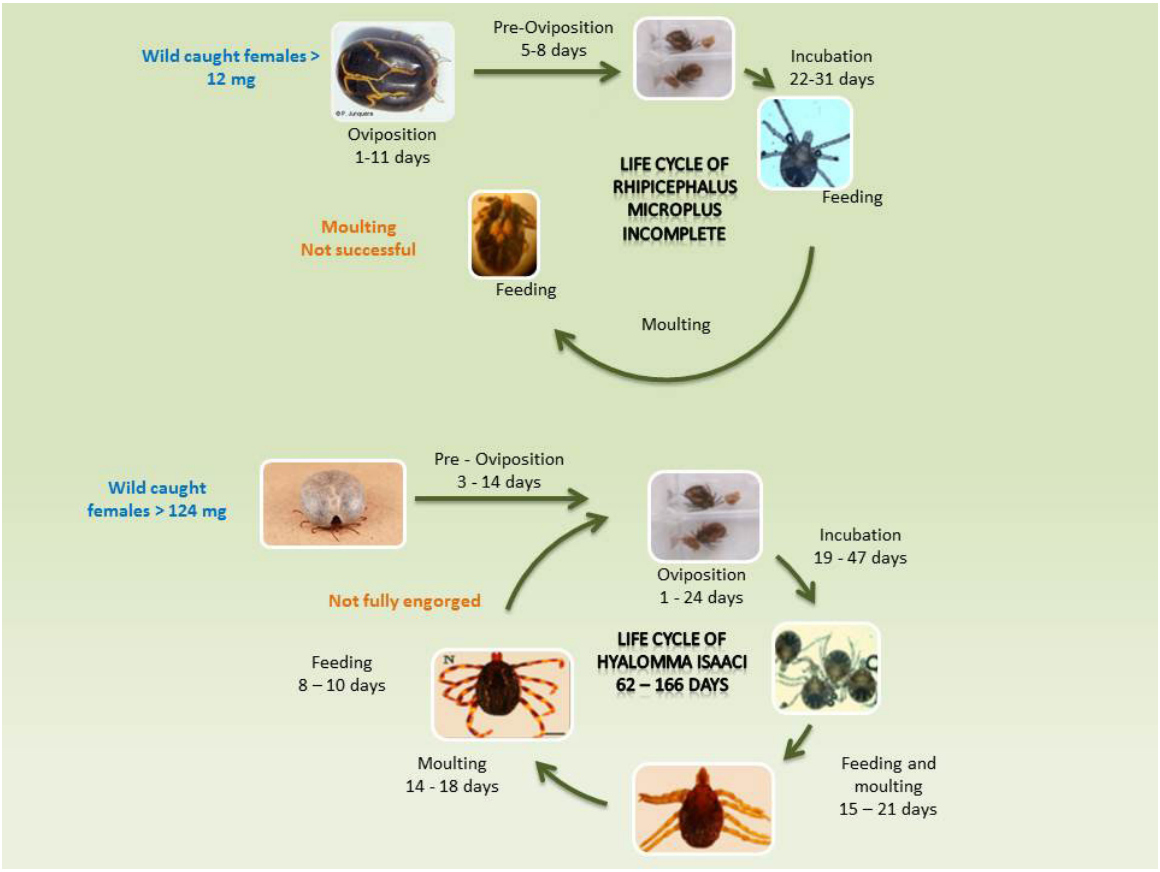
RESEARCH ARTICLE

Experimental aspects of life cycles of two hard tick species, *Rhipicephalus (Boophilus) microplus* and *Hyalomma isaaci* (Acari: Ixodidae), on New Zealand white rabbits

K.O. Bandaranayaka, U.I. Dissanayaka and R.S. Rajakaruna*



Highlights

- Two-host life cycle of *Hyalomma* isaaci was completed within 62 - 166 days.
- One-host life cycle of *Rhipicephalus microplus* was incomplete.
- Parasitic phase of *R. microplus* was confined to larval engorgement to moulting into nymphs.
- Nymphs or adults of *R. microplus* did not feed on New Zealand white rabbits.

Ceylon Journal of Science 50(1) 2021: 47-56
DOI: http://doi.org/10.4038/cjs.v50i1.7846

RESEARCH ARTICLE

Experimental aspects of life cycles of two hard tick species, *Rhipicephalus (Boophilus) microplus* and *Hyalomma isaaci* (Acari: Ixodidae), on New Zealand white rabbits

K.O. Bandaranayaka[1,2], U.I. Dissanayaka[1,2] and R.S. Rajakaruna[1*]

[1]Department of Zoology, University of Peradeniya, Peradeniya, 20400, Sri Lanka.
[2]Postgraduate Institute of Science, University of Peradeniya, Peradeniya, 20400, Sri Lanka.

Received: 01/08/2020; Accepted: 13/01/2021

Abstract: *Hyalomma isaaci* and *Rhipicephalus microplus* are two common hard tick species infesting buffalo and cattle. Biological data on the ticks' life cycle were collected by experimental infestation on New Zealand white rabbits (*Oryctolagus cuniculus*) under controlled conditions (70% - 80% Relative humidity and 27 ± 1 ℃ Temperature). Life cycle parameters including latency period, oviposition, feeding, moulting periods were determined and the Mean Reproductive Efficiency Index (REI) and Reproductive Aptitude Index (RAI) were calculated. Females of *H. isaaci* and *R. microplus* started oviposition after 3 - 14 days and 5 - 8 days, respectively. *Hyalomma isaaci* laid 15 - 6,166 (1600.8 ± 1940.9) eggs in 1 - 24 days while *R. microplus* laid 19 - 1,535 (434.2±344.8) eggs in 1 - 11 days. Emerged larvae of *H. isaaci* spent 19 - 47 days in incubation and moulted on-host. Nymphs dropped after feeding for 15 - 21 days, subsequently taking 14 - 18 days to moult into adults. Females fed for 8 - 10 days, and reached a maximum engorgement weight of 127.0 mg. The non-parasitic phase of *R. microplus* was 29 - 57 days, while the parasitic phase was confined only to larval engorgement and moulting into nymphs taking of 8 - 14 days. The REI/RAI was 7.1/3.6 and 8.3/5.1 for *H. isaaci* and *R. microplus,* respectively. *Hyalomma isaaci* completed its two-host life cycle within 62 - 166 days while *R. microplus* could not complete its life cycle because the nymphs did not feed on rabbits and therefore, none of them moulted into adults. The New Zealand white rabbit was not an appropriate laboratory host for the mature stage of *R. microplus* even though it was a suitable host for *H. isaaci*.

Keywords: *Hyalomma isaaci; Rhipicephalus microplus*; hard ticks; cattle; life cycle.

INTRODUCTION

Hyalomma (Euhyalomma) marginatum Koch 1844 was formerly considered to be a complex group consisting of four subspecies: *Hyalomma marginatum marginatum* Koch, (1844), *Hyalomma marginatum rufipes* Koch, (1844), *Hyalomma marginatum turanicum* Pomerantzev, (1946), and *Hyalomma marginatum isaaci* Sharif, (1928) (Horak *et al*., 2002). Apanaskevich and Horak in 2008 re-evaluated the group of *H. marginatum* and assigned the four subspecies in the species level giving the species names of *H. marginatum*, *H. rufipes*, *H. turanicum* and *H. isaaci*. *Hyalomma isaaci* has been reported from all over Asia, including India, Afghanistan, China, Myanmar, Nepal, Pakistan, and Vietnam from cattle, buffalo, goat, and birds (Sharif, 1928; Kaiser and Hoogstraal, 1963; Seneviratne, 1965; Kolonin, 1992; Robbins *et al*., 2002; Gosh *et al*., 2007; Prakasan and Ramani, 2007). In Sri Lanka, *H. isaaci* infests cattle and buffalo (Kaiser and Hoogstraal, 1963; Seneviratne, 1965; Liyanaarachchi *et al.,* 2015). Human infestations of *H. isaaci* have been reported from Sri Lanka, especially related to otoacariasis in Kandy, Ratnapura, and Anuradhapura districts (Dilrukshi, *et al*., 2004; Liyanaarachchi *et al.,* 2015; Ariyarathne *et al*., 2016). Available literature for the Indian population of *H. isaaci* suggests that it has either a three-host or a two-host life cycle, which it completes in a minimum of 77 days (Apanaskevich and Oliver, 2013) or 45 - 57 days (Ponnadurai *et al*., 2013) under laboratory conditions.

