Life cycle of Hyalommadromedarii ticks (Acari: *Ixodidae*) on Sheep under experimental conditions

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ABSTRACT

Aims: This experiment aimed to study the life cycle of *Hyalomma dromedarii*~~(*H. dromedarii*)~~ fed on sheep under experimental conditions.
Place and Duration of Study: The study was conducted between June and October 2018 at Atbara Veterinary Research Laboratory, Atbara, River Nile state, Sudan.
Methodology: Nine sheep were purchased from the local market and used as hosts for feeding *H. dromedarii* (larvae, nymphs and adults) using the ear bags method. After feeding, detached engorged larvae, nymphs and adults were maintained under laboratory conditionsat 25°C and relative humidity (R.H)75%. All information including feeding periods, the number of engorged and detached ticks (larvae, nymphs and adults), pre-moulting and moulting periods, preoviposition and preeclosion periods were recorded daily.
Results:93.3%of larvae (1400/1500) successfully complete their feeding during a period between 3-17 days at 34.5°C and (R.H) 47%. The pre-moulting and moulting periods of larvae were between 12 -13 days and 2-3 days, respectively.
Regarding the nymph stage, 96% of nymphs (288/300) completed their feeding~~Concerning the nymph stage, 96% of nymphs (288/300) successfully completed their feeding during a period~~ between 3-10 days at 27.4°C and (R.H) 56%. The nymph took between 13-15 days as pre-moulting period, while the moulting period was 3-4 days. All adult ticks, 100% (30/30) completed their feeding successfully on sheep during a period between 4-10 days at temperatures ~~ranged~~ ranging between 33-35°C and (R.H)~~ranged~~ ranging between 36-41%. The preoviposition and preeclosion period varied between 4~~–~~6 days and 16-21 days, respectively. The average of engorged ticks' weight, number of eggs produced and the weight of mass eggs were 396.6 mg, 3530 eggs and 217.84 mg, respectively. Regarding hatchability, the results revealed that more than 97.3% of eggs were able to hatch and produce larvae.
Conclusion: Our study showed that *H. dromedarii*behaved as a three-host tick and completed its life cycle between 94-140 days.

Keywords: Hyalomma dromedarii, life cycle, sheep, Sudan.

1. INTRODUCTION

Globally, ticks are considered as significant arthropod vectors due to the wide variety of pathogens they can transmit to animals and humans [1]. Therefore, the information on the distribution patterns, biological life cycles, and the influence of the season and climate, as well as habitat factors of ticks is a key to identifying disease foci. Adding to that, this information could be used later to formulate prevention and control measures [2].

Sudan has one of the largest livestock populations in Africa. It has a total livestock population of 132.442 million heads constituting 41million heads of sheep, 32 head of goats, 31 head of cattle and 4.8 head of camels [3]. These animals generally provide meat and milk

for local consumption and meat and live animals for export, which contributes to foreign currency earnings [4].

In Sudan, more than 70 tick species were identified including the most economically important ticks in Africa [5]. Among these ticks, ~~*Hyalomma dromedarii* (~~*H. dromedarii*~~)~~ has been identified in different parts of Sudan and it primarily feeds on camels [6]. However, it also infests other domestic animals such as cattle, sheep, goats, and equines [7, 8, 9, 10, 11]. In cattle, this tick may play role in the transmission of *Theileria annulata*~~(*T. annulata*),~~particularly where camels are reared alongside cattle [2, 12]. *H. dromedarii* can transmit different types of pathogens such as the Crimean–Congo hemorrhagic fever virus, theileriosis in camels and cattle (*T. annulata*) and Q fever [7, 13, 14, 15].

The life cycle of *H. dromedarii* has been investigated in several studies [16, 17]. This tick follows different types of life cycles to avoid stress conditions. In an early study, they pointed out that *H. dromedarii* tick is a three-host species changing to two-host as their density on the host becomes high [18]. ELGhali [19] reported that this tick on cattle and sheep behaves as a three-host tick, while 60% of them changed to two-host when they fed on rabbits. Alahmed and Kheir [17] reported that this tick behaved as a two-host tick when fed on rabbits. A similar finding was also documented in Northern Sudan where they found that this tick behaved as a two-host tick when fed on camels [20].

