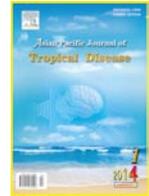




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Biology of *Dermacentor marginatus* (Acari: Ixodidae) under laboratory conditions

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PEER REVIEW

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Comments

This is an important study on biology of *D. marginatus* under controlled laboratory conditions on different developmental stages including egg, larvae, nymphs and adult males and females. The results are useful and interesting which prepare a basic knowledge of tick that lead us to control ticks effectively. The control of tick infestations and pathogens they transmit constitute a priority to improve human and animal health.

Details on Page S288

ABSTRACT

Objective: To investigate and survey the biology of *Dermacentor marginatus* (*D. marginatus*) under laboratory conditions.

Methods: In this investigation, *D. marginatus* adult ticks were collected from sheep in Semnan province. Then various developmental stages of *D. marginatus* including larvae, nymphs and adult males and females under laboratory condition were bred and their biology was scrutinized.

Results: The requisite time to complete the life cycle of *D. marginatus* under controlled laboratory conditions for larvae (26 °C, 75% relative humidity) and nymph (26 °C, 95% relative humidity) moulting, was on average 92 d (range 75–104 d), including preoviposition and egg incubation (22.5 d), larvae incubation (20.5 d), nymphal stage (28 d) along with male maturation (21 d). The index of conversion efficiency and the index of reproduction efficiency in females were 0.397 and 8.300, respectively.

Conclusions: Although in this investigation, there was no meaningful correlation between preoviposition period and the weight of female ticks which were laid successfully. The significant linear relationship was fully observed between the weight of engorged female of *D. marginatus* and the number of eggs laid. The mean of preoviposition period from 5.4 d in autumn to 34.2 d in spring increased. The minimum weight of ticks with laying capacity was 69 mg and lighter ticks (21 mg) either did not lay or if they did their eggs did not hatch.

KEYWORDS

Bionomics, *Dermacentor marginatus*, Ixodid tick, Life cycle

1. Introduction

Dermacentor marginatus (Sulzer, 1776) (*D. marginatus*) is a cosmopolitan tick which is considered as a three host tick. This tick was frequently reported from the northern parts of Africa (Morocco, Algeria and Tunisia), southern parts of Europe to the north of France, from the north of Turkey and Syria to Russia and China^[1]. *D.*

marginatus was also distributed in north, northwest and west of Iran (Azarbaeijan, Ardabill, Zanjan, Semnan and Kordestan provinces)^[2]. This tick commonly dwells in plain, shrubbery and broadleaf forests. In some part of Southern Europe, it can be found in mountainous regions at an altitude of 3 500 meters whereas its preferable elevation is 800–1000 m^[3]. Domestic and wild animals are suitable hosts of adult stage while immature stages are

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ectoparasites of smaller mammals^[1]. Ticks can transmit some pathogen agents and are responsible for severe harm to human health and economic losses to livestock industries^[4,5]. *D. marginatus* carries *Rickettsia slovaca*, *Francisella tularensis*, *Cocci* *bornuti* and encephalitis virus^[6]. Marguez introduced *D. marginatus* as an important vector of *Anaplasma phagocytophilum* and *Borrelia burgdorferi*. In addition, infestation of sheep with *D. marginatus* is more observable in thoracic areas but in goats the most engaged part is between horns^[7].

Considering the wide distribution of *D. marginatus* in Iran and Middle East, probing the biology and life cycle of this tick is a matter of high importance. Furthermore, there are no adequate and detailed published data of the biology of *D. marginatus*. Therefore, the current study was conducted to scrutinize the biology of *D. marginatus* at various developmental stages, under controlled laboratory conditions in hope of an accumulating basic knowledge for further investigation.

