

Life cycle of *Hyalomma dromedarii* ticks (Acari: Ixodidae) on cattle under experimental conditions

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ABSTRACT:

This study aimed to study the feeding pattern of *Hyalomma dromedarii* (*H. dromedarii*) on cattle to find whether it can play a role in the transmission of *Theileria annulata* among cattle under the field conditions. The life cycle of *H. dromedarii* had been studied on cattle from March to July at a range temperature of 34.5 - 38.5°C and range relative humidity of 21 - 47 %. Our study showed that *H. dromedarii* completed its life cycle in 97- 131 days and behaved as a three-host tick. Out of 1500 larvae, 1486 larvae (99.1%) were successfully complete their feeding on cattle during a period between 3 and 17 days at 38.5°C and relative humidity (R.H.) 21%. The larvae took between 14 and 16 days as premoult period, while the moulting period was 2-3 days. Regarding the nymph stage, 98.7% of nymphs (296/300) successfully completed their feeding on cattle during a period between 3 and 10 days at 35.4°C and R.H. 26%. The nymph took between 14 and 16 days as premoult period, while the moulting period was 3-4 days. All adult tick 100% (30/30) successfully completed their feeding on cattle during a period between 4 and 10 days at a temperature range between 34.5°C - 35.5°C and R.H. range between 28 - 47%. The preoviposition and preeclosure period varied between 4 - 6 days and between 16 -20 days respectively. Our result showed that the average of engorged ticks' weight, number of eggs produced and the weight of eggs was 0.7886 g, 7718 eggs and 0.504055 g respectively. Regarding hatchability, the results revealed that more than 93.65% of eggs were able to hatch and produced larvae. In conclusion, since *H. dromedarii* behaved as a three-host tick during this experiment it can participate in the transmission of *Theileria annulata* between cattle in field.

Keywords: Life cycle, Acari: Ixodidae, *Hyalomma dromedarii* ticks.

Introduction:

Ticks belonging to the family ixodidae represent a group of highly specialized blood sucking arthropods that are obligate temporary parasites of mammals, birds or reptiles (Balashov, 1968). Eleven genera of ticks and 63 species of tick infesting a variety of animal species, including birds and reptiles, have been identified in the Sudan (Hoogstraal, 1956).

According to Hoogstraal, (1956); Karrar et al., (1963) and Osman et al., (1982) ticks infesting livestock in the Sudan are mainly *Hyalomma anatolicum*, *Hyalomma dromedarii*, *Hyalomma rufipes*, *Hyalomma impressum*, *Hyalomma impeltatum*, *Hyalomma truncatum*, *Rhipicephalus evertsi evertsi*, *Rhipicephalus sanguineus* group, *Rhipicephalus praetextatus*, *Rhipicephalus decoloratus*, *Rhipicephalus annulatus*, *Amblyomma lepidum* and *Amblyomma variegatum*. More than 70 tick species were identified in the Sudan including the most economically important ticks in Africa (Hassan, 2003).

Tropical theileriosis, caused by *Theileria annulata*, is one of the most prevalent and killing tick-borne diseases among cattle (Losos, 1986). *Theileria annulata* is endemic in northern Sudan, hindering all efforts at upgrading cattle for milk production. *Hyalomma anatolicum* and *H. detritum* are the most important vectors for *Theileria annulata* (Daubney and Said, 1951). Um El-Hassan et al. (1983) concluded that *Hyalomma anatolicum* is considered to be the main vector for the disease in the Sudan. Salih et al., (2005) reported a high salivary glands' infection rate (61.8%) in *Hyalomma anatolicum* in River Nile State, Northern Sudan. However, the disease was transmitted experimentally through several tick species of the genus *Hyalomma* such as: *H. excavatum* (Daubney and Said, 1951), *H. dromedarii* (Mazlum, 1969), *Hyalomma rufipes* (Jongejan et al., 1983) and *H. marginatum* (Sayin et al., 2003). *H. truncatum* immature stages feed on rodents, so, it plays no role as a vector in the epidemiology of the disease in nature (Magano et al., 2000). Furthermore, tropical theileriosis had been transmitted

successfully under experimental conditions by Taha, (2009) through *Hyalomma rufipes* and *Hyalomma dromedarii* ticks. Alahmed and Kheir, (2003) found that *Hyalomma dromedarii* behaves as two host tick on rabbits, and camel (El Ghali, 2005).

