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Research article

# Drop-off rhythms and survival of *Hyalomma anatolicum* (Acari: Ixodidae) fed on crossbred (Friesians x Zebu) calves in Sudan

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## HIGHLIGHTS

- *Hyalomma anatolicum* larvae behaved as 3-host life cycle when fed on cross-bred calves at zero grazing system.
- The drop-off rhythms of *H. anatolicum* mostly occurred at night.
- Unfed larvae were more resistant to desiccation than unfed nymphs.
- The ability of unfed stages to survive completes nearly ten weeks.

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ABSTRACT

*Hyalomma anatolicum* is one of the most economically important ticks in the Sudan. It is the main vector of tropical theileriosis in cattle and other diseases in different species. The study aims to investigate the drop-off rhythms and survival periods of tick stages fed on crossbred calves. Larvae, nymphs and adults fed on cross-bred calves (male, 3–6 months old, Friesians x Zebu) kept under zero grazing system. Drop-off rhythms of engorged stages were studied under field conditions while the survival periods of unfed stages were investigated under field and laboratory conditions (27 °C and 85% R.H.). Significantly high numbers of engorged larvae dropped at night between 20H:00 and 07H:00. (March, April and August). Engorged nymphs dropped late evening and early night 16H:00–20H:00. (September), while insignificant number of engorged females dropped between 10H:00 and 14H:00 (October). Survival durations of unfed *H. anatolicum* stages were longer under laboratory conditions than in the field. These were 99.36 ± 1.24 and 13.12 ± 0.68 days for larvae, 63 ± 1.33 and 16 ± 0.87 days for nymphs and 90 ± 3.6 and 45 ± 2.7 days for adults, respectively. Under field condition survival studies were conducted in May, August and December 2016 for larvae, March, May, August and December 2016 for nymphs and in October 2016 for Adults. In August, 70% of unfed larvae and nymphs survived for three weeks and 2.5 weeks, respectively, in May, 16% of unfed larvae survived for one week while no unfed nymphs survived for more than 4 days. This finding may indicate that larvae are more resistant to desiccation than nymphs. The abnormal changes on the alloscutum of *H. anatolicum* desiccated nymphs and females were observed being shrunken and truncated posteriorly, a finding which opens new avenues for further studies. For control strategies more studies are recommended on the dropping to allow ticks drop in an area not suitable for further development as one of the control strategies and the survival studies also is exploited in rotational grazing of animals.

## 1. Introduction

The genus *Hyalomma* is considered as prominent vectors of the domestic animal and human pathogen agents as well as hematophagous parasites of all terrestrial animals [1]. Hassan and Salih reported that *H. anatolicum* is one of the most economically important ticks in the Sudan with *Amblyomma variegatum, A. lepidum, Rhipicephalus decoloratus and R. annulatus* [2]. In Khartoum State, the annual production losses due to tropical theileriosis in cattle in Khartoum State estimated to be between US $ 4 and 6 million, and 85% of dairy farm investigated experienced clinical theileriosis with 22% and 30% mortality among newly born calves and heifers respectively [3], while Malignant ovine theileriosis (MOT) causes morbidity and mortality among sheep in Sudan [4].

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*Hyalomma anatolicum* is considered as prominent vectors of domestic animal and human pathogen and, identified from different geographical regions in the north, central, western and eastern Sudan [5]. In Sudan, the climate ranges from arid in the north to tropical wet and sub-humid in the south, desert regions in central and northern Sudan are among the driest and the sunniest places on Earth [6]. *H. anatolicum* is a xerophilic species thriving in semi-desert conditions in Northern Sudan; it was established in Eastern and Central Sudan [2, 7] and spread to western and south parts due to the animal movement and deforestation, although it does not inhabit high humid zones. It is able to propagate inside cattle pens and do not infest cattle kept under out-grazing or nomadic systems [2]. It is the main vector of tropical theileriosis in the Sudan that affects Kenana and Butana types of cattle in Sudan [8], Malignant Ovine Theileriosis of sheep and goats [9, 10] as well as equine piroplasmosis caused by *Theileria equi* and *Babesia caballi* [11], and Crimean-Congo Hemorrhagic fever virus (CCHFV) [12]. Drop-off rhythms of engorged ticks may either be nocturnal or diurnal. In nest-dwelling ticks, detachment rhythms tend to be coordinated with circadian rhythms of activity of the hosts, in order to end up in the nest where the hosts shelter [13]. Nocturnal dropping may help ticks avoid being preyed upon [14]. Amin speculated that the response of feeding ticks to the changes on the level of corticosterone of host's blood was one of the regulating mechanisms [15]. In contrast, Rechav pointed out that there was no significant effect on host movement and supported the effect of light and/or darkness influence of the drop-off rhythms [16]. Collected data on drop-off of engorged ticks can be used in their control strategies [17].

Survival of ticks is negatively correlated with air temperature and positively with relative humidity (R.H.) [17, 18]. During the host finding phase, ticks tend to avoid dehydration and their water balance against the drying power of atmosphere in which the activity of water is normally much below that of body [19]. El Ghali et al. reported that body weight changes in *H. anatolicum* were affected by both temperature and R.H. the maximum water uptake occurs under the lowest air temperature and highest R.H. while the maximum loss occurs at the highest air temperature and the lowest R.H. when survival periods shortened at 40 °C and 63% R.H [18]. Punyua pointed out that the season in which ticks were exposed in the field was an important factor determining survival rates [20]. There was an initial rapid uptake of water at night by the dehydrated ticks and water lost during the day was replaced at night. Drop-off rhythms and longevity of *H. anatolicum*, the vector of *Theileria annulata* has not been studied in Sudan. The purpose of the study was to determine and establish the drop-off rhythms of fed stages and survival periods of unfed stages of *H. anatolicum* when fed on calves (cross-bred) kept under zero grazing system and under laboratory and field conditions in Khartoum State, Sudan.

## 2. Methods

### 2.1. Study site and experimental animals

These experiments were conducted in animal enclosures at the Department of Parasitology, Faculty of Veterinary Medicine, University of Khartoum at Shambat. The calves' experimental animals were brought from Khartoum University Farm. They were males, 3–6 months old ages and cross-bred (Friesians x Zebu). They were kept, under zero grazing system, in small pens (3 × 2 × 2 meters dimensions) for one week of acclimatization. The calves were fed on concentrates and grass fodder and had free access to drinking water. They were examined for blood parasites in blood smear and lymph node biopsy smears. No parasites were detected in all the smears. For field survival studies, the sites of tick release were buildings made of concrete blocks in shade. The meteorological data for the site of the study are shown in Table (1). For studies under laboratory conditions, desiccators were adjusted at 85% humidity provided by saturated salt of Potassium Chloride (KCl). The desiccators were placed inside a cooled incubator adjusted at 27 ± 1 °C.

### 2.2. Drop-off rhythms of engorged ticks

Drop-off rhythms study was carried out according to standard method described by Bailey [21], using ear bags (dimension of the bags (sleeves), 10 cm diameter x 25 length). Approximately 1500–2000 unfed larvae, 200 nymphs and 15 males and 15 females were fed in the different sets of three calves (3–6 months old, crossbred, Friesians x Zebu). These tick stages were initially collected as fully engorged *H. anatolicum* females that had fed on cattle and a colony was established under laboratory conditions. Dropped engorged ticks were collected in transparent polyethylene bags by shaking the ears every 2 h starting from 07H:00–20H:00 for two consecutive days for dropped larvae and nymphs and three consecutive days for dropped females after the onset of dropping. The dropped ticks from each ear of each animal were separately counted recording day, time of dropping and the animal number. These experiments were carried out in March, April and August 2016 for engorged larvae, September 2016 for engorged nymphs and October 2016 for engorged females. Meteorological data of the study site are shown in Table 1.