In 1934, Minning divided the Genus *Boophilus* into three subgenera, including numerous species using morphological characters. Later, several authors have revised the genus, limiting the number of species to three (Cooley, 1946; Anatos, 1950; Hoogstraal, 1979; Arthur, 1960): *B. annulatus* (Say, 1821), *B. decoloratus* (Koch, 1884), and *B. microplus* (Canestrini, 1888). Much later, in 2002, Barker and Murrel classified *Boophilus* as a subgenus of *Rhipicephalus.* Currently, the clade consists of five species, namely *R. microplus, R. annulatus, R. decoloratus, R. kohlsi* and *R. geigyi*. *Rhipicephalus microplus* infests cattle, buffalo, horses, donkeys, goats, sheep, deer, pigs, dogs, and wildlife in tropics and subtropics (Seneviratna, 1965; Prakasan and Ramani, 2007; Diyes and Rajakaruna, 2015; Liyanaarachchi *et al*., 2015). There are few reports on human infestation of *R. microplus* in Cuba (de la Cruz *et al*., 1991), Argentina (Guglielmone *et al*., 1991), China (Kuo-Fan, 1991), and Sri Lanka (Seneviratne, 1965, Dilrukshi *et al.*, 2004). The taxonomical status of boophilids in Sri Lanka is rather unclear (Seneviratna, 1965; Dilrukshi, 2006). According to Seneviratna, in 1965 Sri Lankan boophilids consist of two species: *R. microplus* and *R. annulatus,* of which *R. microplus* being the widely distributed species. *Rhipicephalus microplus* has a one-host life cycle where larvae, nymph, and adult feed on the same host (Wall and Shearer, 2001). Laboratory studies show that it takes 29 - 37 days (Joydhar *et al*., 2010) to two

**Corresponding Author's Email:* rupikar@pdn.ac.lk
https://orcid.org/0000-0001-7939-947X

months (Amaraal *et al*., 2012; Benitez *et al*., 2012; Wall and Shearer, 2001) to complete the parasitic phase of the life cycle on rabbits and cattle under controlled conditions.

The number of hosts and the biological parameters in the life cycle of a tick is important in describing the disease dynamics of the infectious agents they carry. Moreover, many live laboratory-raised ticks are required for studies of transmission dynamics, maintenance, infectivity, virulence, and pathogenicity of tick-borne pathogens. Therefore, protocols on maintaining laboratory tick colonies of many epidemiologically important hard tick vectors and descriptions on biological and reproductive parameters of tick life cycle together with general laboratory setup, suitable host animals, tick feeding protocols, and environmental requirements necessary for the maintenance of these colonies are essential. This study was conducted to describe the biological and reproductive parameters of the life cycle of the Sri Lankan population of *H. isaaci* and *R. microplus* under laboratory conditions following the protocols in Bandaranayake *et al.* (2016a and 2016b) with appropriate modifications where necessary.

MATERIALS AND METHODS

Gravid females of *H. isaaci* and *R. microplus* were collected from cattle and buffaloes from Polonnaruwa, Kandy, and Matale districts in Sri Lanka and brought live to the laboratory. Ticks were identified to the species level using the taxonomic description of Seneviratne (1965) and the identifications were confirmed by Dmitry Apanaskevich, the Assistant Curator of the U.S. National Tick Collection at the Department of Biology, Georgia Southern University, USA. Females were weighed using an electrical balance (to the nearest 0.1 mg) and individuals were placed in separate plastic chambers. The lid of the chamber was kept slightly open to allow air movement. Ticks were allowed to lay eggs, and the life cycles of the two tick species were studied under laboratory conditions at 70% - 80% of relative humidity and 27 ± 1 °C of temperature inside an incubator.

Initiation of the life cycle

Each gravid female was assigned a number and was categorised according to their weight, as in Table 1. Oviposition of these females was monitored daily until they died. The eggs laid by each female were collected daily around 0900 hours, counted, and separated into clutches of 100 eggs. The weight of the females was measured soon after they died. The eggs were incubated in a glass incubator until hatched. The following information was collected during the procedure: pre-oviposition time (the period from the detachment from the host body to initiation of oviposition), oviposition time (the period from the first egg elimination to the last egg elimination), the total number of eggs laid by each female, period from the elimination of the last egg to the death and variation of daily egg-laying in different female weight categories (as in Table 1) from the first egg elimination to the last egg elimination. The Reproductive Efficiency Index (REI; Number of eggs per mg of female weight) was calculated for each female tick separately.

Table 1: Weight categories of *Hyalomma isaaci* and *Rhipicephalus microplus* gravid females collected from the field

Weight category	Weight (mg)	
	H. isaaci	*R. microplus*
W1	<100.0	<25.0
W2	101.0 - 200.0	26.0 - 50.0
W3	201.0 - 300.0	51.0 - 75.0
W4	>301.00	76.0 - 100.0
W5	_	>101.0

Eggs

The eggs were examined daily under a dissecting microscope to check whether they have hatched or wilted. During this phase, incubation time (the period from oviposition to the first larval appearance) and variation of daily eclosion from the first egg elimination to the last egg elimination were recorded. Then percent eclosion (percentage of hatched eggs with respect to the total number of eggs laid by each female) and Reproductive Aptitude Index (RAI; the number of hatched eggs/ larvae per mg weight of female) were calculated for each female.

Feeding stages

The larvae that hatched out were fed on New Zealand white rabbits (*Oryctolagus cuniculus*). A feeding chamber was glued to the rabbit's body. For this, two circular patches of 32 mm diameter were shaved in the rear end of the rabbit on both sides of the vertebral column. Then, a bottomless plastic vial with a screw cap was glued onto the shaved patch of the rabbit using a mild adhesive. Then, the larvae that were starved for 24 h were introduced to this feeding chamber, and the body of the rabbit was covered with a cloth so that the rabbit was unable to remove the vial while grooming.

Parasitic phase of *Hyalomma isaaci*

For *H. isaaci*, 300 - 400 larvae from a pooled egg collection from 20 females were introduced to the feeding chambers, and the feeding duration was monitored daily. Once the engorged larvae moulted into nymphs, they got reattached to a different site of the host body and started feeding. Nymphs that detached after feeding were collected, weighed, and kept in an incubator for moulting.

Soon after the nymphs moulted, weight and sex were recorded. In this phase the time taken for feeding and moulting of larvae and nymphs were recorded. Then, the percentage of the nymphs moulted (calculated as the number of moulted nymphs with respect to all the dropped nymphs from the host after feeding), male: female ratio of the moulted adults, and the time and the size difference between males and females of moulted adults were calculated. Only the females were considered to record the feeding time for adults because males showed intermittent feeding, taking short blood meals from time to time, and detaching. Nine females were chosen randomly to monitor the feeding time. A single female was introduced to a feeding vial and allowed to feed. The feeding period of

each female was monitored until they dropped off the host.

Parasitic phase of *Rhipicephalus microplus*

To study the parasitic phase of *R. microplus*, 800 - 1,000 larvae from a pooled egg collection of 20 females were used in the feeding chamber. Since *R. microplus* was a one-host tick, larvae and nymphs tend to moult on the host itself. During the procedure, the feeding period and moulting of each stage were monitored.

