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Laboratory studies of the life cycle and behaviour of Amblyomma variegatum **(Fabricius, 1794)** (Acarina: Ixodidae)

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Abstract

Amblyomma variegatum is a 3-host tick parasitising livestock in Nigeria. It is of great economic importance because it is a known vector of disease-causing microorganisms, such as *Dermatophilus congolensis, Theileria mutans and Cowdria ruminantium*. It has a long life cycle which in the laboratory at 25 °C and the relative humidity of 85 % lasts 160—200 days. It was observed that the longest developmental period occurred at the egg stage (eggs are laid on the ground). This makes this stage the most vulnerable, considering that they are the ones more exposed to climatic and environmental hazards.

Ključne riječi: Amblyomma variegatum (Fabricius, 1794) *(Acarina: Ixodidae)*, životni ciklus, ponašanje.

Key words: Amblyomma variegatum (Fabricius, 1794) *(Acarina: Ixodidae)*, life cycle, behaviour.

Introduction

Amblyomma variegatum is an ectoparasitic ixodid tick of livestock. It is the commonest and most abundant tick in Nigeria (*Strickland*, 1961; *Mohammed*, 1974; *Dipeolu*, 1975), Adults and immature stages are found in cattle. This tick is of great economic importance as it is a known vector of various disease-carrying microorganisms. *A. variegatum* is a known vector of *Dermatophilus congolensis* (*Macadam*, 1964), *Theileria mutans* and *Cowdria ruminantium* (*Balashov*, 1972; *Mohammed*, 1974). *Mohammed* (1974) estimated that at least 75 % of cattle in Nigeria are infected with this parasite. *Dipeolu* and *Ogunji* (1977) observed that the state of engorgement of the females was important in determining whether they would lay and deposit eggs or not. They found out that a minimum weight of 1 gm must be attained by the females if they are to lay viable eggs. They also observed that under quasi-natural conditions, the life cycle of *A. variegatum* took up 152 days. *Norval* (1974) also observed that *A. hebraeum* required 169—238 days to complete its life cycle at 26 °C and the relative humidity of 90 %. Under similar conditions, however, *Boophilus decoloratus* required only 67—86 days (*Londt*, 1973).

The aim of this work was to provide information on the life cycle of *A. variegatum* under controlled laboratory conditions of 25 °C and 85 % relative humidity. Observations were made on its habits and the effect of the photoperiod on oviposition.

Material and methods

Fifty (50) *A. variegatum* females at various stages of engorgement were detached from cattle at the Veterinary Cattle Control Post, Bodija, Ibadan. The engorged ticks collected were weighed (using Mettler Analytical Balance H 15) in the laboratory and only those having attained the weight of 1 gm or more were used for the experiment. Each tick was weighed, placed in a universal bottle which was then plugged with cotton wool. The bottle was labelled to indicate the weight of the tick and the date of collection. The bottles were incubated at the normal laboratory temperature of 25 °C and the relative humidity of 85 %. Daily observations were made and data recorded on the duration of preoviposition, oviposition, mortality and preeclosion periods. The course of oviposition was monitored by weighing the eggs removed at 5-day intervals. Engorged females were collected at weekly intervals for a period of 6 months. The larvae and nymphs were fed on the ears of rabbits. The rabbits used were about 6 months old and had not previously been exposed to ticks. During feeding, the larvae were confined to the host in cloth bags attached to the skin of the ear with an adhesive plaster. Engorged instars were removed from the cloth bags and placed in the universal bottles and incubated in dessicators at 25 °C and 85 % relative humidity. The method of counting eggs was that used by *Dipeolu* and *Ogunji* (1977).

In another experiment, 60 engorged female ticks were divided into 3 sets. They were all weighed and each set (one tick per universal bottle), were placed in three separate dessicators and maintained at 25 °C and 85 % relative humidity. Each set was subjected to three different photoperiodic conditions as shown below: 1. LL—continuous light for 24 hours (using an electric bulb); 2. DD—continuous darkness for 24 hours (put in a dark cupboard); 3. LD—12 hours light, 12 hours darkness. Daily observations were made and the preoviposition period and pattern of oviposition recorded. The course of oviposition was monitored by weighing the eggs removed at 5-day intervals.

Results

Preoviposition period. The preoviposition period based on fifty (fully engorged and partially engorged) females which weighed over 1 gm lasted between 9—14 days (mean 11.5 ± 3.68). The largest number of females (35 %) laid eggs on the thirteenth day, while 50 % of females laid eggs after twelve days (Table 1).

Table 1. Preoviposition periods of replete females at 25 °C and relative humidity of 85 %

Preoviposition period (days)	9	10	11	12	13	14
Number of replete females concerned	1	6	8	18	22	1

Mean preoviposition period = 11.5 ± 3.66 (Mean ± S.D.)

Table 2. Laying period for *A. variegatum* females at 25 °C and relative humidity of 85 %

Laying period (days)	25	26	27	28	29	30	31	32	33	34	35
Number laid	2					38					10
Percentage laid	4					76					20

Mean oviposition period = 30 ± 7.35 (Mean ± S.D.)