### 2.3. Survival of unfed ticks

About 500 newly hatched larvae, 10 newly moulted nymphs and 5 newly moulted adults (after hardening period of the stages) were placed in 14, 12 and 20 glass test tubes (measuring 100 mm diameter x 16 cm length), respectively. These tubes were closed by cotton wool prior to transporting them to the site of release under cool and humid conditions. Similar numbers from the respective batches of each stage were placed in an incubator set at 27 ± 1 °C and 85% R.H. In the field, the survival periods were monitored (to count alive and dead ticks) every 24 h, every week and every month for unfed larvae (during May, August and December 2016) unfed nymphs (during March, May August and December 2016) and unfed adults (during October 2016), respectively. Meteorological data of the study site are shown in Table (1). In the laboratory, monitoring was carried out every three days, every week and every month for larvae, nymphs and adults respectively. The numbers of dead larvae were estimated while and the number of dead nymphs and dead adults were counted and recorded individually for each tube and each site of release.

### 2.4. Meteorological data of Shambat

Shambat meteorological data for the months of January to December 2016 and January and February 2017 were obtained from the Ministry of Environment, Natural Resources and Physical Development Meteorological Authority, Sudan Government [22] (Table1).

### 2.5. Statistical analysis

Data obtained from various experiments were subjected to an appropriate general linear model (GLM) procedure of the statistical analysis using the statistical analysis system (SAS) (version 9.1) package. The SAS was used to perform analysis of variance (ANOVA). Mean separations were performed using Ryan- Einot- Gabriel-Welsch Multiple Q test (REGWQ) [23]. Mean numbers of dropped engorged stages were counted every 2 h of the day starting from 07H:00–20H:00 and the meteorological data were compared for months March, April, and August 2016 for larvae, September and October 2016 for nymphs and female respectively, to enable determination of the effect of the meteorological

**Table 1.** Shambat meteorological data for the months of March to December 2016.

| Day | Max-T | Min-T | RH | Rain | Day | Max-T | Min-T | RH | Rain |
|---|---|---|---|---|---|---|---|---|---|
| 1-Mar | 40 | 17.5 | 21 | 0 | 1-Apr | 33.7 | 18.5 | 17 | 0 |
| 2-Mar | 41 | 16.5 | 23 | 0 | 2-Apr | 37.8 | 17.5 | 23 | 0 |
| 3-Mar | 40 | 16 | 24 | 0 | 3-Apr | 40 | 17 | 21 | 0 |
| 4-Mar | 37.5 | 17 | 25 | 0 | 4-Apr | 39.6 | 17 | 22 | 0 |
| 5-Mar | 39 | 19 | 21 | 0 | 5-Apr | 39.6 | 17.5 | 16 | 0 |
| 6-Mar | 38 | 18.5 | 17 | 0 | 6-Apr | 40.5 | 20 | 16 | 0 |
| 7-Mar | 37.8 | 15.5 | 20 | 0 | 7-Apr | 39 | 20 | 17 | 0 |
| 8-Mar | 40 | 15 | 17 | 0 | 8-Apr | 42 | 21 | 18 | 0 |
| 9-Mar | 42 | 17.5 | 22 | 0 | 9-Apr | 42 | 23 | 16 | 0 |
| 10-Mar | 44.5 | 17.5 | 16 | 0 | 10-Apr | 42 | 19 | 15 | 0 |
| 11-Mar | 44.5 | 20 | 20 | 0 | 11-Apr | 45 | 21 | 22 | 0 |
| 12-Mar | 44.5 | 20 | 22 | 0 | 12-Apr | 43.5 | 22 | 19 | 0 |
| 13-Mar | 43.5 | 24.5 | 28 | 0 | 13-Apr | 42.8 | 22 | 19 | 0 |
| 14-Mar | 41 | 19.5 | 32 | 0 | 14-Apr | 34.7 | 21 | 19 | 0 |
| 15-Mar | 39.7 | 22 | 28 | 0 | 15-Apr | 38 | 18 | 17 | 0 |
| 16-Mar | 37 | 16.5 | 28 | 0 | 16-Apr | 38 | 16.5 | 21 | 0 |
| 17-Mar | 36.5 | 22.5 | 19 | 0 | 17-Apr | 39.5 | 18.5 | 21 | 0 |
| 18-Mar | 38.5 | 18 | 16 | 0 | 18-Apr | 41 | 25 | 19 | 0 |
| 19-Mar | 39.5 | 19.5 | 22 | 0 | 19-Apr | 40.2 | 26 | 22 | 0 |
| 20-Mar | 40.2 | 19.5 | 23 | 0 | 20-Apr | 39.7 | 24 | 20 | 0 |
| 21-Mar | 39.3 | 20 | 20 | 0 | 21-Apr | 41 | 21 | 12 | 0 |
| 22-Mar | 36.5 | 20.5 | 20 | 0 | 22-Apr | 43 | 21 | 15 | 0 |
| 23-Mar | 36.3 | 16 | 21 | 0 | 23-Apr | 42.2 | 24 | 19 | 0 |
| 24-Mar | 39 | 16 | 15 | 0 | 24-Apr | 42.5 | 23.7 | 17 | 0 |
| 25-Mar | 44.5 | 15.7 | 19 | 0 | 25-Apr | 42.5 | 23 | 18 | 0 |
| 26-Mar | 43.5 | 18 | 20 | 0 | 26-Apr | 44.5 | 21.5 | 19 | 0 |
| 27-Mar | 45.5 | 19 | 18 | 0 | 27-Apr | 44 | 24 | 17 | 0 |
| 28-Mar | 43.5 | 25 | 25 | 0 | 28-Apr | 43.8 | 25 | 21 | 0 |
| 29-Mar | 37.5 | 24 | 16 | 0 | 29-Apr | 41.8 | 28 | 15 | 0 |
| 30-Mar | 38 | 23 | 15 | 0 | 30-Apr | 42.8 | 27 | 22 | 0 |
| 31-Mar | 36 | 23 | 16 | 0 | | | | | |
| 1-May | 42.8 | 28 | 22 | 0 | 1-Jun | 44.5 | 24 | 20 | 0 |
| 2-May | 43.5 | 27.5 | 19 | 0 | 2-Jun | 46 | 25 | 20 | 0 |
| 3-May | 43 | 27.5 | 21 | 0 | 3-Jun | 46 | 28 | 15 | 0 |
| 4-May | 43.5 | 26 | 20 | 0 | 4-Jun | 46 | 24 | 22 | 0 |
| 5-May | 42 | 26.5 | 26 | 0 | 5-Jun | 44 | 25 | 23 | 0 |
| 6-May | 40 | 25 | 39 | 0 | 6-Jun | 39 | 26 | 31 | 0 |
| 7-May | 39.5 | 25.5 | 39 | 0 | 7-Jun | 43 | 27.5 | 24 | 0 |
| 8-May | 42 | 27 | 25 | 0 | 8-Jun | 42.5 | 27 | 30 | 0 |
| 9-May | 43 | 24 | 22 | 0 | 9-Jun | 43.2 | 27.5 | 27 | 0 |
| 10-May | 43 | 27.4 | 23 | 0 | 10-Jun | 43.5 | 26 | 34 | 0 |
| 11-May | 43 | 26 | 30 | 0 | 11-Jun | 43.6 | 27.5 | 28 | 0 |
| 12-May | 43 | 25 | 29 | 0 | 12-Jun | 46 | 24 | 24 | 0 |
| 13-May | 42 | 25 | 20 | 0 | 13-Jun | 45.2 | 27.5 | 26 | 0 |
| 14-May | 43 | 24 | 25 | 0 | 14-Jun | 39 | 24.5 | 31 | 0 |
| 15-May | 45 | 24.5 | 20 | 0 | 15-Jun | 40.5 | 26 | 36 | 0 |
| 16-May | 43.5 | 26 | 23 | 0 | 16-Jun | 42 | 24 | 32 | 0 |
| 17-May | 44 | 27 | 26 | 0 | 17-Jun | 36 | 27 | 40 | 0 |
| 18-May | 43 | 28 | 30 | 0 | 18-Jun | 40 | 26 | 48 | 0 |
| 19-May | 44 | 29 | 28 | 0 | 19-Jun | 36 | 25 | 45 | 0 |
| 20-May | 45.5 | 30 | 26 | 0 | 20-Jun | 37 | 25.5 | 45 | 0 |
| 21-May | 42.6 | 28 | 28 | 0 | 21-Jun | 42 | 25 | 35 | 0 |
| 22-May | 44 | 25.2 | 27 | 0 | 22-Jun | 37 | 27 | 35 | 0 |
| 23-May | 45.5 | 24 | 24 | 0 | 23-Jun | 39.5 | 28 | 37 | 0 |
| 24-May | 42.2 | 27 | 27 | 0 | 24-Jun | 41 | 27 | 34 | 0 |
| 25-May | 44 | 28 | 20 | 0 | 25-Jun | 38.5 | 25 | 35 | 0 |
| 26-May | 41.5 | 28 | 20 | 0 | 26-Jun | 40 | 27 | 35 | 0 |
| 27-May | 40.5 | 24 | 20 | 0 | 27-Jun | 38.5 | 28.5 | 38 | 0 |