Larvae from eight females from the two tick species were kept without a blood meal to monitor their survival duration. The day of the first and the last larva died was noted down.

Statistical analysis

Since REI did not show a normal distribution (Anderson-Darling normality test), the relationship between female weight and REI was assessed using Pearson's correlation. The lowest weight for oviposition was determined using regression analysis. Percent eclosion, RAI, and female weight did not show a normal distribution (Anderson-Darling normality test) and, therefore, the relationship of the total number of eggs, RAI, and percent eclosion with the female weight was analysed using Spearman's correlation and regression analyses. The data on moulting periods and weights of nymphs were not distributed normally (Anderson-Darling normality test); hence, the relationship among categories of male and female was assessed using a Mann-Whitney U test. All analyses were performed using MINITAB (version 17).

Ethical clearance

All the study protocols were read and approved by the Ethical Review Committee at the Postgraduate Institute of Science, University of Peradeniya, Sri Lanka.

RESULTS

A total of 89 females of *R. microplus* (n = 51) from cattle in Kandy and Matale Districts and *H. isaaci* (n = 38) from cattle and buffalo in Kandy and Polonnaruwa districts were collected from the wild.

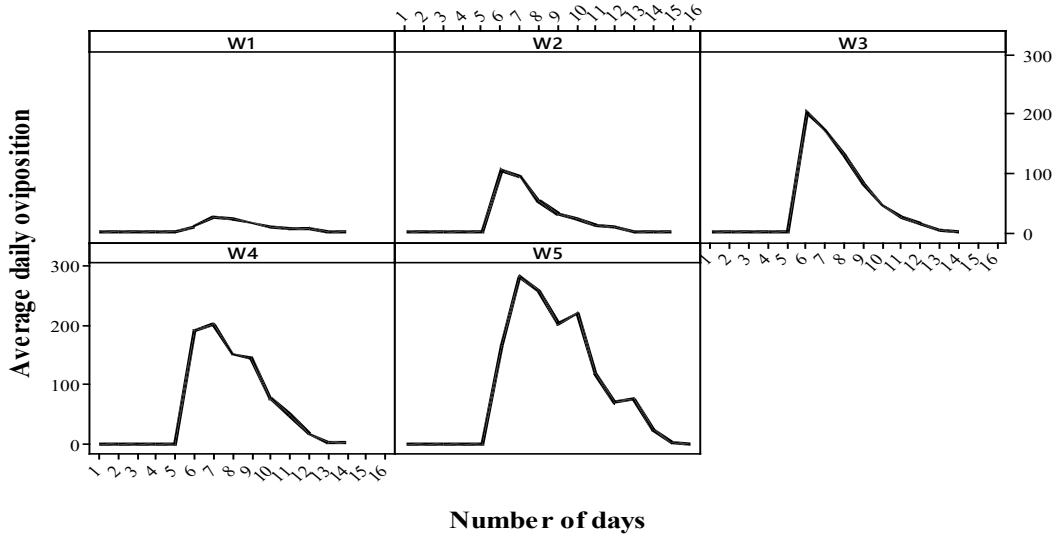


Figure 1: Oviposition patterns of *R. microplus* females under four weight categories (W1 to W5).

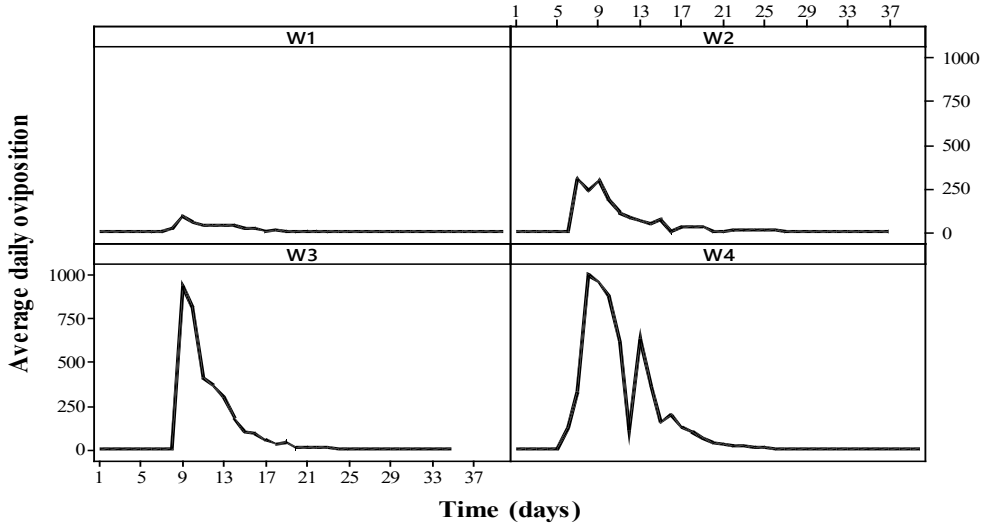


Figure 2: Oviposition patterns of *H. isaaci* females under four weight categories (W1, W2, W3 and W4).

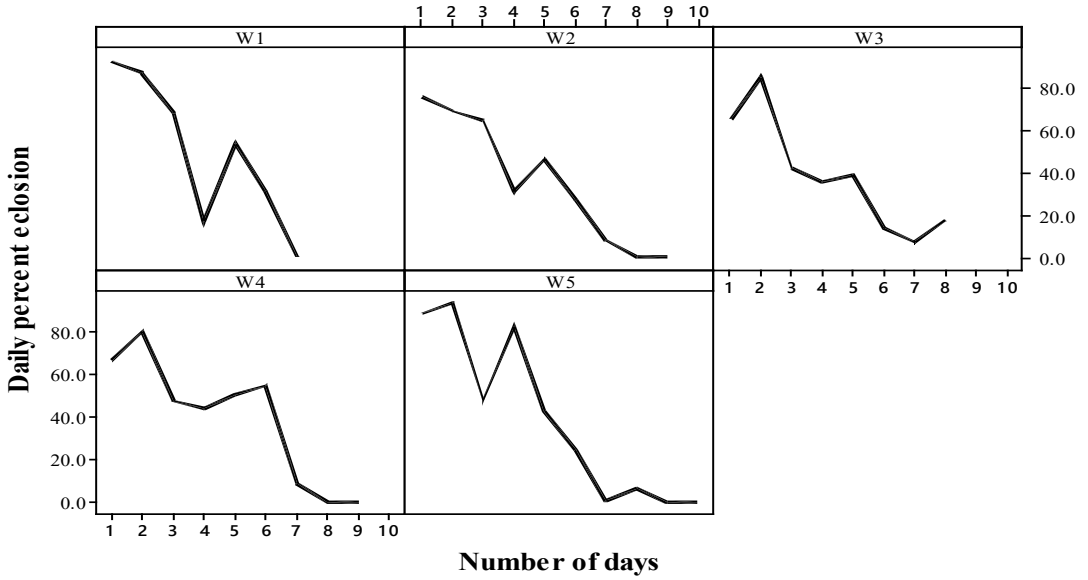


Figure 3: Daily eclosion patterns of *R. microplus* females under four weight categories (W1 to W5).