The life cycle of *Hyalomma dromedarii* tick on cattle has not been studied previously. Therefore, the current study is designed to follow the life cycle of *Hyalomma dromedarii* ticks fed on cattle, which may reflect the role of this tick in the transmission of tropical theileriosis under field conditions.

Materials and Methods:

Experimental Animals: Nine Butana cattle from Atbara Animal Breeding Research Station were used as hosts for the feeding of *Hyalomma dromedarii* (three animals per each stage; larvae, nymph and adult) as described previously (Bailey, 1960).

Source of ticks and laboratory maintenance of ticks: Three engorged females of *Hyalomma dromedarii* were collected from camel at El Damer Market and identified according to Hoogstraal, (1956). Ticks were maintained under laboratory conditions as described in section (2.7) to oviposition (Winston and Bates, 1960).

Feeding of larvae: Three cattle were infested with larvae of *H. dromedarii* (approximately 500 larvae per animal (250 larvae in each ear)) as described by Baily (Bailey, 1960). Detached larvae were maintained under laboratory conditions as described in section (2.7).

Feeding nymphs: Three cattle were infested with nymph of *H. dromedarii* (approximately 100 nymphs per animal (50 larvae in each ear)) as described by baily (Bailey, 1960). Detached nymphs were maintained under laboratory conditions as described in section (2.7).

Adult feeding: Three cattle were infested with adult of *H. dromedarii* (approximately 20 adult per animal (10 adult (5 females and 5 males) in each ear) as described by baily (Baily, 1960). Adult ticks were collected after their detachment, weighed separately using a sensitive balance (Germany) and classified based on their sex. Out of them, five females were placed in specimen tubes and maintained under laboratory conditions as described in section (2.7) until oviposition. Firstly, the egg conversion ratio (ECR) of each female was counted using the following formula:

Egg conversion ratio (ECR) =
$$\frac{\text{The weight of whole egg mass}}{\text{The weight of engorged female}}$$

Secondly, the mass of 250 eggs of each engorged female was weighed (Ouhelli and Pandey, 1984). Then the average weight of a single egg was determined by dividing the weight of 250 eggs by the number of eggs (250).

2.6 Egg Hatching

In each new specimen tube, 250 eggs were placed and kept under laboratory conditions as explained in section (2.7) until hatching. The numbers of hatching larvae were counted using a microscope. Hatchability was calculated as a percentage (total number of larvae divided by the total number of eggs in each tube).

2.7 Handling of experimental animals, ticks (larvae, nymphs and adult), and data

Before feed step, cattle were treated with dexamethazone as an immunosuppressant (Ahmed, 1999). Cattle were kept at temperature range between 34.5- 38.5°C and relative humidity between 21-47 % during this experiment. All ticks (larvae, nymphs and adults) were maintained in the laboratory using a glass desiccator containing a solution of sodium chloride (40 gm. in 100 ml distilled water) at room temperature 25°C and R.H. 75%. Ventilation of ticks was provided during daily inspection

Engorged larvae, nymphs and adult ticks dropped off animals were collected in polythene bags, and counting of engorged larvae and nymphs was done immediately after repletion using 1 × 8 cm. glass petri-dish placed on an ice bag in order to minimize their activity.

All information such as feeding periods, the number of engorged ticks (larvae, nymph and adult), the number of detached ticks (larvae, nymphs and adult) pre-moulting and moulting periods, preoviposition period and preeclution periods (incubation periods) were recorded daily.

Results:

Tick duration of the whole life cycle:

The results showed that the average mean time of *Hyalomma dromedarii* (*H. dromedary*) that fed on cattle to complete the life cycle was around 112.5 days (range between 95 - 130 days). In this experiment, *H. dromedarii* behaves like a three-host tick.