Oviposition period. The laying period for the fifty replete females ranged from 25—35 days (mean 30 ± 7.35) as shown in Table 2. The largest number of replete females (76 %) laid eggs for thirty days. In forty females the peak of oviposition was on the 10th day, while the peak for the remaining ones was on the 15th day. Most of the eggs (about 75 per cent) were laid within the first fifteen days of oviposition. The egg production curve is characterised by a period of several days when the production is maintained at a high level after which it decreases and the egg laying usually ceases after the 25th to the 35th day (Fig. 1). The total number of eggs laid by individual females ranged from 7,216 — 23,142 (mean 16,822 ± 82.1) eggs. It was observed that injured females died either before oviposition or before completing oviposition.

Snow and *Arthur* (1966) showed that the number of eggs laid by female ticks is a function of the weight of imbibed nutrients rather than the quantity needed for metabolic purposes other than egg production. To test the validity of this assertion in respect to *A. variegatum,* the total weight of eggs produced by each of twenty females was plotted against the body weight. The result (Fig. 2) showed a linear relationship between the weight of female ticks and the weights of the eggs produced. Fig. 2 also shows a positive correlation (r = 0.980;

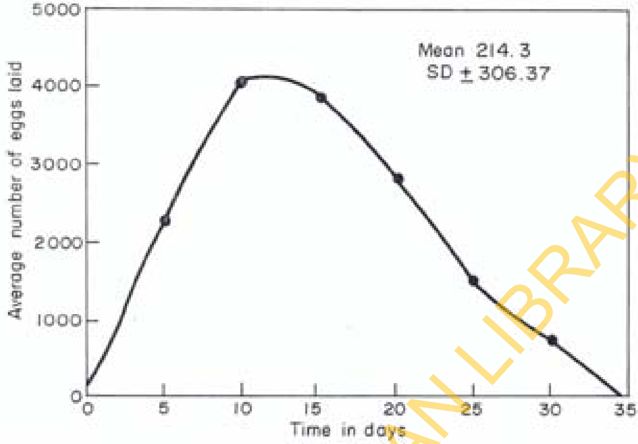

Fig. 1. Oviposition curves of 10 female *A. variegatum* at 25 °C and 85 % relative hummidity.

$P < 0.01$) between the weight of eggs and the weight of engorged females. This confirms earlier findings by *Dipeolu* and *Ogunji* (1977) that the greater the weight of the female, the more eggs it will produce.

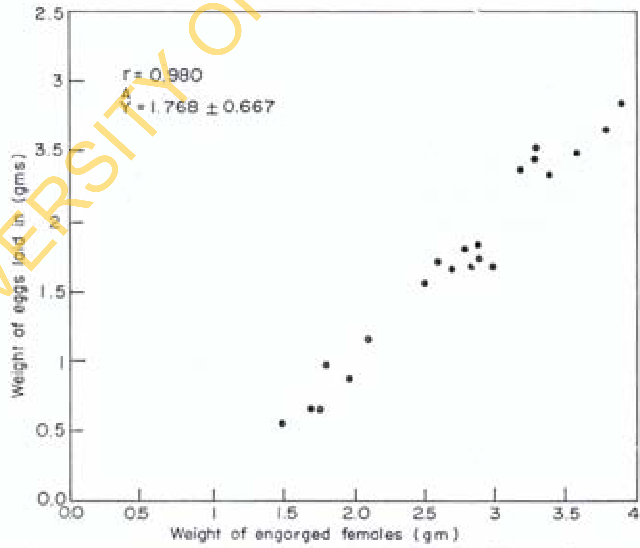

Fig. 2. Relationship between weight of eggs and weight of engorged females *A. variegatum*

Effect of the photoperiod on oviposition. Fig. 3. shows that the photoperiod has no effect on either the preoviposition period or the duration of oviposition. The oviposition curve for the replete females under this experimental condition was very similar to the normal laboratory experiment just recorded. The peaks of oviposition for the three experiments (LL, DD, LD) were also recorded on the 10th and the 15th day of oviposition. All the ticks were dead by the 35th day.

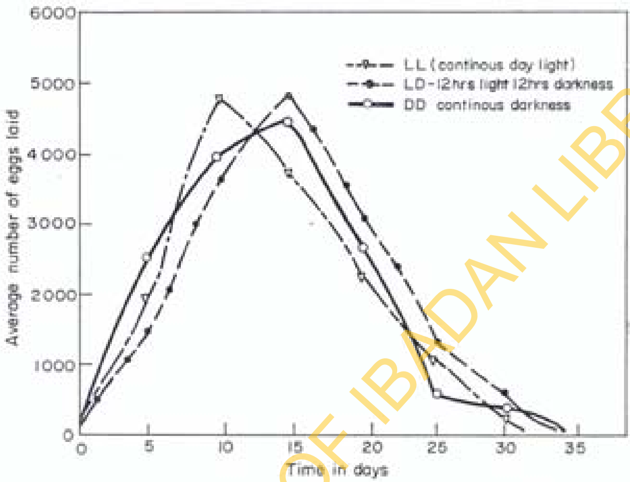

Fig. 3. Oviposition curves of *A. variegatum* females at 25 °C and 85 % relative hummidity under different photoperiodic conditions

Incubation (preeclosion) period. The incubation period based on the eggs of fifty (50) replete females ranged from 58—67 days (mean 60 ± 5.66) as shown in Table 3; 56 % of the eggs hatched on the 60th day. The percentage hatchability was very high with almost all the eggs ecloding into larvae.