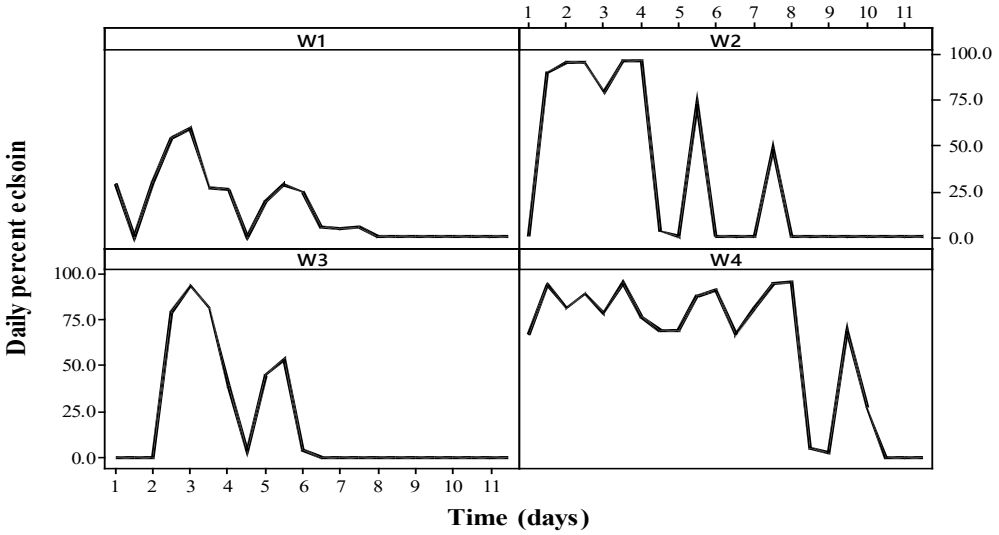


Figure 4: Daily eclosion patterns of *H. isaaci* females under four weight categories (W1, W2, W3 and W4).

Oviposition

The wild-caught females of *H. isaaci* had a latent period of 3-14 (mean ± SD, 7.2 ± 2.4) days prior to oviposition and those of *R. microplus* had 5 - 8 days (mean ± SD, 5.5 ± 0.8). Both species had a similar egg-laying pattern with a peak in the middle of the period and then tapering down (Figures 1 and 2). There was no distinct pattern in the percent eclosion in either of the species (Figures 3 and 4). However, the eggs laid during the early part of the oviposition showed higher hatching success.

Of the females of *R. microplus* collected from the wild, those who weighed less than 12 mg did not oviposit, and the oviposition period took 1 - 11 (mean ± SD, 8.0 ± 2.4) days (Table 2). Females laid 19 - 1,535 (mean ± SD, 434.2 ± 344.8) eggs and underwent a weight loss of 67.9% - 85.7% after oviposition (Table 3). Oviposition of females had shown a single distinct pattern in all weight categories where a short latent period of five days or more, followed by a peak around the 8th - 10th day and then gradually tapering down (Figure 1). On the contrary, the daily eclosion of five weight categories did not have a generalized pattern (Figure 2). However, the eggs laid during the early days of oviposition had shown a higher hatching success (Figure 2).

Of the *H. isaaci* females collected from wild, those weighed less than 22 mg did not oviposit, and oviposition periods took 1 - 24 days (mean ± SD,11.9 ± 5.5), respectively (Table 2). Two distinct patterns of oviposition were observed among different weight category females. Category W1, W2, and W3, which contained the partially engorged females, had a distinct pattern of a short latent period followed by a rapid peak around the 10th day and gradually tapered down (Figure 3). Whereas the Category W4, which contained fully engorged females, had a distinct pattern starting with a short latent period tailed by a peak of oviposition around the 9th day, then a sudden drop from day 13 followed by a second rapid peak around day 15 and finally tapered down (Figure 3). The daily eclosion of four weight categories of *H. isaaci* did not have a generalized

Table 2: Life cycle parameters of the four tick species under controlled laboratory conditions

Tick species	Life stage	Parameter of life cycle	Duration in days		
			Max	Min	Mean ± SD
R. microplus	Female	Pre-oviposition	8	5	5.5 ± 0.8
		Oviposition	11	1	8.0 ± 2.4
		Last oviposition to death	7	1	3.7 ± 2.0
	Egg	Incubation	31	22	24.8 ± 1.5
	Larva to nymph	Feeding to moulting	14	8	10.3 ± 3.0
H. isaaci	Female	Pre-oviposition	14	3	7.2 ± 2.4
		Oviposition	24	1	11.9 ± 5.5
		Last oviposition to death	32	2	13.2 ± 6.6
	Egg	Incubation	47	19	26.5 ± 2.8
	Larva to Nymph	Feeding and moulting period	21	15	17.9 ± 1.8
	Nymph	Moulting period	18	14	15.7 ± 1.1
	*Female	Feeding period	10	8	7.7 ± 2.5

* indicates the adults (females) which were raised in the laboratory

Table 3: Biological parameters of four tick species under controlled laboratory conditions in relation to their reproduction

Tick species	Biological parameter	Max	Min	Mean ± SD
Rhipicephalus microplus	Total number of eggs per female	1535.0	19.0	434.2 ± 344.8
	Engorgement weight of females (egg laying) mg	128.0	12.0	44.8 ± 26.5
	Percent eclosion	79.9	0.0	-
	REI (Reproductive Efficiency Index)	13.5	1.1	8.3 ± 3.4
	RAI (Reproductive Aptitude Index)	8.9	0.0	5.1 ± 2.1
	Percent weight reduction after oviposition	85.7	67.9	-
Hyalomma isaaci	Total number of eggs per female	6166.0	15.0	1600.8 ± 1940.9
	Engorgement weight of females	471.0	22.0	138.9 ± 123.8
	Percent eclosion	84.1	0.0	-
	REI	15.5	0.2	7.1 ± 4.1
	RAI	11.0	0.0	3.6 ± 3.9
	Percent weight reduction after oviposition	77.4	3.5	-
	Percent moulting in to adults	-	-	89.5
	*Engorgement weight of females mg	127.0	93.0	109.7
	*Feeding success of females	-	-	33.3

* indicates the adults (females) which were raised in the laboratory

pattern (Figure 4). Wild Females laid 15 - 6,166 (mean ± SD, 1600.8 ± 1940.9) eggs and experienced a weight loss of 3.5% - 77.4% after oviposition (Table 3).

Reproductive parameters

The incubation periods of eggs of *R. microplus* and *H. isaaci* were 22 - 31 (mean ± SD, 24.8 ± 1.5) days and 19 - 47 days (mean ± SD, 26.5 ± 2.8), respectively (Table 3). Percent eclosion was similar in both species (Table 3). There was a strong positive correlation between the number of eggs laid by a female with the weight of the female in both species (Pearson's correlation analysis, $p<0.001$; Figure 5). Larger females laid a higher number of eggs (Correlation analysis; Spearman correlation; *R. microplus*: $r = 0.980$, $p<0.001$ *H. isaaci*: $r = 0.774$, $p<0.001$). A positive correlations between the weight and percent eclosion was found in *H. isaaci* (rho = 0.891, $p<0.001$) but not in *R. microplus* (rho = 0.484, $p = 110$; Figure 6).

The mean RE and RA indices for *R. microplus* were 8.3 ± 3.4 and 5.1 ± 2.1, for *H. isaaci* they were 7.1 ± 4.1 and 3.6 ± 3.9, respectively (Table 3). Positive correlations were found between REI and female weight (*R. microplus*: rho = 0.753, $p<0.001$, *H. isaaci*: rho = 0.686, $p<0.001$; Figure 7). Moreover, RAI and female weight also had positive correlations in both species (*R. microplus*: rho = 0.601, $p<0.001$, *H. isaaci*: rho = 0.932, $p<0.001$; Figure 7).