2. Materials and methods

2.1. Collection and rearing of ticks

The present survey was performed on adult ticks of Shahmirzad (north of Semnan province, Iran, 35°46'22"N, 53°19'43"E) in sheep from 2011 to 2012. After accumulating and sending specimens to laboratory, identification of genus and species were undertaken by means of stereoscope and light microscope which was equipped with a camera (Memmert D–91126), according to systematic keys of Nabiyani and Estrada–Pena *et al.*^[2,3]. In total 128 *D. marginatus* (71 males and 57 females) were collected during six steps of sampling (61 in spring and 67 in autumn). Regarding the preparation of tick colonies for rearing, ticks were washed in 70% alcohol ethanol (alcoholic bath) by a brush for 20 seconds and dried gently by tissue. In the next step, 52 engorged female ticks (16 female ticks from spring and 36 from fall) were bred.

2.2. Treatment of engorged females

The female ticks were weighed and lodged separately in recyclable plates (7 mL) which were weighed before usage. Some micro pores were contrived in order to provide oxygen to the larvae. Micro pores of the plates were so tiny that they didn't allow the larvae to exit after hatching. Plates which contained ticks and psychrometer were held in vitreous desiccators which contained saturated salt water. Next desiccators were put into incubators equipped with refrigerator. Besides, filament and florescent lamps were utilized to provide appropriate light (12 h dark and 12 h light) with a temperature of (26±1) °C and 70%–75% relative humidity which is suitable for *D. marginatus* breeding.

The ticks were monitored daily and any changes of the ticks were scanned meticulously. The time duration of preoviposition and laying ticks were recorded. After

the oviposition period, the dead ticks were weighed and removed from the plates. Eggs were also weighed and counted (Figure 1). The eggs which in plates were maintained in a vitreous desiccators (26 °C and 75% relative humidity) and every morphological change was scanned and registered during the daily investigation. In addition, some eggs were randomly dissected under stereoscope day by day. Fifteen milligrams of eggs were weighed and counted in order to compute the number of laid eggs^[8].



Figure 1. The egg masses laid by *D. marginatus*.

2.3. Observation on the biology of larvae and nymphs

The time duration of egg hatching (from first egg laid to appearance of first larva) and total time of egg hatching (from appearance of first larva to appearance of newly larva) were recorded respectively. After eggs hatching, 300 larvae were counted, weighed and kept into incubator for 4 d. Afterwards they were put on house mouse [*Mus musculus* (*M. musculus*)] and a New Zealand white rabbit for nourishment purpose (Figure 2). Considering that larva could move freely on *M. musculus*, the rabbit was impeded to access to its head by a cover which was devised on it. On the fourth day, larvae were accumulated from *M. musculus* and their cage as well.



Figure 2. The attached engorged female of *D. marginatus* on rabbit.

M. musculus and rabbit were maintained at (26±1) °C and 55% relative humidity. After the completion of blood sucking of larvae, they were gathered and weighed daily.

For moulting purpose, fed larvae were kept into two incubators with the same temperature (26 °C) but with two different relative humidity (75% and 95%). Nymphal stages were fed on rabbit and *M. musculus* for six days after moulting. Some nymphs were also put into a bag which was made of thin socks. Then the bag was put on the rabbit's ears. When the nymphs moulted to transform to adults, they were weighed and maintained in incubator under laboratory conditions. Daily investigation was performed on the *M. musculus* and rabbit. After detachment of engorged nymph instars from host, they were weighed and kept into desiccator (26 °C and 95% relative humidity).

In the present study two of the following variants, the index of conversion efficiency (CEI) and the index of reproduction efficiency (REI) which are associated to reproduction of ticks, were computed meticulously as follows:

CEI=weight of eggs/replete female tick weight (g)

REI=number of eggs/replete female tick weight (g)

3. Results

3.1. Life cycle of *D. marginatus*

The time taken to complete the life cycle of *D. marginatus* under controlled laboratory condition and for larvae (26 °C, 75% relative humidity) and nymph (26 °C 95% relative humidity) moulting was on average 92 d (range 75–104 d), including preoviposition and egg incubation (22.5 d), larvae incubation (20.5 d), nymphal stage (28 d) as well as male maturation (21 days) (Table 1).

