus (Sulzer, 1776) (*D. marginatus*) is a very common tick in the Mediterranean region and it is considered as a three-host tick which has ability of completing the entire life cycle in one year. It has more thermophilic requirements compared to other species which can occur together, such as *Ixodes ricinus* (*I. ricinus*) and *Hyalomma punctuate*. Humidity and temperature play a critical role to ectoparasites life cycle particularly ticks[4]. In the Mediterranean region, cattle, sheep, goat and wild boars can be infested by adult *D. marginatus*. Human are prone to be infested with immature stages while dog may be infested with mature[5-7]. This tick can transmit some significant agents including *Rickettsia slovaca*, *Francisella toularensis*, *Cocsila bornuti* and encephalitis virus[8]. Recently feeding hard ticks under laboratory conditions seem to be effective

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and efficient for purpose of maintaining. Despite of the fact that *D. marginatus* is widely distributed in our country there is not enough and valuable information about morphometric and biology of this tick in different reign of Iran[9]. In addition, studies of transmission, maintenance, infectivity, virulence, and pathogenicity of tick-borne agents require the use of large numbers of live laboratory-raised ticks. And this is not possible without enough knowledge about tick life cycle. Thus, current survey was performed to study the weight and the morphometric character of mouth part of different developmental stages of *D. marginatus* in the life cycle.

2. Materials and methods

The present study was carried out on *D. marginatus* which were isolated from sheep in Shahmirzad and suburb which is located in north of Semnan City (35°46′ N Latitude and 52°21′ E Longitude). The identification of *D. marginatus* was undertaken by means of stereoscope and light microscope according to systematic keys of Nabiyan *et al*. and Estrada-Pena *et al*[2,9]. Nourished female ticks weight and their length of body, capitulum and mouth parts were measured. After laying eggs and breeding, the weight of all developmental larva stages and the length of mouth parts were measured carefully. All measurements in the present study were recorded by aid of AxioVision software.

3. Results

3.1. Morphometric characteristics of eggs

The investigated eggs appeared from light to dark brown, round to oval shape with waxy luster. The mean of dimension was 566 μm×436 μm (Figure 1). A total of 15 mg of eggs were counted (*n*=312) and the mean of 100 eggs weight of *D. marginatus* was calculated to 5 mg.

Figure 1. Eggs of *D. marginatus*.

3.2 Morphometric characteristic of larval stage

Six-legged larva of *D. marginatus* had tiny scutum and eyes while they did not have spiracle and genital pore. A rise in temperature provoked them to show a high activity while their motility cease by drop of temperature. The length of unfed larva, hypostome and capitulum were recorded (690±10) μm, (75±5) μm and (172±7) μm, respectively (Figure 2). The weight of larvae (*n*=300) was calculated to be 6 mg. Therefore, the weight per larva was calculated to be 0.02 mg (Tables 1 and 2). The weight of 100 larvae was computed 50 mg. Furthermore, the mean of length and weight of replete larva were (1400±30) μm and 0.5 mg, respectively.

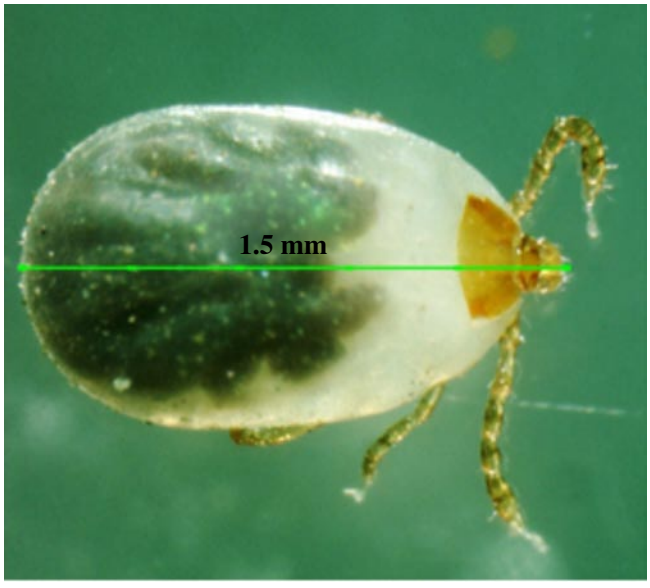

Figure 2. Replete larva of *D. marginatus*.