Feeding and moulting of larvae:

A total of 1486 out of 1500 (99.1%) larvae were able to complete their cycle on cattle (Table 1). Larvae needed between 3 to 17 days to complete their feeding. The majority of larvae ended their feeding on day six. Their pre-moulting period varied between 14 to 16 days, while the moulting period varied between 2 to 3 days.

Table (1): Feeding and developmental periods of larvae of *Hyalomma dromedarii* fed on cattle

Ticks	Animals			Total
	Cattle No. 1	Cattle No. 2	Cattle No. 3	
Total No. of applied larvae	500	500	500	1500
No. of engorged larvae (fed larvae)	496 (99.2%)	498 (99.6%)	492 (98.4%)	1486 (99.1%)
Feeding period of larvae	3 - 17 days	3 - 16 days	3 - 17 days	3 - 17 days
Pre-Moulting period of larvae	14 - 16 days	14 - 16 days	14 - 16 days	14 - 16 days
Moulting period of Larvae	2 - 3 days	2 - 3 days	2 - 3 days	2 - 3 days

Feeding and molting of nymphs:

Out of 300 nymphs, 296 (98.7%) were able to complete their cycle on cattle. The feeding period of nymphs ranged from 3 to 10 days. Their pre-moulting period varied between 14 - 15 days, while the moulting period varied between 3-4 days (Table 2).

Table (2): Feeding and developmental periods of nymph of *Hyalomma dromedarii* fed on cattle

Ticks	Animals			Total
	Cattle No. 1	Cattle No. 2	Cattle No. 3	
Total No. of applied nymph	100	100	100	300
No. of engorged nymph (fed nymph)	99 (99%)	98 (98%)	99 (99%)	296 (98.7%)
Feeding period of nymph	3 - 10 days	3 - 10 days	3 - 10 days	3 - 10 days
Pre-Moulting period of nymph	14 - 15 days	14 - 15 days	14 - 15 days	14 - 15 days
Moulting period of nymph	3 - 4 days	3 - 4 days	3 - 4 days	3 - 4 days

Feeding of adults:

As shown in Table 3, all ticks (100%) were able to complete their cycle on cattle and the feeding period ranged between 4 and 7 days.

Table (3): Feeding and developmental periods of females of *Hyalomma dromedarii* fed on cattle

Ticks	Animals			Total
	Cattle No.1	Cattle No. 2	Cattle No.3	
Total No. of applied adult females	10	10	10	30
Fed adult females	10 (100 %)	10 (100 %)	10 (100 %)	30 (100 %)
Feeding period	4 - 9 days	4 - 10 days	4 - 10 days	4 - 10 days

The relationships between tick weight, preoviposition, and preecllosion :

The relationships between the weight of engorged female tick, preoviposition, and preecllosion were illustrated in table 3. The weight of engorged females (n=5), which dropped off from cattle, ranged between 0.620 and 0.961 g. The preoviposition periods of these ticks varied from 4 to 6 days, while the preecllosion periods varied between 16 to 20 days.

Regarding the production of eggs, the estimated numbers of eggs produced by one tick ranged between 5461 eggs and 9452 eggs, whereas the weight of eggs mass that produced by one tick ranged between 0.354965 g and 0.61438 g (Table 3). The egg conversion ratio ranged between 57.3% and 63.9% (Table 3).

Table (3): Relationship between the weight of engorged female, the periods of preoviposition and preecllosion, the production of eggs (the masses and numbers of eggs) and ECR.