Table 1

The duration of various developmental stages of *D. marginatus*.

Developmental stages	Period	Days (range)
Egg	Incubation	17.5 (6.0–23.0)
Larva	prefeeding	6.0
	Feeding	5.5 (4.0–7.0)
	Premoulting	9.0 (8.0–10.0)
	Survival of unfed	>50.0
Nymph	Prefeeding	6.0
	Feeding	8.0 (6.0–9.0)
	Premoulting	14.0 (12.0–15.0)
	Survival of unfed	90.0
Female	Prefeeding	8.0
	Feeding	13.0
	Preoviposition	5.0 (4.0–6.0) spring
	Oviposition	16.1 (10.0–24.0)
	Survival of unfed	>210.0

The arithmetical mean of preoviposition, from laying, incubation period (duration between oviposition commence until hatch of first egg) and egg hatching period (hatch of first egg until hatch of the last one) under laboratory conditions [(26±1) °C and 75% relative humidity] for spring and autumn were presented in Table 2. Although sometimes some female ticks laid later compared to other ticks and it was observed that their eggs hatched earlier. Therefore, larvae age after the end of hatching was ranged from 1 to 8 d. The success and failure of oviposition of

female ticks of *D. marginatus* were observed and some parameters such as weight of engorged tick were compared and indicated in Table 3.

Table 2

The duration of various periods related to fed female tick.

Period (day)	Spring	Autumn
Preoviposition	5.41±0.94	34.24±18.29
Oviposition	17.50±0.84	18.50±3.27
Incubation	19.50±1.38	18.60±3.50
Moulting	7.4±0.55	6.60±0.55

Table 3

Comparison of successful and unsuccessful oviposition of *D. marginatus*.

Oviposition	Max weight of engorged tick (mg)	Min weight of engorged tick (mg)	Mean of tick weight (mg)
Successful oviposition	380.0	69.0	166.6
Unsuccessful oviposition	98.0	21.0	41.1

In the beginning of attachment, the larva, nymph and adult fed slowly. As they started feeding progressively, their weight and body size augmented gradually. Initially males and then females started feeding. The average feeding period for female ticks were recorded 13 d (Table 4). The weight of the lightest engorged female that successfully laid and the preoviposition period in the spring with fall season were listed and compared in Table 2.

In addition, the average moulting period of nymphs were recorded 14 d at (26±1) °C and 95% relative humidity (Table 4). The whole larvae which were maintained at 26 °C 75% relative humidity were shrunk and deceased. Those larvae which were kept at 26 °C and 95% relative humidity could molt successfully. The average period of moulting for nourished larvae on rabbit and *M. musculus* were 9 and 8 days respectively (Table 4).

Table 4

The upbringing characteristics of different developmental stages in rabbit and *M. musculus*.

Stages		Prefeeding (d)	Feeding (d)	Moulting (d)
Larva	Rabbit	6	5–7	8–10
	<i>M. musculus</i>	6	4–7	7–9
Nymph	Rabbit	6	7–9	13–15
	<i>M. musculus</i>	6	5–7	12–14
Female	Rabbit	8	12–15	–

Regarding the dwelling organ of larva on host, the larvae selected the internal part of ears and neck in *M. musculus*, while ears and eyes were two preferable organs of fed larvae on rabbit. It was observed that nymphal stages lodged on the ears of rabbit while in the *M. musculus*, ears and nose were two sites that nymphs were found. The average time of feeding period for nymph instars in rabbit and *M. musculus* were 8 and 6 d, respectively (Table 4).

3.2. Oviposition

The oviposition data is listed in Table 5. At the beginning of oviposition, the mass of the eggs were

observed every minute. The maximum size was seen from 2 to 5 d after commence of oviposition. After 5 d, the daily egg mass reduced gradually. During this study two significant variants (the CEI and REI) were computed. The maximum number of these variants for *D. marginatus* were reported 0.547 and 11.38 while the mean of the two mentioned variants were 0.397 and 8.30 respectively (Table 5).