Table 1
Morphometric characteristic of different developmental stages of *D. marginatus*.

Stage	Replete		Unfed	
	Length(μm)	Weight (mg)	Length(μm)	Weight (mg)
Larva	1400±30	5	690±10	0.02
Nymph	4100±100	11±1	1300±50	0.14
Female	12000±600	380	3700±200	66.40

Table 2
Comparison of hypostome length in different developmental stages of of *D. marginatus*, *R. sanguineus* and *I. ricinus* and also the length of capitulum of *D. marginatus* in different stages.

Stage	Length of capitulum (μm)	Length of hypostome (μm)		
	D. marginatus	*D. marginatus*	*I. ricinus*	*R. singenuos*
Larva	172±7	75±5	90	50
Nymph	280±10	135±5	170	120
Male	770±120	300±30	280	270
Female	790±130	370±35	500	370

3.3. Morphometric characteristics of nymphal instars

When larva molted to nymph, imaging and measuring

was carried out. The length of unfed nymph, hypostome and capitulum were (1300±50) μm, (135±5) μm and (280±10) μm, respectively (Figure 3). Eyes were present on eight-legged nymph. Nymphs were without ornamentation scutum. In addition, genital aperture was absent. A spiracle was seen in the posterior of the fourth leg. The mean weight of unfed nymph was 0.14 mg. The weight of 50 replete nymphs was computed 550 mg and the mean of their length and weight (replete nymph) were (4100±100) μm and (11±1) mg respectively (Tables 1 and 2).

Figure 3. Unfed nymphal instar of *D. marginatus*.

3.4. Morphometric characteristics of adult ticks

Isolated adults ticks had a scutum and we could readily observe the characteristic of *D. marginatus*. In this process, the morphometric criteria of fed and unfed female ticks measured meticulously and it is presented in Table 1. The longest length and width in replete female was 12.6 mm×8.4 mm (Figures 4-6). The weight of unfed and replete female was 4.66 mg and 380 mg, respectively.

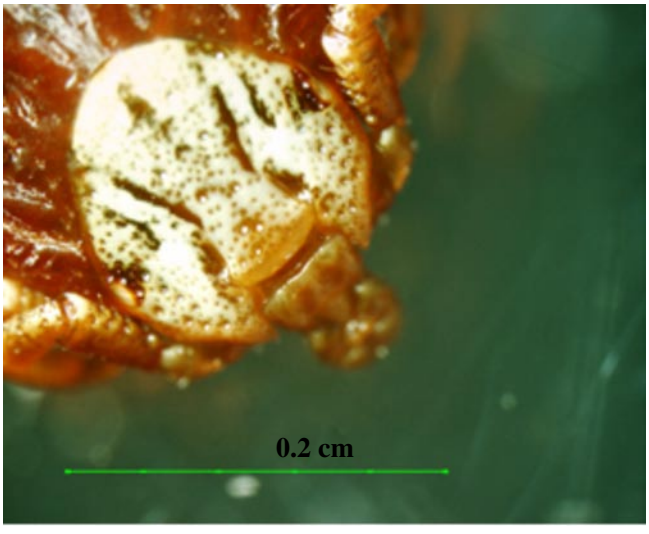

Figure 4. Capitulum of *D. marginatus* (unfed female).

Figure 5. Unfed *D. marginatus* (female).