The much controversy on the type of life cycle of *H. dromedarii* prompted us to study its feeding behavior on sheep and drop-off rhythms. Adding to that in Sudan, the economic importance of *H. dromedarii* is not limited to camels only, but it affects other animal species and their health and productivity are also influenced by tick and tick-borne diseases. ~~As far as I know, no~~ No work has been conducted previously to investigate the life cycle of *H. dromedarii* on sheep in Sudan. Based on the mentioned above, the current work aimed to study the life cycle of *H.dromedarii* under experimental conditions when it is fed on sheep.

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2. MATERIAL AND METHODS

2.1 Study area

The study was conducted at Atbara Veterinary Research Laboratory, Atbara, River Nile state, Sudan. Atbara is the capital city of River Nile state, which is located in the north part of Sudan between latitude 16-22 °N and longitude 30-32 °E.

2.2 Experimental Animals~~:~~

Nine sheep were purchased from the local market of Atbara and used as hosts for feeding *H. dromedarii*. During this experiment, sheep were kept at a temperature range between 27.4-35.5°C and ~~relative humidity (~~R.H.~~)~~ between 28-56%. Before the feed step, sheep were treated with dexamethasone as an immunosuppressant [21]~~ (Ahmed, 1999)~~.

2.3 Source of ticks~~:~~

Three engorged females of *H. dromedarii* were collected from camels at El Damer Market, River Nile State, Sudan and identified according to Hoogstraal [22]. Ticks were maintained under laboratory conditions using specimen tubes and a glass desiccator (containing 40% sodium chloride solution) at 25°C and R.H. 75% to oviposit. The ventilation of ticks was provided during daily inspection (Figure 1).

Figure1: Maintain of ticks (larvae, nymphs and adults) under laboratory conditions ~~using specimen tubes and a glass desiccator (containing 40% sodium chloride solution) at 25°C and R.H. 75%.~~

2.4 Feeding of emerged larvae, nymphs and adults on sheep~~:~~

Before feeding on sheep, the newly emerged larva, nymph and adult were kept under laboratory conditions using specimen tubes and a glass desiccator as described above for 7 days for hardening. The feeding was performed using the ear bags method (Figure 2). Briefly, the ears of sheep were shaved, washed with soap, dried and then disinfected with absolute alcohol. The ticks were placed to feed on sheep inside cotton cloth bags, which were fixed at the base of the ears using adhesive and a blaster. For each stage of *H. dromedarii* (larvae, nymph and adult), three sheep were used. Approximately, 1,500 larvae (500 larvae ~~per~~ /animal), 300 nymphs (100 nymphs ~~per~~ /animal) and 30 adults (5 females and 5 males ~~per~~ /animal) were settled on sheep.

Figure 2: Feeding of ticks ~~(larvae, nymphs and adults)~~ on sheep using the ear bag method~~.~~

2.5 Handling of ticks (larvae, nymphs and adults) in the laboratory

After the feeding, detached engorged larvae and ~~nymph~~ nymphswere maintained under laboratory conditions for moulting using a glass desiccator as described above. The engorged adults were collected after they dropped off (Figure 3), weighed separately using a sensitive balance (Sigma-Aldrich, Germany) and classified based on their sex. Out of them,

five females were placed in specimen tubes and maintained under laboratory conditions as illustrated above to oviposit (Figure 4). Firstly, the egg conversion ratio (ECR) of each female was counted using the following formula:

~~Egg conversion ratio (~~ECR~~)~~=(The weight of whole egg mass)/(The weight of engorged female)

Secondly, the mass of 250 eggs of each engorged female was weighed ~~[23]~~ (Ouhelli and Pandey, 1984) [23]. Then the average weight of a single egg was determined by dividing the weight of 250 eggs by the number of eggs (250). In each new specimen tube, the 250 eggs were placed and kept under laboratory conditions using a glass desiccator as illustrated above until hatching. The numbers of hatching larvae were counted using a microscope and the hatchability was calculated as a percentage (total number of larvae divided by the total number of eggs in each tube).