Table 5

The oviposition data of CEI and REI.

Day	Weight of engorged female (mg)	Weight of egg (mg)	Number	CEI	REI
1	380.0	208.0	4326	0.547	11.38
2	240.0	115.0	2392	0.479	9.96
3	199.0	87.0	1800	0.437	9.04
4	190.0	68.0	1414	0.357	7.44
5	181.5	82.0	1701	0.398	9.37
6	110.0	37.0	770	0.336	7.00
7	83.0	34.0	707	0.409	8.51
8	69.0	24.0	500	0.347	7.24
Mean	181.6	81.9	1701.25	0.397	8.30

REI: Number of eggs/engorged body weight; CEI: Weight of eggs/engorged body weight.

3.3. Temperature impact on egg hatching and emersion of larvae

When the eggs had been refrigerated (2 °C) for 20 d, there was a delay in hatching of the eggs due to the decrease of temperature. Whereas the retransmission of eggs in to incubator (26 °C and 75% relative humidity) caused the restarting of egg hatching and the whole egg also resumed hatching in 48 h.

4. Discussion

The biology and life cycle of *Dermacentor silvarum* (*D. variabilis* (*D. variabilis*)) and *Dermacentor occidentalis* (*D. occidentalis*) under laboratory conditions were described among 34 species of *Dermacentor*[8,9]. Several parameters were utilized to evaluate the reproductive biology of *D. marginatus* in this survey.

In the current survey, the requisite time for complete life cycle of *D. marginatus* under controlled laboratory conditions (26 °C, 75% and 95% relative humidity and 12 h lightness and 12 h darkness) was prolonged 92 d. However, Arthur (1960) indicated that the needed time for complete life cycle of *D. marginatus* was 365 d under natural circumstances and the average time needed for *Rhipicephalus bursa* (*R. bursa*) was 142.45 d[3,10]. The minimum life cycle of *D. silvarum* had a mean duration of 87.5 d (range 74–102 d) under laboratory conditions of (27±1) °C, 70% relative humidity and 6 h lightness and 18 h darkness[8]. In this investigation, the required time for preoviposition, egg incubation and feeding period of larvae had an average of 5, 17.5 and 5.5 d. Arthur mentioned that 7–12 d and 20 d for preoviposition and egg incubation period is required, respectively. Furthermore, he mentioned that the feeding period of larvae is affected

by their age. Thus, the older larvae fed longer compared to younger ones and this period in younger larvae (2–25 d of age) was reported 2–5 d whereas the results of the current study was shorter or almost are inconsistent with Arthur results. It might have been originate from the same prepared humidity and temperature at this survey.

Considering studies were carried out on different species of *Dermacentor*, it seems those species which were inhabited under natural conditions with similar circumstances, have shown similar reactions when they were under laboratory conditions[9]. The life cycle of *D. silvarum* at 27 °C lasted 87.5 d compared to our results 92 d[8]. The habitat conditions of *D. silvarum* with *D. marginatus* are almost similar and it is distributed from south of Russian to south east of Asia. This tick is normally observed in mountainous districts with cold and dry weather at an altitude of 3500 m[1].

D. marginatus are observed in northern areas of Iran and southern skirts of Alborz ranged mountains (from north of Semnan to Azarbaijan) where the weather is cold and dry in spring and summer (Nabiyan, 2008). Estrada–Pena mentioned a vegetation area at an altitude of 800–1000 m as a proper habitat condition for *D. marginatus*[3]. Furthermore, *D. marginatus* and *D. silvarum* were reported from small rodents as domestic and wild herbivores[1].