(*continued on next page*)

**Table 1** (*continued*)

| Day | Max-T | Min-T | RH | Rain | Day | Max-T | Min-T | RH | Rain |
|---|---|---|---|---|---|---|---|---|---|
| 28-May | 40.5 | 24.5 | 18 | 0 | 28-Jun | 42.5 | 28 | 30 | 0 |
| 29-May | 40.5 | 26.5 | 25 | 0 | 29-Jun | 42 | 27 | 29 | 0 |
| 30-May | 41.5 | 25.5 | 18 | 0 | 30-Jun | 41.5 | 25.5 | 28 | 0 |
| 31-May | 43 | 24 | 18 | 0 | | | | | |
| 1-Aug | 33.7 | 27 | 59 | 0 | 1-Jul | 42.5 | 26.5 | 31 | 0 |
| 2-Aug | 33 | 26 | 63 | 0 | 2-Jul | 41.7 | 26.5 | 31 | 0 |
| 3-Aug | 35 | 26.5 | 56 | 9.3 | 3-Jul | 43 | 27.5 | 29 | 0 |
| 4-Aug | 34 | 24.5 | 66 | 0 | 4-Jul | 42.2 | 25 | 31 | 0 |
| 5-Aug | 34.5 | 25.2 | 59 | 0 | 5-Jul | 43 | 25 | 30 | 0 |
| 6-Aug | 31.5 | 26 | 80 | 32 | 6-Jul | 42.6 | 27 | 28 | 0 |
| 7-Aug | 33.5 | 24 | 67 | 0 | 7-Jul | 41.3 | 27.3 | 33 | 0 |
| 8-Aug | 35.5 | 25 | 55 | 0 | 8-Jul | 39 | 27 | 42 | 0 |
| 9-Aug | 34 | 24 | 62 | 0 | 9-Jul | 39.2 | 28 | 42 | 0 |
| 10-Aug | 34.5 | 25 | 54 | 0 | 10-Jul | 38 | 27.5 | 54 | 2 |
| 11-Aug | 34.5 | 25.5 | 56 | 0 | 11-Jul | 31.8 | 25 | 66 | 0 |
| 12-Aug | 36 | 24 | 53 | 0 | 12-Jul | 36.5 | 25.7 | 50 | 5 |
| 13-Aug | 37.5 | 26 | 46 | 22 | 13-Jul | 36.2 | 24 | 53 | 36 |
| 14-Aug | 34.7 | 23 | 72 | 0 | 14-Jul | 35.5 | 23.5 | 63 | 0 |
| 15-Aug | 37.7 | 21.5 | 53 | 0 | 15-Jul | 36 | 22 | 54 | 9 |
| 16-Aug | 39.5 | 25.5 | 43 | 0 | 16-Jul | 33 | 24 | 66 | 0 |
| 17-Aug | 36 | 26.5 | 56 | 0 | 17-Jul | 38.2 | 24 | 46 | 0 |
| 18-Aug | 35.5 | 26 | 56 | 0 | 18-Jul | 38.5 | 27 | 45 | 0 |
| 19-Aug | 37.5 | 26 | 53 | 6.2 | 19-Jul | 36.5 | 24.5 | 50 | 0 |
| 20-Aug | 30 | 22 | 74 | 0 | 20-Jul | 35 | 24.5 | 53 | 0 |
| 21-Aug | 33.5 | 24 | 66 | 0 | 21-Jul | 37.3 | 25.5 | 49 | 0 |
| 22-Aug | 37.4 | 24.5 | 56 | 0 | 22-Jul | 40.5 | 26.5 | 47 | 0 |
| 23-Aug | 36.2 | 26 | 55 | 0 | 23-Jul | 42 | 30 | 35 | 0 |
| 24-Aug | 40 | 26.5 | 46 | 0 | 24-Jul | 38 | 27 | 45 | 0 |
| 25-Aug | 37.5 | 25.5 | 54 | 0 | 25-Jul | 35.2 | 26 | 50 | 0 |
| 26-Aug | 39.5 | 25 | 44 | 0 | 26-Jul | 37.5 | 26.5 | 47 | 0 |
| 27-Aug | 40.5 | 25.5 | 40 | 0 | 27-Jul | 38 | 24.5 | 34 | 20.5 |
| 28-Aug | 40.8 | 27 | 39 | 0 | 28-Jul | 33 | 26 | 67 | 0 |
| 29-Aug | 39 | 27.5 | 43 | 0 | 29-Jul | 33.5 | 22 | 67 | 0 |
| 30-Aug | 36 | 25.5 | 49 | 0 | 30-Jul | 35 | 23 | 60 | 0 |
| 31-Aug | 40 | 25 | 41 | 0 | 31-Jul | 33.5 | 25 | 58 | 0 |
| 1-Aug | 33.7 | 27 | 59 | 0 | 1-Jul | 42.5 | 26.5 | 31 | 0 |
| 2-Aug | 33 | 26 | 63 | 0 | 2-Jul | 41.7 | 26.5 | 31 | 0 |
| 3-Aug | 35 | 26.5 | 56 | 9.3 | 3-Jul | 43 | 27.5 | 29 | 0 |
| 4-Aug | 34 | 24.5 | 66 | 0 | 4-Jul | 42.2 | 25 | 31 | 0 |
| 5-Aug | 34.5 | 25.2 | 59 | 0 | 5-Jul | 43 | 25 | 30 | 0 |
| 6-Aug | 31.5 | 26 | 80 | 32 | 6-Jul | 42.6 | 27 | 28 | 0 |
| 7-Aug | 33.5 | 24 | 67 | 0 | 7-Jul | 41.3 | 27.3 | 33 | 0 |
| 8-Aug | 35.5 | 25 | 55 | 0 | 8-Jul | 39 | 27 | 42 | 0 |
| 9-Aug | 34 | 24 | 62 | 0 | 9-Jul | 39.2 | 28 | 42 | 0 |
| 10-Aug | 34.5 | 25 | 54 | 0 | 10-Jul | 38 | 27.5 | 54 | 2 |
| 11-Aug | 34.5 | 25.5 | 56 | 0 | 11-Jul | 31.8 | 25 | 66 | 0 |
| 12-Aug | 36 | 24 | 53 | 0 | 12-Jul | 36.5 | 25.7 | 50 | 5 |
| 13-Aug | 37.5 | 26 | 46 | 22 | 13-Jul | 36.2 | 24 | 53 | 36 |
| 14-Aug | 34.7 | 23 | 72 | 0 | 14-Jul | 35.5 | 23.5 | 63 | 0 |
| 15-Aug | 37.7 | 21.5 | 53 | 0 | 15-Jul | 36 | 22 | 54 | 9 |
| 16-Aug | 39.5 | 25.5 | 43 | 0 | 16-Jul | 33 | 24 | 66 | 0 |
| 17-Aug | 36 | 26.5 | 56 | 0 | 17-Jul | 38.2 | 24 | 46 | 0 |
| 18-Aug | 35.5 | 26 | 56 | 0 | 18-Jul | 38.5 | 27 | 45 | 0 |
| 19-Aug | 37.5 | 26 | 53 | 6.2 | 19-Jul | 36.5 | 24.5 | 50 | 0 |
| 20-Aug | 30 | 22 | 74 | 0 | 20-Jul | 35 | 24.5 | 53 | 0 |
| 21-Aug | 33.5 | 24 | 66 | 0 | 21-Jul | 37.3 | 25.5 | 49 | 0 |
| 22-Aug | 37.4 | 24.5 | 56 | 0 | 22-Jul | 40.5 | 26.5 | 47 | 0 |
| 23-Aug | 36.2 | 26 | 55 | 0 | 23-Jul | 42 | 30 | 35 | 0 |
| 24-Aug | 40 | 26.5 | 46 | 0 | 24-Jul | 38 | 27 | 45 | 0 |