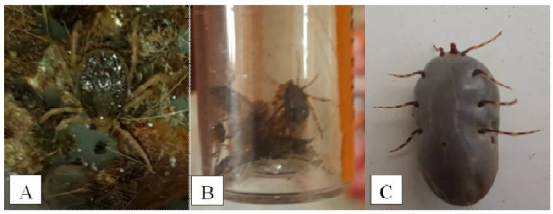

Figure 3: Adult of *H. dromedarii* before hardening (A) ~~and~~ , after hardening (B) and after feeding (engorged females) (C)

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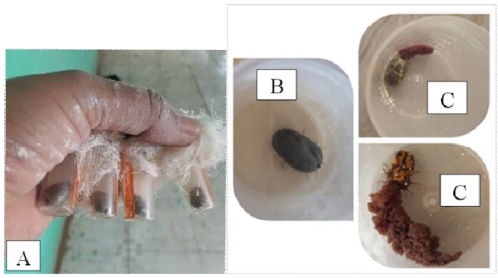

Figure 4: ~~Females tick were placed in specimen~~ Specimen tubes (A & B) ~~and maintained under laboratory conditions using a glass desiccator to oviposit~~ with female ticks (C).

2.6 Handling of data

All information such as feeding periods, the number of engorged ticks (~~larvae, nymph and adult~~all stages), the number of detached ticks (~~larvae, nymphs and adult~~all stages), pre-moulting and moulting periods, preoviposition period and preeclosion periods were recorded

daily. The preoviposition period defines as the interval from the introduction of adult females to a given temperature to when the first eggs were observed, while the preeclosion period knows as the interval from when eggs were first deposited to when larvae were first observed.

3. RESULTS

3.1 The life cycle of ~~*Hyalomma*~~ *H.dromedarii*

In this experiment, *H. dromedarii* that fed on sheep behaved as a three-host tick and took between 94 to 140 days to complete its life cycle. Around 93.3% of larvae (1400/1500) were able to complete their cycle on sheep (n=3). The larvae remained attached to the sheep for 3 to17 days to complete their feeding then they dropped off. They required between 12 to13 days as pre-moulting period and between 2 to 3 days to moult to the nymph stage (Table 1). Out of 300 nymphs, 296 (98.7%) were able to complete their cycle on sheep (n=3). The nymph remained attached to the sheep for 4 to10 days to complete their feeding. The pre-moulting period and the moulting period were 13-15 and 3-4 days, respectively (Table 1).All ticks (100%) were able to complete their cycle on sheep (n=3) and the feeding period ranged between 5 and 14 days (Table 1).

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Table 1: Feeding and developmental periods of larvae, nymph and adult of *H. dromedarii* fed on sheep

	Ticks		
	larvae	**Nymph**	**Adult**
Hardening period (days)	7	7	7
Total No. of attached tick	1500	300	30
No. of engorged detached tick (%)	1400 (93.3%)	288 (96%)	30 (100%)
Feeding period (days) (x ± sd)	3 – 17 (10.0 ± 4.4)	4 – 10 (7.0 ± 2.1)	5 – 14 (9.5 ± 3.0)
Pre-moulting period (days) (x ± sd)	12 – 13 (12.5 ± 0.7)	13 – 15 (14.0 ± 1.0)	-
Moulting period (days) (x ± sd)	2 – 3 (2.5 ± 0.7)	3 – 4 (3.5 ± 0.7)	-

x= mean, sd= standard deviation

3.2 The relationships between tick weight, preoviposition, and preeclosion

Five replete females were collected after detachment, weighed separately and maintained under laboratory conditions. The weight of females (n=5) ranged between 243 and 635 mg. The preoviposition and peeclosion periods were 4-6 and 16-21 days, respectively (Table 2).The estimated numbers of eggs produced by one tick ranged between 1674-5870 eggs, whereas the weight of eggs mass produced by one tick ranged between 83.7—399.16 mg. The egg conversion ratio was between 34.4-62.86% (Table 2).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 93.6 and 97.8 (Table 3).

Table 2: Relationship between the weight of engorged female, the periods of preoviposion and preeclosion, the production of eggs (the masses and numbers of eggs) and ECR.