The required time for complete life cycle of *D. occidentalis* and *D. variabilis* (22–24 °C) were almost two times more than our finding 180–195 and 175–191, respectively[9]. *D. occidentalis* and *D. variabilis* are distributed from Mexico to Canada (north of America) and they have an altitude of 500 m and are normally humid and rainy. The difference between the length of life cycle of *D. occidentalis* and *D. variabilis* and the current study may be varied by different ecological and natural conditions. And rodents and carnivores were reported as host for them[1]. It may be concluded that those conditions prolong the life cycle of above mentioned species. Regarding incubation period of eggs and premoult period of nymphal stage, there is a remarkable difference between *D. marginatus* and two already mentioned species (*D. occidentalis* and *D. variabilis*) that incubation period of their eggs and premoult period of their nymphal stage were recorded two times longer compared to *D. marginatus* and this fact may justify the longer life cycle of *D. occidentalis* and *D. variabilis* versus *D. marginatus*[9].

The minimum duration for the developmental stages of the life cycle of *D. occidentalis*, *D. variabilis*, *D. marginatus*, *D. silvarum* and *Dermacentor andersoni* are almost similar to each other. The minimum time required for completing the period of larvae feeding and nymphal stages in four mentioned species of *Dermacentor* were almost the same and it appears that if proper conditions were prepared for them in the laboratory the life cycle duration in four species would be closer. Though in premoult period of nymphal stage there is a time range difference of 13 to 21 d[9]. In mentioned species, the preoviposition time was longer in comparison with the incubation period of egg. That's because when a female tick was laying, some eggs started hatching and larvae

came out. This trait was observed particularly in *D. silvarum* and *D. marginatus*. According to some studies temperature is an important variant agent in the life cycle of ticks. Therefore when the temperature declines, the required time for the life cycle escalates due to the reduction of metabolic activity of the tick whereas a rise in the temperature until optimum inducement increases the metabolic activity of the tick that leads to decrease of life cycle duration^[11].

There is no doubt that temperature influence in a tick's life cycle is immense. The impact of temperature on the growth and the length of *Rhipicephalus appendiculatus* (*R. appendiculatus*) life cycle proved that at 25 °C *R. appendiculatus* lasted 9 weeks while at 18 °C the life cycle is lasted longer (6 month)^[9,12]. Thus, acquiring the optimum temperature is significant for the growth of the tick under laboratory conditions. In the current study before the end of oviposition period, hatching stage initiated. Therefore, the interval of the first egg laid is more than two weeks from the last egg laid. The minimum weight of ticks which were capable of laying and hatching was 69 mg and lighter ticks (21 mg) either did not lay or if they did their eggs did not hatch.

Drummond and Whetstone (1970) presented the REI, the number of eggs produced per gram of engorged female body weight, and CEI, the weight of eggs produced per gram of engorged female body weight. CEI is a crucial biological parameter for the evaluation of toxins impact on ticks both in the field and under laboratory conditions. CEI is not affected by conditions and it does not vary from season to season^[13]. Ixodidae family has an affluent reproductive capacity^[14]. The significant linear relationship was precisely observed between the weight of engorged female of *D. marginatus* and the number of eggs laid. As the increase of female tick weight, increases the CEI, it can raises until a specific weight, since over passing from the special weight does not lead to increase of CEI anymore^[13]. Moreover, REI can be used instead of CEI. Aforementioned relationship was also reported for the following species: *R. bursa*, *Hyalomma anatolicum anatolicum*, *Rhipicephalus sanguineus*, *Rhipicephalus oculatus*, *D. variabilis*, *Amblyomma inornatum* and *Amblyomma hebraeum*^[15–17].