(*continued on next page*)

**Table 1** (*continued*)

| Day | Max-T | Min-T | RH | Rain | Day | Max-T | Min-T | RH | Rain |
|---|---|---|---|---|---|---|---|---|---|
| 25-Aug | 37.5 | 25.5 | 54 | 0 | 25-Jul | 35.2 | 26 | 50 | 0 |
| 26-Aug | 39.5 | 25 | 44 | 0 | 26-Jul | 37.5 | 26.5 | 47 | 0 |
| 27-Aug | 40.5 | 25.5 | 40 | 0 | 27-Jul | 38 | 24.5 | 34 | 20.5 |
| 28-Aug | 40.8 | 27 | 39 | 0 | 28-Jul | 33 | 26 | 67 | 0 |
| 29-Aug | 39 | 27.5 | 43 | 0 | 29-Jul | 33.5 | 22 | 67 | 0 |
| 30-Aug | 36 | 25.5 | 49 | 0 | 30-Jul | 35 | 23 | 60 | 0 |
| 31-Aug | 40 | 25 | 41 | 0 | 31-Jul | 33.5 | 25 | 58 | 0 |
| 1-Sep | 40.5 | 27 | 41 | 0 | 1-Oct | 40 | 27 | 41 | 0 |
| 2-Sep | 40.2 | 27 | 37 | 0 | 2-Oct | 41 | 27 | 41 | 0 |
| 3-Sep | 35.8 | 26 | 53 | 0 | 3-Oct | 39.4 | 27 | 44 | 0 |
| 4-Sep | 37.5 | 25 | 52 | 0 | 4-Oct | 37 | 25 | 45 | 0 |
| 5-Sep | 40.4 | 26 | 43 | 0 | 5-Oct | 39.5 | 25 | 36 | 0 |
| 6-Sep | 42.5 | 27 | 36 | 0 | 6-Oct | 41 | 26 | 35 | 0 |
| 7-Sep | 41 | 28.5 | 38 | 0 | 7-Oct | 41.5 | 25 | 34 | 0 |
| 8-Sep | 37 | 26 | 44 | 0 | 8-Oct | 41.4 | 25 | 37 | 0 |
| 9-Sep | 39 | 26 | 47 | 0 | 9-Oct | 42 | 24 | 24 | 0 |
| 10-Sep | 40 | 26 | 40 | 12 | 10-Oct | 41.3 | 25 | 35 | 0 |
| 11-Sep | 43.2 | 23 | 60 | 0 | 11-Oct | 41 | 24.5 | 30 | 0 |
| 12-Sep | 38.5 | 24 | 47 | 0 | 12-Oct | 41 | 23 | 35 | 0 |
| 13-Sep | 41 | 27 | 41 | 0 | 13-Oct | 41.5 | 24 | 35 | 0 |
| 14-Sep | 40 | 25 | 41 | 9 | 14-Oct | 41.5 | 21 | 32 | 0 |
| 15-Sep | 41.5 | 26 | 35 | 0 | 15-Oct | 40.2 | 23 | 36 | 0 |
| 16-Sep | 42 | 24 | 58 | 0 | 16-Oct | 41.2 | 24 | 32 | 0 |
| 17-Sep | 31.5 | 23 | 61 | 0 | 17-Oct | 41.5 | 23.8 | 29 | 0 |
| 18-Sep | 37 | 23 | 49 | 0 | 18-Oct | 41 | 23 | 27 | 0 |
| 19-Sep | 39.4 | 25.5 | 48 | 0 | 19-Oct | 40.5 | 25 | 31 | 0 |
| 20-Sep | 40 | 28 | 42 | 2 | 20-Oct | 41 | 25 | 25 | 0 |
| 21-Sep | 36 | 23 | 53 | 0 | 21-Oct | 40 | 23.5 | 29 | 0 |
| 22-Sep | 40.5 | 24.5 | 39 | 0 | 22-Oct | 39 | 25 | 28 | 0 |
| 23-Sep | 40.5 | 25 | 35 | 0 | 23-Oct | 39.5 | 25 | 31 | 0 |
| 24-Sep | 40.5 | 27 | 39 | 0 | 24-Oct | 40.5 | 26 | 28 | 0 |
| 25-Sep | 39.5 | 25 | 43 | 0 | 25-Oct | 41 | 26 | 30 | 0 |
| 26-Sep | 39.5 | 22.5 | 28 | 0 | 26-Oct | 39 | 24 | 27 | 0 |
| 27-Sep | 40 | 25 | 31 | 0 | 27-Oct | 36 | 22 | 34 | 0 |
| 28-Sep | 39.5 | 26 | 32 | 0 | 28-Oct | 37.2 | 25 | 23 | 0 |
| 29-Sep | 40.5 | 26 | 41 | 0 | 29-Oct | 39.5 | 25.5 | 29 | 0 |
| 30-Sep | 41.5 | 26 | 33 | 0 | 30-Oct | 39.4 | 25.5 | 31 | 0 |
| | | | | | 31-Oct | 40 | 23 | 34 | 0 |
| 1-Nov | 41 | 26 | 28 | 0 | 1-Dec | 37 | 19 | 28 | 0 |
| 2-Nov | 39.5 | 26.5 | 32 | 0 | 2-Dec | 35 | 19.5 | 27 | 0 |
| 3-Nov | 38 | 22 | 32 | 0 | 3-Dec | 35 | 19 | 31 | 0 |
| 4-Nov | 38 | 23 | 31 | 0 | 4-Dec | 36.6 | 19.5 | 37 | 0 |
| 5-Nov | 37.5 | 24 | 30 | 0 | 5-Dec | 37 | 22 | 35 | 0 |
| 6-Nov | 36 | 23 | 34 | 0 | 6-Dec | 36 | 22 | 33 | 0 |
| 7-Nov | 36.3 | 23 | 34 | 0 | 7-Dec | 33.4 | 16 | 29 | 0 |
| 8-Nov | 36.5 | 21 | 32 | 0 | 8-Dec | 34 | 14.5 | 36 | 0 |
| 9-Nov | 38 | 20 | 34 | 0 | 9-Dec | 34 | 16.8 | 38 | 0 |
| 10-Nov | 38 | 21.5 | 34 | 0 | 10-Dec | 34.1 | 19 | 39 | 0 |
| 11-Nov | 38.2 | 23.5 | 36 | 0 | 11-Dec | 33.8 | 20.5 | 37 | 0 |
| 12-Nov | 38.5 | 23 | 32 | 0 | 12-Dec | 34 | 19.5 | 38 | 0 |
| 13-Nov | 38.3 | 22.5 | 33 | 0 | 13-Dec | 33.4 | 18 | 29 | 0 |
| 14-Nov | 38.5 | 22 | 33 | 0 | 14-Dec | 32.6 | 17 | 23 | 0 |
| 15-Nov | 38.3 | 20 | 34 | 0 | 15-Dec | 29.6 | 15.5 | 31 | 0 |
| 16-Nov | 38 | 20 | 29 | 0 | 16-Dec | 30 | 14.8 | 38 | 0 |
| 17-Nov | 37.5 | 19.9 | 31 | 0 | 17-Dec | 31.5 | 15 | 36 | 0 |
| 18-Nov | 38 | 20 | 31 | 0 | 18-Dec | 33.5 | 15.5 | 36 | 0 |
| 19-Nov | 37.5 | 22 | 34 | 0 | 19-Dec | 32.5 | 17.5 | 37 | 0 |
| 20-Nov | 38 | 23 | 28 | 0 | 20-Dec | 32 | 17 | 37 | 0 |
| 21-Nov | 38 | 24 | 23 | 0 | 21-Dec | 31.5 | 18 | 33 | 0 |