The mean RE and RA indices for *R. microplus* were 8.3 ± 3.4 and 5.1 ± 2.1; for *H. isaaci* they were 7.1 ± 4.1 and 3.6 ± 3.9, respectively (Table 3). Positive correlations were

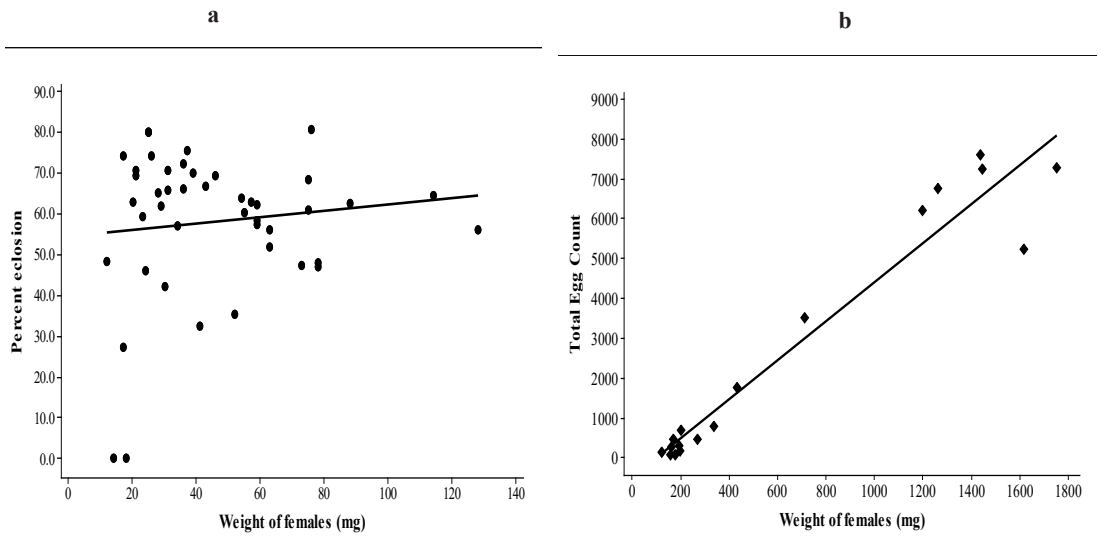


Figure 5: Total egg counts vs. weight of *R. microplus* females (regression fit; a:R^2 - 95.4%: Y= 0.079X + 10.12) and *H. isaaci* females (b:R^2 - 85.0%: Y= 0.066X + 48.78).

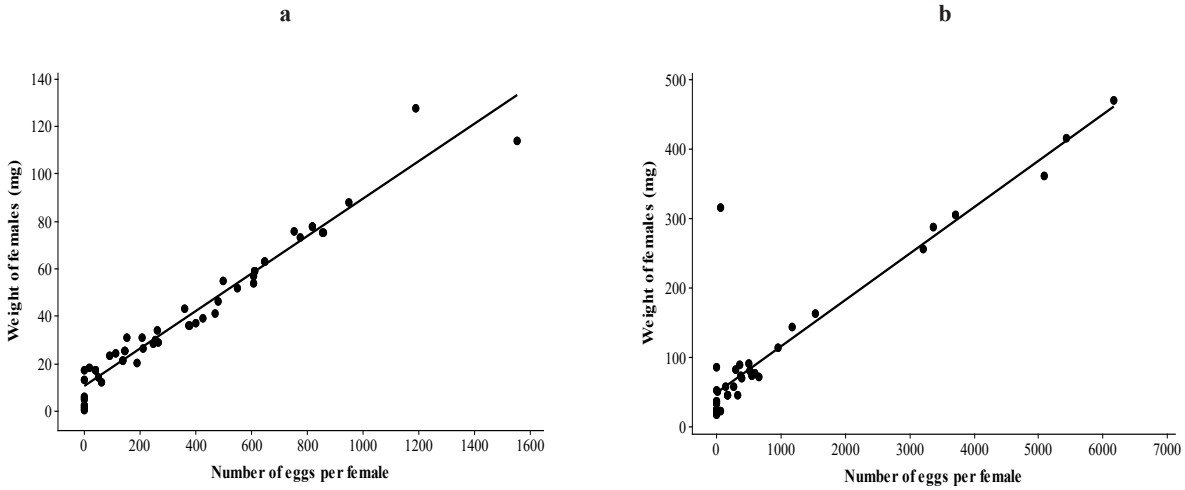


Figure 6: Percent eclosion vs. weight of females of *R. microplus* (a: Regression fit; R^2 - 1.4%), of *H. isaaci* (b: Regression; R^2 - 66.7%).

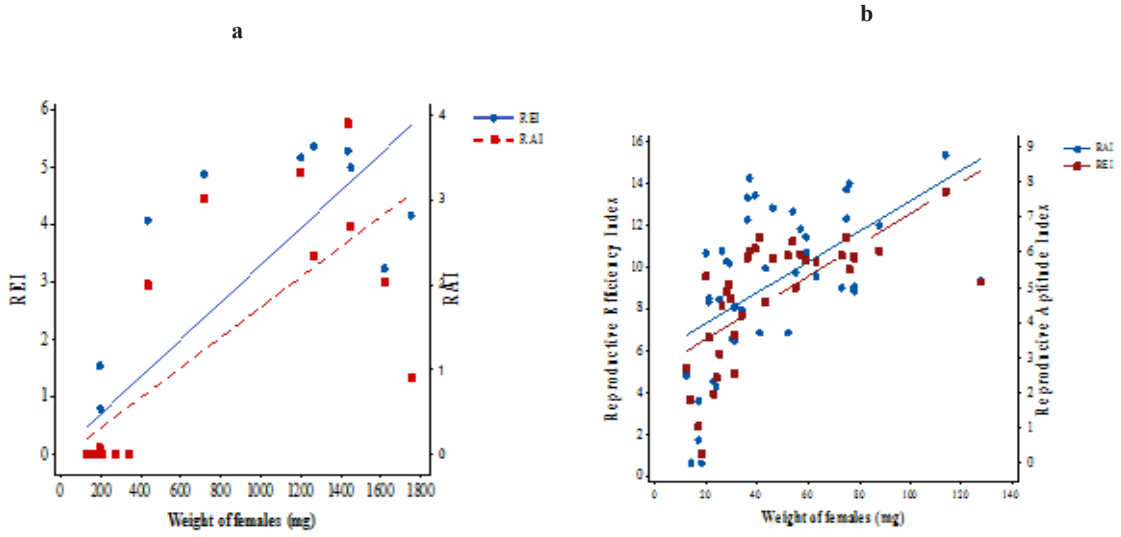


Figure 7: Reproductive Efficiency Index (REI) and Reproductive Affinity Index (RAI) vs. weight of females of *R. microplus* (a: Regression fit; R^2 - 46.4% and R^2 - 29.6% respectively), *H. isaaci* (b: Regression fit; R^2 - 82.4% and R^2 - 88.3% respectively).

found between REI and female weight (*R. microplus*: rho = 0.753, p<0.001, *H. isaaci*: rho = 0.686, p<0.001; Figure 7). Moreover, RAI and female weight also had positive correlations in both species (*R. microplus*: rho = 0.601, *p*<0.001, *H. isaaci*: rho = 0.932, p<0.001; Figure 7).

Parasitic phase of the life cycles

Larvae of the two species got attached and fed successfully on New Zealand white rabbits. They remained attached to the host and moulted. Newly emerged nymphs got reattached to the host and started feeding. Feeding of larvae and nymphs and the moulting of larvae for *R. microplus* took 8 - 14 (mean ± SD, 10.3 ± 3.0) days (Table 3). Even though nymphs of *R. microplus* fed on New Zealand white rabbits, none of them moulted into adults, and therefore *R. microplus* did not complete its life cycle on rabbits. However, all three stages (larvae, nymphs, and female) of *H. isaaci* successfully fed on rabbits where larvae moulted into nymphs on the host. This confirms a two-host life cycle. Nymphs dropped off to moult once they were fully engorged. There was no difference in the moulting period of nymphs that became females (15.5 ± 1.7 days) or males (15.8 ± 0.9 days; W = 289.5, *p* = 0.3492). Moreover, there was no difference in the engorgement weight of nymphs that moulted into males or females (W = 342.0, *p* = 0.2811). Adult females of *H. isaaci* fed for 8 - 10 (mean ± SD, 7.7 ± 2.5) days (Table 3). The male to the female sex ratio of laboratory-reared *H. isaaci* was 2:3 in the adult population. *Hyalomma isaaci* fed on the rabbit and took 62 - 166 days to complete the life cycle under controlled laboratory conditions (Table 3).