The maximum rate of REI in our study was 11.38 and it is close to *D. silvarum* REI (11.09). In addition, the CEI rate in our survey was 0.547. As a result of detaching female ticks which were bred from sheep before completing their feeding, the mean of the CEI in the present research was less compared to other studies^[8,9]. In the Ixodidae family, the CEI is relatively similar: 0.508 in *R. bursa*, 0.57 in *Amblyomma incisum* (*A. incisum*) and 0.56 in *Hyalomma truncatum*^[10,15,16]. Szabo et al. studied on *A. incisum* and recorded the number of 6821 eggs per gram of engorged female tick^[15]. In our investigation, no meaningful correlation was observed between preoviposition period and the weight of female tick which had laid successfully. Because sometimes some of the lighter female ticks commenced to lay and also finished their oviposition earlier. The average preoviposition period of ticks which were accumulated in spring and autumn were 5.41±0.94 and

34.24±18.29, respectively. We concluded that the reason of the short period of preoviposition in spring is diapause phenomenon that genetically is a determined stage of suppressed development, which is a significant adaptive mechanism for dormancy during periods of undesirable environmental circumstances^[17]. The importance of diapause is due to the synchronization of the life cycle with the favorable season.

Another variant which plays prominent role concerning tick breeding is humidity. It is noteworthy that this major factor does not influence the length of tick's life cycle. In fact, humidity has a vital role for a tick rearing because if humidity drops the mortality rate of ticks will quickly increases in each stage^[18]. In the present survey when we put replete larvae in the desiccators which contains female ticks and their eggs, all larvae died whereas when we add distilled water instead of saturation salt water in order to prepare humidity (more than 95%) it was observed that 95% of larvae and nymphal stages molted successfully. In this investigation the average preoviposition period soared from 5.4 d in autumn to 34.2 d in spring soared. In the current study, employing *M. musculus* and rabbit as a host of larvae and nymphal stages, there is no reasonable difference between feeding and moulting period.

According to Sanches report there was a meaningful difference for feeding period, weight and laying output in *Amblyomma brasiliensis* which were fed on pig and rabbit^[19]. In the present survey, feeding duration in both *M. musculus* and rabbit was equal because these hosts are natural host of *D. marginatus*.

Finally, our study presents a new data on biology of *D. marginatus* under controlled laboratory conditions. 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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Comments

Background

The family Ixodidae, hard ticks (Arachnida: Acari: Parasitiformes) encompasses nearly 700 species in two major phylogenetic and morphological groups including the Prostriata and the Metastricata. They are considered as

obligatory blood-sucking ectoparasites living on a wide variety of hosts including mammals, birds and sometimes reptiles. Ticks are vectors of causative agents of diseases not only for humans but also animals. Annually they impose a remarkable economical losses and damages to livestock industries. Investigations on this important family at various developmental stages under controlled laboratory conditions increases basic knowledge of researchers and it plays a significant role in order to design an effective and efficient control programs.

Research frontiers

The study was carried out in order to provide comprehensive information regarding biology of *D. marginatus* under controlled laboratory conditions by breeding and monitoring the different developmental instars on house mouse and rabbit. The data embraces the different phases including the needed time for completing life cycle of the tick, feeding of females, oviposition, preoviposition, incubation of eggs and larvae, nymphal stage, nymph and also computation of two major indexes CEI and REL.

Related reports

The study mentioned and compared similar surveys on tick biology under field and laboratory conditions including *D. silvarum*, *Hyalomma truncatum*, *R. appendiculatus*, *R. bursa*, *Amblyomma cajennense*, *A. incisum* and *Amblyomma brasiliense*.

Innovations & breakthroughs

There is a limited data concerning *D. marginatus* despite of its noticeable distribution in the world and country. In addition, this is the first study on biology of the tick under controlled laboratory condition and it can fill the lack of enough information about this hard tick.

Applications

Collecting these data may be useful and apply for the development of novel control methods for the prevention of transmission of tick-borne pathogens and tick infestations to human and animals. And also preparing biology information of ticks will contribute us to understanding of the biology of the tick-pathogen interface.

Peer review

This is an important study on biology of *D. marginatus* under controlled laboratory conditions on different developmental stages including egg, larvae, nymphs and adult males and females. The results are useful and interesting which prepare a basic knowledge of tick that lead us to control ticks effectively. The control of tick infestations and pathogens they transmit constitute a priority to improve human and animal health.

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