(*continued on next page*)

**Table 1** (*continued*)

| Day | Max-T | Min-T | RH | Rain | Day | Max-T | Min-T | RH | Rain |
|---|---|---|---|---|---|---|---|---|---|
| 22-Nov | 37.7 | 22 | 32 | 0 | 22-Dec | 33.5 | 15 | 34 | 0 |
| 23-Nov | 36.8 | 24.5 | 33 | 0 | 23-Dec | 35.5 | 16 | 37 | 0 |
| 24-Nov | 34.5 | 20 | 23 | 0 | 24-Dec | 34.6 | 17 | 41 | 0 |
| 25-Nov | 30 | 18.3 | 28 | 0 | 25-Dec | 35.2 | 18 | 27 | 0 |
| 26-Nov | 32.2 | 18 | 31 | 0 | 26-Dec | 30.6 | 17 | 32 | 0 |
| 27-Nov | 33.2 | 18 | 24 | 0 | 27-Dec | 30 | 16.5 | 32 | 0 |
| 28-Nov | 35.6 | 14 | 29 | 0 | 28-Dec | 30.5 | 17 | 34 | 0 |
| 29-Nov | 35.6 | 18 | 31 | 0 | 29-Dec | 31 | 17.5 | 30 | 0 |
| 30-Nov | 35.4 | 19 | 33 | 0 | 30-Dec | 32.5 | 15 | 30 | 0 |
| | | | | | 31-Dec | 34.5 | 17 | 35 | 0 |

Min-T = Ambient minimum temperature.
Max-T = Ambient maximum temperature.
R.H= Ambient relative humidity.
Rain = Total rainfall.

data of the month of release and drop off rhythms of engorged stages. Correlation analysis was performed to relate survival periods with meteorological values.

### *Ethical statement*

All procedures described in this experiment were approved by Faculty Research Ethic committee at University of Khartoum.

## 3. Results

### *3.1. Drop-off rhythms of Hyalomma anatolicum fed on calves*

Engorged larvae dropped between 20H:00 and 07H:00 in a very highly significant ($P \leq 0.001$) number (Figure 1A). The dropped larvae were very highly significant in August and April, between 20H:00 and 07H:00 in comparison with that of March which was not significantly different in time of dropping (Figure 2). It was found that all larvae (100%) behaved as 3-host ticks. Nymphs dropped in a very highly

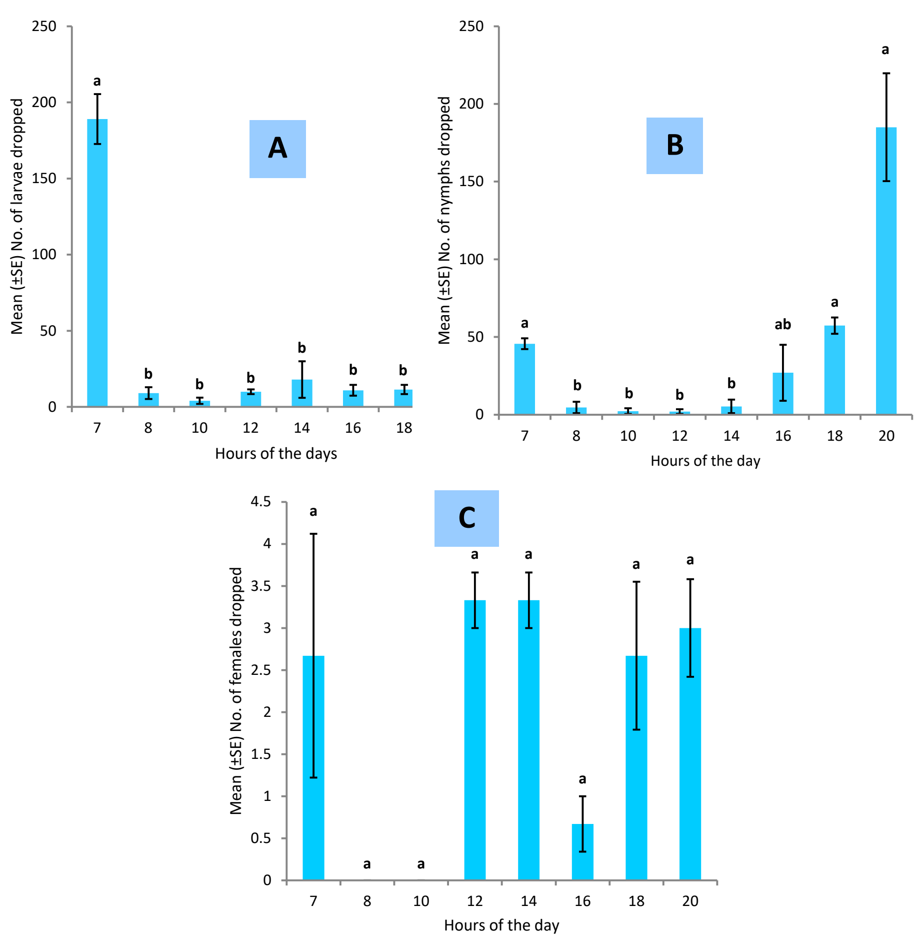


Figure 1. Mean (±SE) number of dropped engorged **A**: larvae, **B**: Nymphs and **C**: females of *H. anatolicum* from calves in different hours of the day in October 2016 at Shambat, the same letters are not significantly different at 5% level according to REWGQ range test.