No. of tick	Weight of engorged female (mg)	Periods (days)		Egg		
		Preovipostion	Preeclosion	No. of eggs	weight of all eggs mass (mg)	ECR (%)
A	635	5	19	5870	399.16	62.86
B	243	5	19	1674	83.7	34.4
C	558	4	16	4637	278.22	49.86
D	271	5	19	2685	161.1	59.4
E	276	6	21	2784	167.04	60.52
(x ± sd)	396.6±184.9	5±0.7	18.8±1.8	3530±1689.4	217.84±122.8	-

Egg conversion ratio (ECR) = the weight of all egg mass (mg)/ the weight of engorged female (mg), x= mean, sd= standard deviation

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Table 3: The hatching percentages among eggs that laid by female ticks fed on sheep

No. of tick	Total No. of eggs	Total No. of hatched larvae	Percentage of hatchability (%)
A	5870	5740	97.8
B	1674	1620	96.8
C	4637	4528	93.6
D	2685	2590	96.5
E	2784	2690	96.6
(x ± sd)	3530±1689.4	3433.6±1663.7	-

x= mean, sd= standard deviation

4. DISCUSSION

The current study investigated the life cycle of *H. dromedarii*, which included preoviposition periods, preecolosion periods, feeding periods of larvae, nymphs and adults, pre-moulting and moulting periods of larvae and nymphs and hatchability of eggs. During this experiment, sheep were kept at a temperature ranged between 27.4-35.5°C and relative humidity between 28 - 56%. While all the ~~stage~~ stages of the tick ~~(larvae, nymphs and adults)~~ were maintained under laboratory conditions (temperature 25°C and R.H. 75 %.) after they dropped from sheep.

In this study, ~~*Hyalomma dromedarii* (~~*H. dromedarii*~~)~~ that fed on sheep behaved as a three-host tick. An analogous finding was reported in an early study conducted by Delpy and Gouchey in 1937 ~~(cited in hoogstraal~~ [22]~~)~~, who stated that *H. dromedarii* is a three-host tick and changes to two-host under stress conditions such as heat. Likewise, ELGhali [19] documented that *H. dromedarii* on sheep and cattle is a three-host tick. In contrast, this tick behaved as a two-host tick when fed on camels under field conditions in Sudan and on rabbits under laboratory conditions [17, 20]. In other studies performed in Iran, they found that *H. dromedarii* behaved as a one-host tick on camels [24]. It is well known that *H.dromedarii* can behave as a one-, two- or three-host tick depending on ~~a number of~~ several factors such as the host availability and the environmental conditions [25]. This variation in feeding patterns also exists in other species of *Hyalomma* such as *H. anatolicum excavatum*, which is a two-host life cycle and may change into a three-host life cycle depending on which host the ticks feed on [26].

In the current study, the feeding periods of larvae and nymphs on sheep ranged between 3–to17 and 4-to10 days, respectively. This is ~~in line~~ similar with the results obtained by Siroký et

al, [27] who found that the feeding periods of *H. aegyptium* (a three-host tick) larva ranged between 3 to 9 days, while the nymphs required between 5 to 10 days to engorge. In other studies, in which it was investigated the feeding periods of *H. dromedarii* fed on camels and rabbits (the tick behaves as a two-host tick and the larvae fed, moulted and continued as nymphs on the same host (larval-nymphal)), they it was reported that the larval-nymphal feeding periods ranged between 16 to 27 and 11 to 16 days, respectively [17, 20]. Therefore These results showed a differences between the life cycle of *H. dromedarii* fed on sheep and those fed on camels and rabbits, whereas the larval-nymphal feeding period was shorter in ticks fed on rabbits compared with those fed on camels and sheep (larval and nymph feeding periods together).

Regarding the feeding period of adult ticks, our results showed that the adults required between 5 to 14 days to complete their feeding on sheep. Compared with other investigations in which they reported that the adult needed between 5 to 9 and 10 to 14 days to engorge and dropped off from camels and rabbits, respectively [17, 19]. Obviously, there is a variant in the feeding periods of tick (larvae, nymphs and adults) among sheep, camels and rabbits. This is may be attributed to the fact that the blood feeding of the tick on the host is affected by several factors such as the tick density, host defenses, ages of the host and the species of the host [28]. Moreover, a variation in atmospheric temperature has a significant impact on tick survival, activity and development [29]. Thus under natural conditions, the atmospheric temperature would probably have an influence on the duration of the feeding.