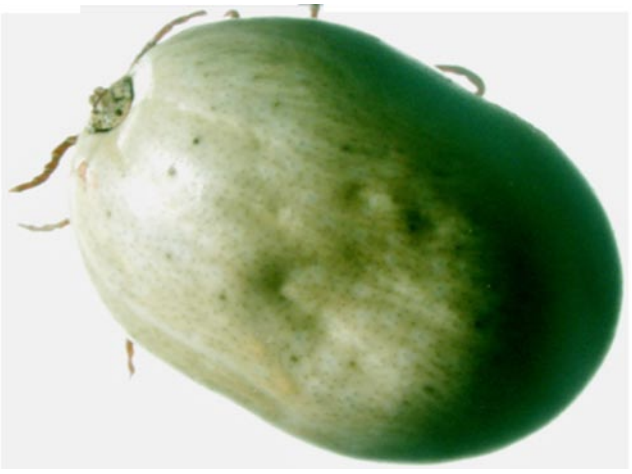

Figure 6. Engorged *D. marginatus* (female).

4. Discussion

In the current study the weight of fed nymph and unfed female of *D. marginatus* were 0.5, 11 and 4.6 mg, respectively; while Liu reported the same criteria of *Dermacentor silvarum* (*D. silvarum*) 0.42, 64.6, unfed female 6.06 and unfed male 4.78 mg, respectively. The weight of engorged larval instars and unfed adult tick in these two species are nearly close to each other whereas the weight of fed nymphal instars of *D. silviyarum* was 64.6 mg which is six times heavier than of nymphal of *D. marginatus* compared to our findings[10]. According to Sanches *et al*., fed adult, larva and nymph of *Amblyomma brazilensis* were 862, 1.3, 18.2 mg[11]. The replete nymph in *Hyalomma asiaticum*, *Hyalomma scholzei* and *Ixodes hexagonus* were 27, 6.01 and 6.01 mg, respectively[7,12].

In another survey, the weight of unfed larva, nymph and female of *Htruncatum truncatum* were 0.02, 0.19, 11.1 mg while nourished of them were 0.4, 17.29 and 532.8 mg that indicates 20, 91 and 48 times increase in their weights[13]. According to different studies, the weights of fed nymph in most of hard ticks was 6-27 mg whereas fed nymph of *D.*

silvarum was 64.6 mg[10]. In the present study the weight of fed nymph was 11 mg that our results are correspondent to other investigations. Surprisingly, we could easily observe a weight losing in every stage after molting. According to aforementioned fact, the unfed adult of *D. marginatus* was 2.5 times lighter compared to fed nymph of this species. This remarkable point is observable regarding weight of unfed nymph and fed larva and also about unfed larva in comparison to primitive weight of eggs.

In the current investigation, the weight of 100 eggs was calculated 5 mg. However, the weight of 100 unfed larvae was 2 mg (0.02 mg per larva). The weight of unfed larva of *D. silvarum* and *Hyalomma truncatum* were 0.04 and 0.02 mg, respectively[10,13]. The length of nourished larva and nymph of *D. marginatus* were two and three times longer than unfed ones, respectively. In the current study the length of replete female were 12 mm while Ionita *et al*. (2008) reported 17.3 mm for fed female and this difference may attributed to two significant points: firstly, probably the blood sucking of females ticks were not completed in the current study. Secondly may it is correlated to different climate conditions in two areas that it requires more studies in order to confirm this fact[12].

In the present research the length of hypostome in unfed larva, nymph and fed male and female were recorded (75±5) μm, (135±5) μm, (300±30) μm and (370±35) μm. Therefore, the length of hypostome of female is longer than male. The length of capitulum of unfed larva, nymph and fed male and female of *D. marginatus* were (172±7) μm, (280±10) μm, (770±120) μm and (790±130) μm, respectively. In another study, this range for capitulum of female and male of *D. marginatus* were 774-948, 700-900 μm which is in agreement to our findings[12,14].

Finally, this study provides basic and new data on life cycle of *D. marginatus* under controlled laboratory conditions which are useful for survey on transmission, maintenance, infectivity, virulence, and pathogenicity of tick-borne agents and it is not possible without enough knowledge about tick life cycle under standard conditions. Furthermore, increase of bionomic knowledge of ticks play a major role for purpose of tick control programs particularly in respect to the strategic acaricidial application. However, more field and laboratory studies are needed to understand the biological feature of tick species in order to prepare a comprehensive data concerning bionomic knowledge of ticks that leads us to design better control programs.

Conflict of interest statement

We declare that we have no conflict of interest.

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