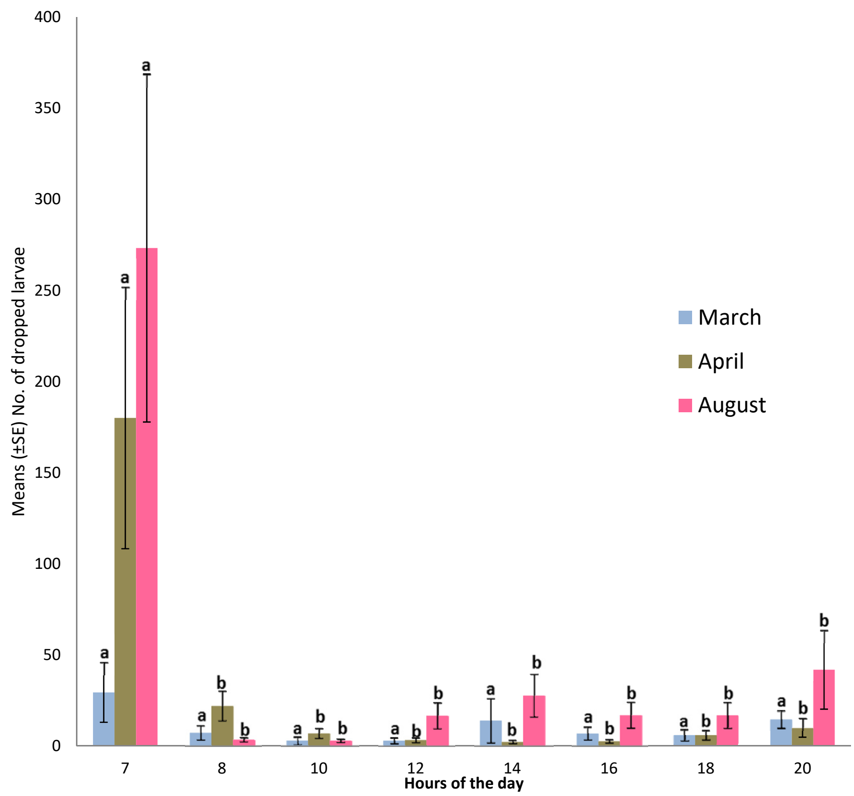


Figure 2. Mean (±SE) numbers of dropped engorged larvae of *H. anatolicum* in March, April and August 2016 at Shambat, the same letters are not significantly different at 5% level between hours of the day in the same month, according to REWGQ range test.

significant ($P \leq 0.001$) numbers between 16H:00 and 20H:00 (Figure 1B). For females, dropping, there was no significant difference ($P > 0.05$) among numbers dropped in the morning, afternoon, and at night. The highest mean number dropped was insignificant at 10H:00–12H:00 and 12H:00–14H:00 (Figure 1C). Females started dropping on day five of application with notable peaks on days five and day six (38.8% and 26.5%, respectively). They continued dropping with very few numbers until day 10. Although there was no significant difference ($P > 0.05$) between the mean weights of females dropping on different days, but, females of the highest mean weights (302.4 ± 17.8 mg) dropped on the first day (day 5) (Figure 3). It was noticed that males did not drop until the end of monitoring periods.

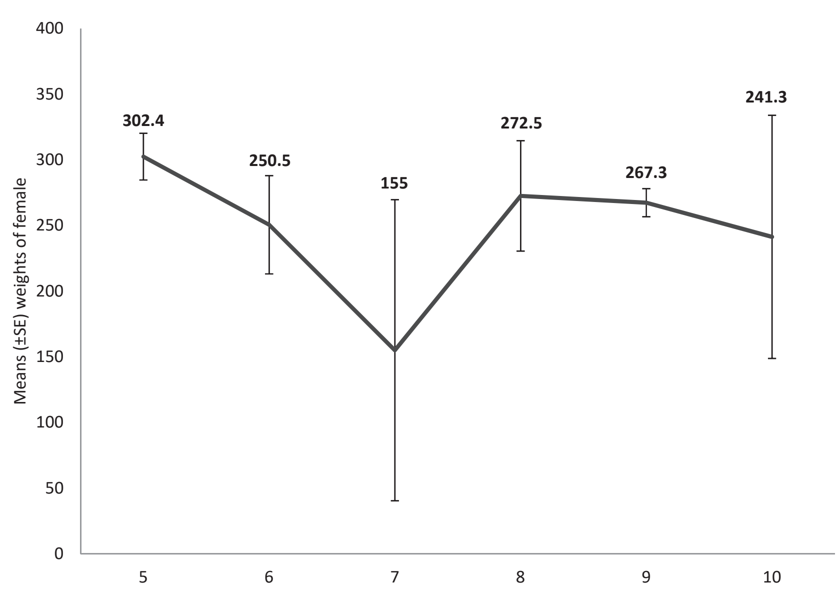


Figure 3. Mean (±SE) weights of engorged females of *H. anatolicum* dropped on different days after feeding on calves in 2016 at Shambat.

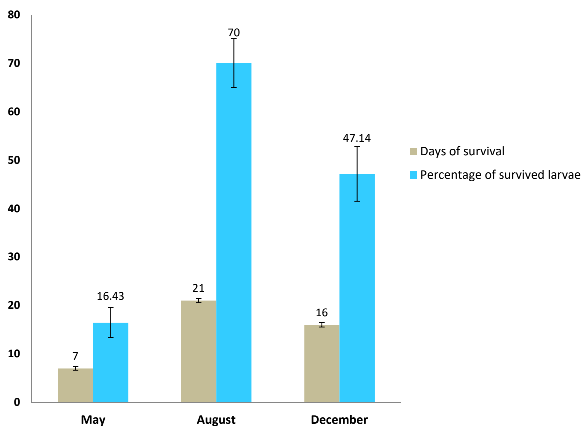


**Figure 4.** Means (±SE) number of days and percentage of survival of unfed larvae of *H. anatolicum* in May, August and December 2016 under field conditions in 2016 at Shambat.