DISCUSSION

The study shows that *H. isaaci* successfully completed its two-host life cycle within 62 - 166 days, but the females hatched in the laboratory did not successfully feed on New Zealand white rabbits, whereas *R. microplus* single-host life cycle was incomplete. The parasitic phase of *R. microplus* life cycle was confined only to larval engorgement and moulting into nymphs. Nymphs or adults did not feed. Rabbits may not be a suitable host for the nymphal and adult stages of the cattle tick. Even the feeding of larvae of *R. microplus* was also not very successful because the recovery rate of nymphs was about one-tenth. A study done by Amaral *et al*. (2012) shows that the rabbits are not suitable to continue a life cycle but only suitable to raise stages of *R. microplus* because of the low recovery rate in each stage. However, higher recovery rates of *R. microplus* nymphs, as well as adults feeding rabbits, have been reported elsewhere (Nirjhar *et al.,* 2000: Joydhar *et al*., 2010).

There was a minimum weight of the wild-caught females of the two tick species that laid eggs. Females of less than a certain weight (12 mg for *R. microplus* and 22 mg for *H. isaaci*) didn't oviposit in both species. Studies have shown that there should be 10% of its mean engorgement size to oviposit (Balashov, 1972). For instance, *Haemaphysalis longicornis,* has a minimum weight of 28.4 mg for oviposition, representing 10.4% of its mean weight (Yano and Shiraishi, 1989). Since these wild-caught females were forcefully removed from the natural host, their mean engorgement weight may have been lower than if they were allowed to drop off naturally. Studies have shown that the mean engorgement weights of cattle tick can be as high as 251 ± 120 mg (Benitez *et al.,* 2012) and 82.3 ± 3.9 mg (Nirjhar *et al.,* 2000) in females which are allowed to feed on cattle and drop off naturally.

The oviposition in both tick species followed the general ixodid pattern. Here two distinguished oviposition patterns were observed where the partially engorged females had a sigmoidal type oviposition pattern, and fully engorged females had a pattern where there were two peaks during the mid-days of oviposition. Dipeolu *et al.* (1991) describe a similar pattern in *Amblyomma variegatum* in fully and partially engorged females. In contrast, Guglielmone *et al.* (1991) and Shoukry *et al*. (2000) describe a sigmoidal type oviposition pattern in fully engorged *R. microplus* and *Hyalomma schulzei*. Despite the extent of the engorgement of the female and the species, all females exhibited a period of latency.

Pre-oviposition and oviposition periods for *R. microplus* were 5.5 ± 0.8 days and 8.0 ± 2.4 days, respectively. Similar results have been reported in other studies of Joydhar *et al*. (2010), who reported 5.5 ± 0.1 days and 6.0 ± 0.1days and Amaral *et al.* (2012) recorded 3.85 ± 1.80 days and 5.55 ± 1.8 days while Benitez *et al.* (2012) recorded 3 ± 0 days and 6.7 ± 1.4 days of pre-oviposition and oviposition periods, respectively. Although Gallardo and Morales (1999) reported a similar pre-oviposition period of 4.74 days for *R. microplus* and their oviposition period is as long as 23.49 days. Some authors report an oviposition period of 12 - 14 days (Wall and Shearer, 2001). Pre-oviposition and oviposition periods can vary substantially depending on the geographic location of the same tick species. Pre-oviposition, oviposition, feeding of immature stages, and moulting periods of *H. isaaci* were similar to those reported in Apanaskevich and Oliver (2013). Longer periods could be due to the ticks' intrinsic factors, the unsuitability of the environmental conditions that were presented to them in the laboratory, or the disturbance during the collection and transportation of ticks from the habitat to the laboratory. The percent weight loss of the females of the two species was different where *H. Isaaci* showed a wider range of 3.5% to 77.4%, whereas that of *R. microplus* was 67.9% to 85.7%. *Haemaphysalis cuspidata* experienced an average weight loss of 67% (Geevarghese and Mishra, 2011).

The egg incubation period of *R. microplus* was 22 - 31 days. This is consistent with the findings of Amaral *et al.* (2012; 19 - 26 days). However, Joydhar *et al*., in 2010 have reported shorter incubation periods of 9 - 18 days. The incubation period of 19 - 47 days of *H. isaaci* in this study was comparable to that described by Apanaskevich and Oliver (2013; 24 - 33 days) but with a longer period for the Sri Lankan population. Divergent temperatures to the ticks' optimal incubation temperature during experimental studies could result in prolonged incubation periods (Satyanarayana *et al.,* 1981). The extent of developmental periods is greatly governed by temperatures (Branagan, 1973; Punyua, 1984; Short *et al.,* 1989), and the stamina of

free-living stages of ticks is greatly influenced by humidity (Berkvens, 1992). Consequently, the prolonged incubation periods could be a collective effect of both temperature and humidity.

The Reproductive Efficiency Index (REI) of females of both species was low: 1.1 - 13.5 in *R. microplus* and 0.2 - 15.5 in *H. isaaci*. A very high REI of 1.95 - 89.37 and 2.30 - 88.5 (Amaral *et al.,* 2012) and 8.53 ± 3.96, (Benitez *et al.,* 2012) have been reported in *R. microplus* while others have reported similar values such as 6.6 - 12.5 (Benetaez *et al*., 2012; Gaxiola-Camacho, 2009). The REI of *H. isaaci* was comparable to that of previous studies, for instance, *H. rufipes* has a REI of 10.63 (Chen *et al*., 2012), *H. asiaticum* has a REI of 8.82 (Chen *et al*., 2009), and *Hyalomma rufipes* has 10.53 (Chen *et al*., 2012). The Reproductive Affinity Index (RAI) was lower than REI for both species. If all the laid eggs did not hatch, the RAI shows a lower value than REI (Gaxiola-Camacho, 2009). The low REI lower limit of the two species could be because the wild-caught females may not be fully engorged as they were forcefully removed from the natural host. Percent eclosions for both species were higher where more than half of the eggs laid were hatched. Percent eclosion of *R. microplus* was 58.9%, which is close to the percentage reported in other studies (Nirjhar *et al.,* 2000; Joydhar *et al*., 2010; Benitez *et al.,* 2012). Percent eclosion for *H. isaaci* was 62.9%. Varying percentages have been reported for other *Hyalomma* species: *H. Dromedarii* of 82.0 - 94.1% and 56.2 - 58.9% (ELGhali and Hassan, 2010) and *H. impeltatum* of 84% (Logan *et al.,* 1989).

Larger females of *H. isaaci* had a higher reproductive output where positive correlations were observed in the total number of eggs per female, total eclosion per female, REI and RAI with the female weight. Larger females had a higher reproductive output according to much previous literature (Yano and Shiraishi, 1989; Gallardo and Morales, 1999; *Chen et al*., 2009; Gaxiola-Camacho, 2009; Siroky *et al*., 2011; Chen *et al*., 2012), which is common among many ixodids (Uspensky and Ioffe-Uspensky, 1999) and other animal taxa (Peters, 1983; Honek, 1993). However, some reproductive parameters like eclosion are highly associated with extrinsic factors like temperature and relative humidity (Berkvens, 1992; Barros-Battesti *et al*., 2000; Cardoso *et al.,* 2006; Adejinmi and Akinboade, 2011). Studies have shown that REI and RAI are higher during the dry season (Gaxiola-Camacho, 2009). The reproductive success of an engorged female is directly related to her capacity to feed, heavier females how higher reproductive output. The success or failure in egg viability has important biological ramifications (Gaxiola-Camacho, 2009). In the models that calculate reproductive success, females, not only egg-laying values but also hatchability and final larvae production are considered (Price, 1977).