In this study, the pre-moulting period of larvae and nymphs that detached from sheep varied between 12 to 13 and 13 to 15 days, respectively, at 25°C. At the same temperature, Alahmed and Kheir [17] found that the pre-moulting period of *H. dromedarii* nymphs fed on rabbits was longer (14-24 days). Compared with other species of *Hyalomma*, these periods are considered long as the per-moulting periods of H. anatolicum reared at 28 °C and relative humidity R.H. 85% were 5 and 9 days for larva and nymphs, respectively [30]. The moulting periods of larvae and nymphs in this experiment were 2-3 and 3-4 days, respectively. These periods were shorter compared to that of *H. marginatum* reared at 28 °C (4-10 days for larvae and 15—24 days for nymphs), and of *H. dromedarii* fed on rabbits (14-24 days for nymphs) [17, 31]. Clearly, the pre-moulting and moulting periods are various among *Hyalomma spp* and within the same tick species and they are significantly affected by the host species.

In this experiment, the engorgement weight of replete *H. dromedarii* females (n=5) ranged between 243 to 635 mg (396.6±184.9 mg). This weight is less than the weight of replete *H. dromedarii* females fed on rabbits (981±218 mg) and camels (ranged between 840-600 mg) [17, 20]. Moreover, our finding is less compared with *H. anatolicum* fed on rabbits (482.92±88.08 mg) [32]. These findings concluded that engorged females of *H. dromedarii* fed on sheep have the smallest sizes compared with other others. Again, this variation could be linked with the variation of the host.

In the current study, the preoviposition periods varied between 4 to 6 days (5±0.7 days). This finding is in agreement with previous reports in which the researchers reported that the preoviposition period of *H. dromedarii* was 4.4 days (as mean) at 29°C [16]and between 5 to8 days at 25°C [17]. A similar duration was also observed in other tick species such as *H. marginatum* (3-5 days) and *H. anatolicum* fed on rabbits (4.8±0.42 days) [32, 33]. Regarding the preeclosion period, our study showed that the tick required between 16 and 21 days (18.8±1.8 days). This duration was shorter compared with data that presented in other studies in which the preeclosion periods of *H. dromedarii* detached from rabbits and camels were between 56 —to68 and 25 to48 days, respectively [17, 20]. The long preeclosion

period (mean 21.3±1.16 days) was also observed in *H. anatolicum*~~fed on rabbits~~[32]. Generally, these periods could be varied particularly when the temperature and humidity are shifting [2].

In the current study, the engorged female of *H. dromedarii* that weighed 243 mg (tick no. B, the smallest weight) gave the smallest number of eggs (1674 eggs) and the hatchability was 96.8%. While the engorged female of *H. dromedarii* that weighed 635 mg (tick no. A, the biggest weight) gave the biggest number of eggs (5870 eggs) and the hatchability was 97.8%. The relationship between the female weight, the number of eggs and hatchability has been investigated in a number of studies. Clearly, there is a strong relationship between the weight of engorged females and the ~~numbers~~ numberof eggs, as increases in the females' weight were associated with an increase in the number of eggs. This result is in accordance with a previous study in which the total number of eggs that were produced was raised in a line with the body weight of *Rhipicephalus sanguineus*, as the tick used the sucking blood for ovarian development and egg maturation [34]. On other hand, it seems to be the hatchability of eggs in this study does not affect by other elements such as engorged females' weight.

5. CONCLUSION

In conclusion*, H. dromedarii* completed its three-host life cycle (larvae, nymph, and adult) on sheep within 94 to 140 days at a temperature ~~ranged~~ rangingbetween 27.4-35.5°C and ~~relative humidity (~~R.H.~~)~~ between 28-56%. This experiment probably explains how *H. dromedarii* follows different types of life cycles to survive under various conditions. Host species, temperature and humidity are vital parameters in the life cycle of *H. dromedarii*. Additionally, this study could give a ~~hind~~ hintthat probable *H. dromedarii* may play a valuable role in the transmission of disease among sheep under natural conditions. However, this outcome needs more investigation in order to confirm our hypotheses.

ETHICAL APPROVAL

All animal procedures were carried out following the ethical standards established by the Institutional Ethics Committee of Sudan University of Science and Technology, Sudan (SUST/DSR/IEC/EA/2018).

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