### 3.2. Survival periods of unfed H. anatolicum

There was highly significant difference (P ≤ 0.001) between the mean number of survival days of unfed larvae in the field during May, August and December. In August, larvae survived longer than December and May (7.0 ± 0.36 days). The mean percent survival of unfed larvae in May, August and December was very highly significantly (P ≤ 0.001) different (Figure 4) . Survival days of unfed larvae were very highly significantly positively correlated with R.H. (r = 0.46, P ≤ 0.001) and rainfall (r = 0.42, P ≤ 0.001) but negatively insignificant with minimum air temperature (r = -0.2, P˃0.05) and highly negatively significantly to maximum air temperature (r = -0.23, P ≤ 0.01). Percent survival of unfed larvae was very highly positively correlated with R.H. (r = 0.25, P ≤ 0.001), but it was insignificantly correlated to rainfall (r = 0.2, P˃0.05) and minimum air temperature (r = -0.14, P˃0.05) and very highly significantly with the maximum temperature (r = -0.27, P ≤ 0.001) (Table 2). The mean survival weeks of nymphs were very highly significantly different (P ≤ 0.001) under field and laboratory conditions (Figure 5b). There was a very highly significant difference between mean number of survival weeks for nymphs (P ≤ 0.001) and mean number of nymphs mortality (P ≤ 0.001)under field conditions in May (RH: 24.5%, Temp: min 26.2° - max 42.2° C) and March (RH: 20.9%, Temp: min 19.1° - max 40.1° C) compared with August (RH: 55.4%, Temp: min 25.4° - max 39.5° C) and September (RH: 42.9%, Temp: min 26.2° - max 42.2° C) which were not significantly different (Figure 6). In May, unfed nymphs did not survive more than a mean of one week but in March they lived for 1.5 ± 0.29 weeks and 2.5 ± 0.17 weeks in August and September (Figure 6). The mean survival period was the least in May (no nymphs survived) followed by September and March which were very highly significantly different (P ≤ 0.001) from that of August (Figure 6). There was very highly significant correlation (P ≤ 0.001) between mean numbers of survival weeks of nymphs in different months (March, May, August and September) and maximum air temperature, R.H. and rainfall (Table 2). Unfed adults survived for mean of 1.50 ± 0.09 months in the field which was very highly significantly different (P ≤ 0.001) among the three months that required for survival under laboratory conditions (Figure 5c). It was observed that unfed *H. anatolicum* nymphs and females showed anatomical changes in the alloscutum (=the part of the body that is not covered with scutum expanding during feeding). This part became elongated, shrunken and truncated posteriorly (Figure 7).

## 4. Discussion

*Hyalomma anatolicum* was fed on calves kept under zero grazing system. Most engorged larvae dropped at night between 20H:00 and 07H:00. and a fewer numbers dropped during the morning hours and day time while most nymphs dropped in a significant high numbers between 16H:00 and 20H:00. Insignificant different numbers of females dropped between 10H:00 and 14H:00 and early night at 16H:00–20H:00. Serdyukova in 1945 working in Tadzhikistan, observed that *H. anatolicum* larvae, nymphs, and females fed on cattle dropped nocturnally [15]. In India, *H. anatolicum* females mostly dropped at night in the cowshed [24], where the emerging larvae found it easy to seek and attach on to the hosts [25]. In the current study, larvae dropping in high numbers at night and some nymphs and adults at early hours of the night are in line with the finding of Serdyukova who observed that *H. anatolicum* stages dropped in the dark hours. Minshull (1982) stated that the light was the most important oscillator governing the drop-off rhythms more than the oscillator from the host and ticks [17]. It was observed; in this study that dropping seasonally had no pattern. This finding is in line with the fact that season does not affect detachment of engorged ticks [14, 17]. It was noticed that male ticks did not drop which might indicate that mating of *H. anatolicum* male occurs with several females. According to Chiera and Punyua, male staying longer on the host is capable of mating with several females [26].

Air temperature plays a crucial role in regulation of the tick life cycle. Mortality rates were related to the water content of the air [27]. According to Asebe et al. data on survival of the various stages without feeding will determine how long cattle should be excluded from the pastures [28]. Survival periods of ticks were negatively correlated with air temperature and positively with the relative humidity [17, 29]. In the current study, the high negative correlation of air temperature and highly significantly (P ≤ 0.001) positive correlation of R.H. between survival duration and number of survived unfed ticks. The survival days were longer under laboratory than under field conditions. Ali et al. found that 85% R.H. was critical equilibrium humidity (CEH) of *H. anatolicum* at higher R.H. *H. anatolicum* immature stages gain water by absorbing from atmosphere but at low R.H., they lose water through evaporation to the surroundings [30].

This study showed that 16% of larvae survived nearly less than one week in May while 70% of larvae survived for three weeks and 47% for two weeks in August and December, respectively. The shortest survival period was recorded in May. This may be due to the fact that the temperature might have affected the CTT (critical transition temperature) at which a sharp increase in water loss occurs [31], and low R.H. which was below CEH led to the larvae losing water to the atmosphere. In contrast, in August, survival period of larvae was longer than in December because of low R.H. in the latter month. Sweatman stated that longevity was shortened with increasing of saturation deficit [32].

No nymphs survived in May for more than one week probably due to the effect of high temperature which might be within the possible range of CTT, whereas in March half of them survived for a mean of 1.5 weeks because of the slight decrease of ambient temperature. However, in August and September they survived for 2.5 weeks with mean of 7.39 and

**Table 2.** Correlation analysis between climatic factors and survival parameters of unfed larvae, nymphs and adults of *H. anatolicum* in 2016 at Shambat.

| Parameters | Climatic factors | | | |
|---|---|---|---|---|
| | Min-Temp | Max-Temp | R.H. | Rain |
| Survived larvae (%) | -0.143 ns | -0.265*** | 0.257*** | 0.198 ns |
| Days | -0.21 ns | -0.217** | 0.462*** | 0.419*** |
| Survived nymphs | -0.82 ns | -0.43*** | 0.335*** | 0.391*** |
| Weeks | 0.16 ns | -0.339*** | 0.392*** | 0.340*** |

***P ≤ 0.001, ns = not significant.
Number of observation = 165.
Min-Temp = Ambient minimum temperature.
Max-Temp = Ambient maximum temperature.
R.H= Ambient relative humidity.
Rain = Total rainfall.

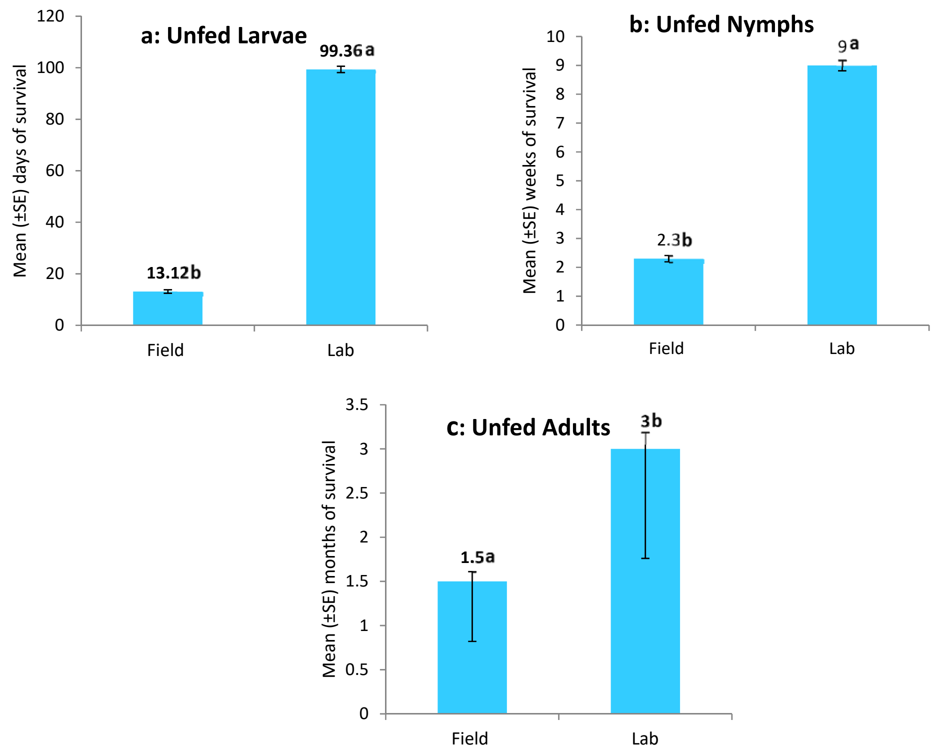


**Figure 5.** **a:** Means (±SE) number of days of survival of unfed larvae, **b:** Mean (±SE) number of weeks of survival of unfed nymphs and **c:** Mean (±SE) number of months of survival of adults of *H. anatolicum* under field and laboratory conditions in 2016 at Shambat, the same letters are not significantly different at 5% level according to REWGQ range test.