No. of tick	Weight of engorged female (g)	Periods (days)		No. of eggs	Egg weight of all eggs mass (g)	ECR (%)
		Preoviposition	Peecllosion			
A	0.961	6	20	9452	0.61438	63.93
B	0.788	4	16	8317	0.540605	68.60

C	0.620	5	18	5461	0.354965	57.25
D	0.687	4	16	5915	0.443625	64.57
E	0.887	4	16	9445	0.5667	63.89
Average	0.7886	4.6	17.2	7718	0.504055	63.648

Egg conversion ratio (ECR) = The weight of all egg mass (g)/ The weight of engorged female (g)

Hatchability:

The hatchability of eggs was calculated by dividing the number of larvae that hatched by the total number of eggs. The hatchability percentage ranged between 93.65 and 99.47 (Table 4)

Table (4): The hatching percentages among eggs that laid by female ticks fed on cattle

No. of tick	Total No. of eggs	Total No. of hatched larvae	Percentage of hatchability (%)
A	9452	9402	99.47
B	8317	7920	95.23
C	5461	5201	95.24
D	5915	5635	95.27
E	9445	8845	93.65

Discussion:

In any tropical environment ticks are active the year round. It is only the magnitude of that activity that changes in relation to the prevailing seasonal conditions (Punyua *et al.*, 1991). *Hyalomma dromedarii* (*H. dromedarii*) is a very characteristic tick with a cosmopolitan distribution, which is closely associated with camels. It is well adapted to extreme dryness of habitat and to camel hosts.

The present study dealt with the developmental periods of *H. dromedarii*, which included preoviposition periods, oviposition periods, pre-eclosion periods, hatchability of eggs and feeding and moulting periods of larvae and nymphs. During this experiment, cattle were kept at temperature range between 34.5- 38.5°C and relative humidity between 21- 47 %. While the ticks stage (larvae, nymphs and adults) were maintained under laboratory conditions (temperature 25°C and R.H. 75 %) after their dropped from cattle. In this experiment, *Hyalomma dromedarii* (*H. dromedarii*) feeding on cattle behaved as three-host. Similar finding was reported by Delpy and Gouchey, 1937 (cited in Hoogstraal 1956), who stated that *H. dromedarii* is a three-host tick and changes to two-host under stress of heat in order to avoid larval desiccation. Likewise, Das and Subramanian, (1972) stated that *H. dromedarii* fed on sheep and cattle is a three-host tick and 60% changed to two-host when fed on rabbits. In contrast, Hoogstraal, (1956) reported that this tick is a two-host life cycle under field condition and the change of host usually occurs after nymphal-adult moulting and rarely after larval-nymphal moulting. In other studies, they reported that *H. dromedarii* is a two host tick on rabbits (Alahmed and Kheir, 2003) and also on camels (El Ghali, 2005). Dipeolu and Adeyefa, (1984) showed that ticks which fed on sheep and horses were not as biologically viable as those of the same species which fed on cattle. The question that has given rise to much controversy on the type of life cycle of *H. dromedarii* prompted us to study its feeding behavior and drop-off rhythms under field conditions.

In the current study, the feeding periods of larvae on cattle was ranged between 3 and 17 days and for nymphs between 3-10 days. Previous studies investigated the larval-nymphal feeding periods of *H. dromedarii*, they found that this period ranged between 16 - 27 days on camels (El Ghali, 2005) and between 11- 16 days on rabbits (Alahmed and Kheir, 2003). These results present some fundamental differences between the biology of the tick *H. dromedarii* fed on cattle and those fed on camels and rabbits. Generally, the larval-nymphal feeding periods were shorter in ticks fed on rabbits compared with those fed on camels.

In this study, the pre-moulting period of larvae detached from cattle varied between 14 - 16 days, whereas the pre-moulting periods of nymphs 14 - 15 days at room temperature 25°C. Alahmed and Kheir, (2003) reported that the pre-moulting periods of nymphs were 14 -24 days at 25°C and between 10 -15 days at 36°C. Another study performed by El Ghali, (2005) found that the pre-moulting periods of nymphs were longest in January, around 20.7 days under the shade and 14.4 days under the sun. Moreover, the author reported an increase in the mean number of nymph pre-moulted with an increase in the mean ambient of temperature, humidity and rain. Furthermore, the longer pre-moulting periods of *H. dromedarii* nymphs was observed by Hagraas and Khalil (1988) as this

periods ranged between 18 and 19 days at 25°C and R.H 84%. Clearly, temperature and humidity are important factors that effect on the pre-moulting and moulting period.