Females of *H. isaaci* fed successfully on rabbits and took 8 - 10 days for feeding, but some studies have shown a longer period of 6 - 24 days (Apanaskevich and Oliver, 2013). The presence of males triggers better feeding of females, and the females tend to take short blood meals or sometimes do not feed at all in the absence of males on the host (Weiss and Kaufman, 2004). In the present study, only females were used for feeding and this could be a reason for showing a shorter feeding period.

CONCLUSION

The study shows that New Zealand white rabbit is a suitable laboratory host for immature stages of both tick species but not for the mature stages of *R. microplus*.

ACKNOWLEDGEMENT

Financial assistance is provided by the National Research Council, Sri Lanka (Grant No. 11-44).

DECLARATION OF CONFLICT OF INTEREST

The authors declare no competing interests.

REFFRENCES

Adejinmi, J.O. and Akinboade, O.A. (2011). Effect of temperature on the oviposition capacity of engorged adult females and hatchability of eggs of dog ticks: *Rhipicephalus sanguineus* and *Heamaphysalis leachi leachi* (Acari: Ixodidae). *African Journal of Biomedical Research* **14**: 35-42.

Amaral, M.A.Z., Prata, M.C.A., Daemon, E. and Furlong, J. (2012). Biological parameters of cattle ticks fed on rabbits. *Revista Brasileira de Parasitologia Veterinária* **21**: 22-27.

Anastos, G. (1950). The scutate ticks, or Ixodidae of Indonesia. *Entomologica Americana* **30**: 77-105.

Apanaskevich, D.A. and Horak, I.G. (2008). The genus *Hyalomma* Koch, 1844: V. re-evaluation of the taxonomic rank of taxa comprising the *H. (Euhyalomma) marginatum* Koch complex of species (Acari:Ixodidae) with re-description of all parasitic stages and notes on biology. *International Journal of Acarology* **34**: 13-42.

Apanaskevich, D.A. and Oliver, J.H. (2013). Life cycle and natural history of ticks, In: Sonenshine, D. E. and Roe, R. M. (Eds), *Biology of Ticks,* Oxford University Press, 198 Madison Avenue, New York, NY 10016. Pp. 62-63.

Ariyarathne, S., Apanaskevich, D.A., Amarasinghe, P.H. and Rajakaruna, R.S. (2016). Occurrence and distribution of tick species associated with human otoacariasis in selected districts of Sri Lanka. *Experiment and Applied Acaralogy* **70**: 99-123; DOI: 10.1007/s10493-016-0056-z. Epub 2016 Jul 6.

Arthur, D. R. (1960). *Ticks. A monograph of the Ixodidae. Part V. The genera Dermacentor*, *Anocentor, Cosmionmmn, Booplzilus, Mnrgnropus*. Cambridge University Press, UK. 251.

Balashov, Y.S. (1972). *Bloodsucking ticks (Ixodoidea).* Vectors of diseases of man and animals. *Miscellaneous Publications of the Entomological Society of America* **8**: 161-376.

Bandaranayaka, K.O., Apanaskevich, D.A., and Rajakaruna, R.S. (2016a). Life cycle of *Amblyomma integrum* (Acari: Ixodidae) under laboratory conditions. *Experimental and Applied Acarology* **69:** 335-345.

Bandaranayaka, K.O., Apanaskevich, D.A., and Rajakaruna, R.S. (2016b). Life cycle of *Nosomma monstrosum* (Acari: Ixodidae) under laboratory

conditions. *Experimental and Applied Acarology* **69**: 97-106.

Barker, S.C. and Murrell, A. (2008). Systematics and evolution of ticks with a list of valid genus and species names, In: Bowman, A. S. and Nuttall, P. A. (Eds), *Ticks: Biology, Disease, and Control,* Cambridge University Press, The Edinburgh Building, Cambridge, UK. Pp. 1-40.

Barros-Battesti, D.M., Onofrio, V.C., Simons, S. M., Bonoldi, V.L.N. and Yoshinari, N.H. (2000). Oviposition and eclosion periods of *Ixodes didelphidis* Fonseca and Aragão, 1951 (Acari: Ixodidae) under laboratory conditions. *Memórias do Instituto Oswaldo Cruz* **95**: 905-908.

Benitez, D., Cetrá, B. and Christensen, M. F. (2012). *Rhipicephalus (Boophilus) microplus* Ticks can complete their life cycle on the water buffalo (*Bubalus bubalis*). *Journal of Buffalo Science* **1**: 193-197.

Berkvens, D.L. (1992). Modelling of *Rhipicephalus annulatus* population dynamics, In: Perry, B.D. and Hansen, J. W. (Eds), *Modelling Vector-borne and Other Parasitic Diseases*, ILRAD, P.O. Box 41921, Nirobi, Kenya. Pp. 47-50.

Branagan, D. (1973). The development periods of the Ixodid tick *Rhipicephalus appendiculatus* Neumann under laboratory conditions. *Bulletin of Entomological Research* **63**: 155-168.

Canestrini, G. (1888). Intorno da alcuni Acari ed Opilonidi dell´America. *Atti Societ à Veneto-Trentina Scienze Naturali Padova Residente Padua* **11**: 100-109.

Cardoso, A.C.B., De Freitas, L.H.T., Daemon, E. and Faccini, J.L.H. (2006). The effect of humidity on the oviposition and larval mortality of *Haemaphysalis leporispalustris* (Packard, 1869) (Acari: Ixodidae) under laboratory conditions. *Brazilian Journal of Veterinary Parasitology* **15**: 58-64.

Chen, Z., Li, Y., Liu, Z., Yang, J. and Yin, H. (2012). The life cycle of *Hyalomma rufipes* (Acari: Ixodidae) under laboratory conditions. *Experimental Applied Acarology* **56**: 85-92.

Chen, Z., Yu, Z., Yang, X., Zheng, H. and Liu, J. (2009). The life cycle of *Hyalomma asiaticum* kozlovi Olenev, 1931 (Acari: Ixodidae) under laboratory conditions. *Veterinary Parasitology* **160**: 134-137.

Cooley, R.A. (1946). The genera *Boophilus*, *Rhipicephalus* and *Haemaphysalis* (Ixodidae) of New world. *National Institute of Health Bulletin* **187**: 1-54.

de la Cruz, J., Socarras, A.A. and Garcia, M.J. (1991). Acari causing zoonoses in Cuba. *Revista Cubana de Ciencias Veterinarias* **22**: 101-105.

Dilrukshi, P.R.M.P., Yasawardene, A.D.K.S.N., Amerasinghe, P.H. and Amerasinghe, F.P. (2004). Human otoacariasis: a retrospective study from an area of Sri Lanka. *Transaction of Royal Society of Tropical Medicine and Hygiene* **98**: 489-495.

Dipeolu, O.O., Amoo, A.O. and Akinboade, O.A. (1991). Studies on ticks on Veterinary importance in Nigeria: intrinsic factors influencing oviposition and egg-hatch of *Amblyomma variegatum* under natural conditions. *Folia Parasitologia* **38**: 63-74.

Diyes, G.C.P. and Rajakaruna, R.S. (2015). Diversity and distribution of tick species infesting goats with two new host records from Sri Lanka. *Journal of National Science Foundation Sri Lanka* **43**: 225-234: DOI: http://dx.doi.org/10.4038/ jnsfsr.v43i3.7951.

ELGhalia, A. and Hassan, S. M. (2010). Life cycle of the camel tick *Hyalomma dromedarii* (Acari: Ixodidae) under field conditions in Northern Sudan. *Veterinary Parasitology* **174**: 305-312.

Gallardo, J.S. and Morales, V.S.J. (1999). *Boophilus microplus* (Acari: Ixodidae): Preoviposicion, oviposicion, incubation de los huevos y geotropism. *Journal Bioagro* **11**: 77-87.