4.39 weeks, respectively. This may be attributed to the fact that the R.H. was relatively high particularly in August when the rainfall increased. Punyua stated that during the rainy season, there was an initial rabid uptake of water at night and water lost during the day was compensated at night [20]. In May and March unfed stages could not regain water to the limit that ensures their survival because the R.H. was below the CEH. Lees reported that during desiccation uptake of water can be exaggerated and rather more water is gained than has been lost [32]. This finding confirms that August and September may be the preferable period for attachment of immature stages.

It was found that under laboratory conditions, larvae survived for more than three months while nymphs survived for two months and adults survived for a similar period of that of larvae. In the field, in May, 16% of larvae survived for one week while no unfed nymphs survived for more than 4 days probably due to high temperature. In August, 70% of larvae and nymphs survived for three weeks and 2.5 weeks, respectively. This finding may indicate that larvae of *H. anatolicum* are more resistant to desiccation than nymphs under field and laboratory conditions. The shorter survival periods of nymphs compared with larvae were recorded; it was found that at 20 ± 2 °C, 30 ± 2 °C and 25–30 °C, the larvae lived for 90–148 days, 45–55 days and 74 to 90 while nymphs lived for 29–47 days, 31 days and 36–37 days, respectively [24]. Similar results were obtained by Ahmed (1987 cited in El Ghali *et al.* 2003b) who stated that larvae are slightly more resistant to desiccation than nymphs, and Londt and Whitehead (1972 cited in El Ghali *et al.* 2003b) working on water balance of larvae stated that both dead and live larvae responded similarly [29]. El Ghali and Hassan stated that it is possible that *H. dromedarii* larvae are more resistant to the desiccation than larvae of *H. anatolicum* [33]. This finding is possibly attributed to the fact that all stages of *H. anatolicum* infest cattle in cattle-sheds which may account for its shorter longevity [24]. It is worth mentioning that, *H. anatolicum* nymphs and females showed anatomical changes in the alloscutum. It became elongated, shrunken and truncated posteriorly (Figure 7). This observation may be correlated with desiccation a fact which opens new avenues for further studies.

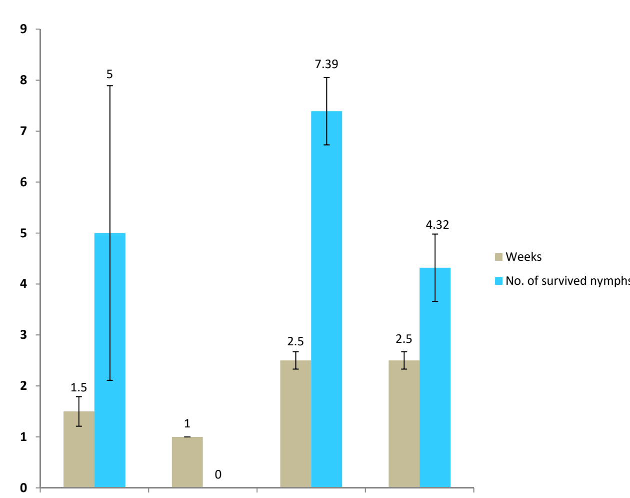


**Figure 6.** Means (±SE) number of weeks and unfed nymphs of *H. anatolicum* in March, May, August and December 2016 under field conditions in 2016 at Shambat.

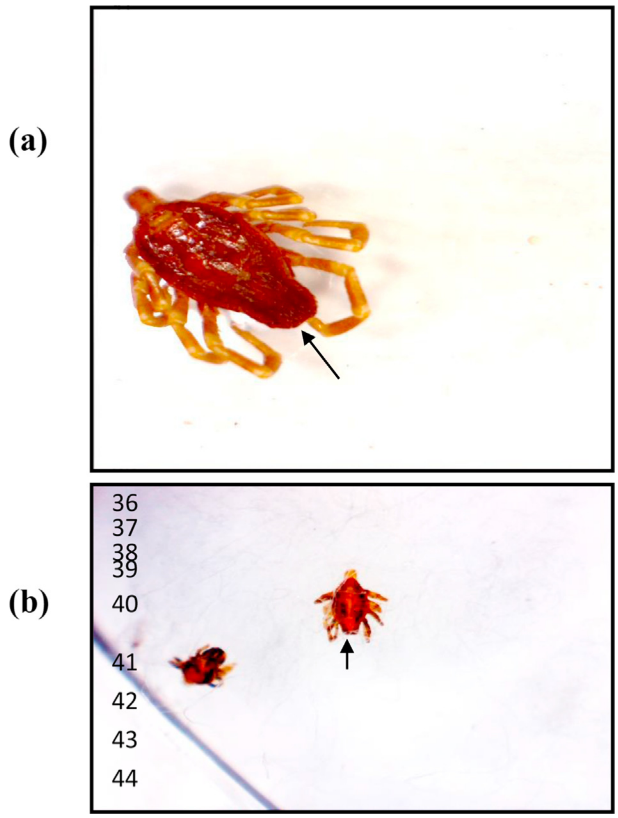


**Figure 7.** Abnormal shape of alloscutum (arrows) of *H. anatolicum* (a) unfed females moulted from engorged nymphs (b) unfed nymphs moulted from engorged larvae fed on calves at Shambat 2016.

## 5. Conclusions

The study concluded that when *H. anatolicum* fed on crossbred (Friesians x Zebu) kept under zero-grazing system, engorged larvae dropped off the hosts in high numbers at night and some nymphs and adults at early hours of the night. This phenomenon is exploited in Australia, in ecologically based strategies of tick control; ticks are allowed to drop in areas not suitable for their further development. It was found that larvae are more resistant to desiccation than nymphs under field and laboratory conditions. Survival periods of unfed ticks under field conditions can be exploited in animal rotational grazing for the tick control strategy. Epidemiological data on tick-borne diseases should be linked to the epidemiology and biology of ticks as a basis for a recommended tick control program. The abnormal changes observed in the alloscutum of unfed nymphs and females may open new avenues in understanding of the desiccation process. Tick stages may be able to survive for more than ten weeks. This study is first of its kind in Sudan, which provides some information about drop-off rhythms and survival periods of *H. anatolicum* stages when fed on calves.

## Declarations

### *Author contribution statement*

Rua K. I. Khogali: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Shawgi M. Hassan: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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### *Data availability statement*

No data was used for the research described in the article.

### *Declaration of interests statement*

The authors declare no conflict of interest.

### *Additional information*

No additional information is available for this paper.

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