In this study, the dropping-off period of *H. dromedarii* from cattle varied between 4 - 10 days. This result is agreement with pervious study in which they found that the adult *H. dromedarii* dropped from camel after 5-9 days (El Ghali, 2005). Alahmed and Kheir, (2003) reported that adult *H. dromedarii* dropped off from rabbits after 10 to 14 days. Obviously, the adult females feeding periods on cattle and camels were shorter than tick fed on rabbits. Tick species, tick stage, host or ecological zone may affect the drop-off timing of the ticks. Variations in the feeding periods may be due to differences in physiological characteristics of strains of a given species of ticks and not due to differences in the atmospheric temperature. Moreover, variations in atmospheric temperature from 5 to 40°C change the body temperature by only 1-1.5°C. Thus under natural conditions, the atmospheric temperature would probably be of little importance in determining the duration of the feeding (Balashov, 1968).

In this experiment, engorgement weight of replete *H. dromedarii* females was found to range between 0.620 - 0.961 g. In previous investigation conducted by El Ghali, (2005) showed that the weight of engorged females fed on camels ranged between 0.600 – 0.840 g. These findings conclude that engorged females fed on cattle have larger sizes compared with females fed on camels.

In current study, the preoviposition periods of *H. dromedarii* fed on cattle varied between 4 - 6 days at room temperatures 25°C. Alahmed and Kheir, (2003) informed that the preoviposition periods ranged between 5 - 8 days at 25°C and between 3 - 6 days at 32°C. Hagra and Khalil, (1988) stated that the preoviposition period of *H. dromedarii* was 4.4 days as mean at 29°C and 75% R.H. The variation among the seasons was also observed by El Ghali and Hassan (2010), who noticed that the preoviposition period was longer in December and April than in August. However, the difference was not significant in the shade, which seems to minimize the effect of heat.

In this study, the preecllosion period of *H. dromedarii* detached from cattle ranged between 16- 20 days. This duration was shorter compared with data presented in other studies in which they found that the preecllosion period of *H. dromedarii* detached from rabbits and from camels ranged between 56 - 68 days (Alahmed and Kheir, 2003) and between 25 -48 days (El Ghali and Hassan, 2010) respectively.

In the present study, engorged female of *H. dromedarii* that weighed 0.620 g (tick No. C, the lowest weight) gave the least number of eggs (5461 eggs) and the hatchability was 95.24%. While the engorged female of *H. dromedarii* that weighed 0.961 g (tick No. A, the highest weight) gave the highest number of eggs (9452 eggs) and the hatchability was 99.47%. The correlation between the female weight and the number of eggs has been investigated in a number of studies. For example, Alahmed and Kheir, (2003) reported that the engorged female of *H. dromedarii* that weighed 0.981 g gave around 9866 eggs and the hatched was 99%. El Ghali and Hassan, (2010) stated that an engorged female of *H. dromedarii* that weighed 0.600 g gave around 6932 eggs and the hatchability was 56.2%, while the other female that weighed 0.840 g gave a high number of eggs (6932 eggs) and the hatchability was 94.1%. Clearly, a strong positive correlation between the engorged weight of the female and the weight of eggs laid, the number of eggs laid and the hatchability of eggs.

Egg conversion ratio of *H. dromedarii* in this study ranged between 57.25% and 68.6%. EL Ghali and Hassan, (2010) reported that the egg conversion ratio (ECR) was associated directly with the season (temperature and humidity). The authors reported that the ECR was higher in August (0.72) than in April and December (0.46 and 0.39, respectively), higher in August under the shade and compared with December under the sun (0.65 and 0.29, respectively).

In conclusion, *H. dromedarii* completed its three-host life cycle (larvae, nymph, adult and egg) on cattle within 95 - 130 days at a mean temperature of 34.5–38.5°C and a relative humidity range of 21 - 75% . This experiment could give a hind that probable *H. dromedarii* may play a valuable role in the transmission of *T.annulata* among cattle under natural conditions. However, this outcome needs more investigation in order to confirm our hypotheses.

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