Gaxiola-Camacho, S., García-Vázquez, Z., Cruz-Vázquez, C., Portillo-Loera, J., Vázquez-Peláez, C., Quintero-Martínez, M. T. and Rosario-Cruz, R. (2009). Comparison of efficiency and reproductive aptitude indexes between a reference and field strains of the cattle tick *Rhipicephalus (Boophilus) microplus*, in Sinaloa, Mexico. *Revista Brasileira de Parasitologia Veterinária* **18**: 9-13.

Geevarghese, G. and Mishra, A.C. (2011). *Haemaphysalis ticks of India*. Elsevier publications. Jamestown road, London NW1 7BY **1**: 86-103.

Ghosh, S., Bansal, G.C., Gupta, S.C., Ray, D., Khan, M.Q., Irshad, H., Shahiduzzaman, M., Seitzer, U. and Ahmed, J. S. (2007). Status of tick distribution in Bangladesh, India and Pakistan. *Parasitology Research* **101**: S207-S216.

Guglielmone, A.A., Mangold, A.J. and Garcia, M. D. (1991). The life cycle of *Amblyomma parvum* Aragao, 1908 (Acari: Ixodidae) under laboratory conditions. *Experimental and Applied Acarology* **13**: 129-136.

Honek, A. (1993). Intraspecific variation in body size and fecundity in insects: a general relationship. *Oikos* **66**: 483-492.

Hoogstraal, H. (1979). The epidemiology of tick-borne Crimean-Congo haemorrhagic fever in Asia, Europe, and Africa. *Journal of Medical Entomology* **15**: 307-417.

Horak, I.G., Camicas, J.L. and Keirans, J.E. (2002). The Argasidae, Ixodidae and Nuttalliellidae (Acari: Ixodida): a world list of valid tick names. *Experimental and Applied Acarology* **28**: 27-54.

Joydhar, N.B., Haq, M.S., Rahman, M.H. and Sarkar, A.K. (2010). Laboratory studies on the life cycle of *Boophilus microplus. International Journal of Biological Research* **2**: 13-18.

Kaiser, M.N. and Hoogstraal, H. (1963). The Hyalommaticks (Ixodoidea, Ixodidae) of *Afghanistan. Journal of Parasitology* **49**: 130-139.

Koch, D. L. (1844). Systematische Uebersicht fiber die Ordnung der Zecken. Arch. f. Naturg., Berlin, Jahrg. X, Bd.I, 217-239.

Kolonin, G.V. (1992). Fauna of ticks (Ixodidae) of vertebrates of Vietnam. In: Sokolov, V.E. (Ed), *Zoological Research in Vietnam*, Nauka, Moskva Pp. 242-280.

Kuo-Fan, T. 1991. Acari: Ixodidae. *Economic Insect Fauna of China* **39**: 1-359.

Liyanaarachchi, D., Rajakaruna, R.S., Dikkumbura, A.W. and Rajapakse, R.P.V.J. (2015).Ticks infesting wild and

domestic animals and humans of Sri Lanka with new host records. *Acta Tropica* **142**: 64-70.

Logan, T.M., Linthicum, K.J., Bailey, C.L., Watts, D.M. and Moulton, J.R. (1989). Experimental transmission of Crimean-Congo hemorrhagic fever virus by *Hyalomma truncatum* Koch. *Americn Journal of Tropical Medicine and Hygiene* **40**: 207-212.

Minning, W. (1934). Beitrtige zur Systema- tik und Morphologie der Zeckengatiung *Boophilus* Curtice. Z. *Parasitenkunde* **7**: l-43.

Nirjhar, N.B.D., Talukder, M.H., Kibria, A.K.M.G. and Rahman, M.H. (2000). Laboratory studies on the life cycle of *Boophilus microplus* on rabbit. *Bangladesh Veterinarian* **17**: 16-20.

Peters, R.H. (1983). *The Ecological Implications of Body Size*. Cambridge University Press, Cambridge, Pp. 329.

Pomerantzev, B.I. (1946). Ticks (Ixodidae) of the USSR and neighbouring countries. Opred. Faune SSSR Zool. Inst. Akad. Nauk SSSR, Vol. 26, Izd. Akad. Nauk SSSR, Moscow, Leningrad, 28 pp. (In Russian).

Ponnudurai, G., Harikrishnan, T.J. and Rani, N. (2013). Observations on the bionomics of common ixodid ticks of ruminants in Tamil Nadu, India. *Journal of Veterinary Parasitology* **27**: 12-15

Prakasan, K. and Ramani, N. (2007). Tick parasites of domestic animals of Kerala, South India . *Asian Journal of Animal and Veterinary Advances* **2**: 74-80.

Price, P.W. (1977). General concepts on the evolutionary biology of parasites. *Evolution* **31**: 405-420.

Punyua, D.K. (1984). Developmental periods of *Rhipicephalus appendiculatus* Neumann (Acarina: Ixodidae) under field conditions. *Insect Science and its Applications* **5**: 247-250.

Robbins, R.G., Platt, S.G. and Keirans, J.E. (2002). First report of *Hyalomma marginatum isaaci* Sharif (Acari: Ixodida: Ixodidae) from the Union of Myanmar, with a concurrent collection of *H. hussaini* Sharif. *Proceedings of the Entomological Society of Washington* **104**: 1061-1063.

Satyanarayana, N.V.V., Rao, B.V. and Ramakrishna, P.R. (1981). Brief note on the biology of *Amblyomma integrum* Karsch, 1987. *Indian Veterinary Journal* **58**: 112-117.

Say, T. (1821). An Account of the Arachnids in the United States. *Journal of the Academy of Natural Sciences of Philadelphia* **2**: 59-83.

Seneviratne, P. (1965). The Ixodidea (Ticks) of Ceylon. Part 2 and 3. *Ceylon Veterinary Journal* **13**: 28-45.

Sharif, M. (1928). A revision of the Indian Ixodidae with special reference to the collection in the Indian Museum. *Records of Indian Museum* **30**: 217-344.

Short, N.J., Floyed, R.B., Noval, R.A. I. and Sutherst, R.W. (1989). Development rates, fecundity and survival of development stages of the tick *Rhipicephalus appendiculatus*, *Boophilus decolaratus* and *B. microplus* under field conditions in Zimbabwe. *Experimental and Applied Acaralogy* **6**: 123-141.

Shoukry, A., El-Kady, G.A. and Diab, F.M. (2000). Bionomics of ticks collected from Sinai Peninsula, Egypt: Biology of *Hyalomma schulzei* (Olenev) (Acari: Ixodidae) under laboratory conditions. *Egyptian Journal of Biology* **2**: 49-56.

Siroky, P., Erhart, J., Petrzelcova, K. J. and Kamler, M. (2011). Life cycle of tortoise tick *Hyalomma aegyptium* under laboratory conditions. *Experimental Applied Acarology* **54**: 277-284.

Uspensky, I. and Ioffe-Uspensky, I. (1999). The Relationship between engorged female weight and egg number in Ixodid ticks: A Biological interpretation of Linear Regression parameters. *Acaralogia* **4**: 9-16.

Wall, R.L. and Shearer, D. (2001). Veterinary Ectoparasites: Biology, Pathology and Control. Iowa state University press, A Blackwell Science Company, 2121 S state.

Weiss, B.L. and Kaufman, W.R. (2004). Two feeding-induced proteins from the male gonad trigger engorgement of the female tick, *Amblyomma hebraeum*. *Proceedings of the National Academy of Sciences USA* **101**: 5874-5879.

Yano, Y. and Shiraishi, S. (1989). Feeding pattern, mating and oviposition in female *Haemaphysalis longicornis* Neumann (Atari: Ixodidae). *Journal of the Faculty of Agriculture, Kyushu University* **33**: 287-29.