Table 3. Incubation periods of the eggs of *A. variegatum* at 25 °C and relative humidity of 85 %

Incubation period (days)	58	59	60	61	62	63	64	65	66	67	68	69
Females concerned	6	8	28		1	1		1		3		

Mean incubation period = 60 ± 5.66 (Mean ± S.D.)

Feeding period for larvae. The feeding period for 200 larvae lasted 7—14 days (mean 8.2 ± 0.02). About 60 percent of the larvae were found to be fully engorged after 7 days of feeding. The engorged larvae were active for over 14 days but stopped being active on the 17th day and settled at the bottom of the universal bottles to moult. The moulting period ranged from 16—20 days (mean 18 ± 0.025).

Feeding period for nymphs. The feeding period for the nymphs ranged from 7—13 days (mean 8 ± 0.022). By the 7th day about 90 % of the nymphs had fully engorged. The moulting period for the nymphs ranged from 20—28 days (mean 26 ± 0.07).

Feeding period for newly emerged adults. The feeding period for the newly emerged adults ranged from 8—12 days (mean 10 ± 0.32).

The duration of the life cycle of *A. variegatum* at 25 °C and 85 % relative humidity was 160—200 days.

Miscellaneous observations. The observations made of the engorged females showed that they were sluggish and had difficulty in moving around. This agrees with the observations made by *Balashov* (1972) that when replete ticks drop off their hosts, their enlarged body prevents extensive movements from an environment that is sometimes harsh and which might limit longevity.

Before the commencement of oviposition, the females were observed to be dark brown but at the end of oviposition, they were yellow with brown spots. The eggs were brown and translucent and usually deposited in batches of compact sticky masses against the upright wall of the universal bottles. Some females (placed on Petri dishes) moved slowly backwards leaving the egg mass strung out like a thin coil.

Once hatched, the larvae were observed to be light grey (ash), tending to congregate just outside their egg shells. They remained in this state for about 4 days before they began to climb the sides of the universal bottles. After seven days of hatching the larvae began to tan and harden and climb to the mouth of the universal bottles in an attempt to escape. It was observed that the unfed larvae could remain active for six months in the laboratory at 25 °C and the relative humidity of 85 %. It was also observed that after storing the larvae in the laboratory under this condition for more than four months and then feeding them on rabbits, they failed to engorge and about 90 % of them were dead when the ear bags were removed after seven days for observation.

Discussion

A. variegatum has a long life cycle which at 25 °C and 85 % relative humidity required 160—200 days to complete. *Dipeolu* and *Ogunji* (1977) observed that under quasi-natural conditions, the life cycle of *A. variegatum* required 152 days to complete. *Norval* (1974) found out that *Amblyomma hebraeum* (maintained in the laboratory at 26 °C and relative humidity 90 %) required 169—238 days to complete its life cycle. Under similar conditions, *Boophilus decoloratus* required 67—86 days (*Londt*, 1973), while *Hyalomma marginatum rufipes* 111—146 days (*Norval*, unpublished). *Arthur* and *Londt* (1973) attributed shorter life cycles in 1- and 2-host ticks to the higher temperatures experienced during the premoult stages when they remain on the host in comparison with those temperatures experienced during the premoult stages in 3-host ticks which develop on the ground. They went on to explain that the blood meal is more rapidly digested and metabolised in 1- than in 3-host ticks. This, they concluded, results in a more rapid transition to the pharate state which thereby reduces the length of the life cycle to the extent that the entire parasitic cycle in *B. decoloratus* requires only 21—23 days. It was observed in this experiment that the longest developmental period occurred at the egg stage. This makes this stage the most vulnerable, considering that they are more exposed to climatic and environmental hazards which can lead to destruction and thereby reducing the population of the eggs produced and the survival of the species. It was also observed that the number of eggs produced by *A. variegatum* was low when compared to their large body size. *Hoogstraal* (1976) said that the use of the high amounts of ingested meal for metabolic purposes other than egg production in ticks is a primitive feature. The relationship between the weight of engorged females and the weight of the eggs produced shows that not all the blood is converted to eggs. To support this view, *Bennett* (1974) found out that *B. microplus* utilizes 20 mg in metabolism exclusive of egg production, *R. e. evertsi* 45 mg (*Rechav* et al., 1977), *Hyalomma m. rufipes* 90 mg, while *A. hebraeum* utilizes 200 mg (*Norval*, 1974). In this experiment, one replete tick weighed 3.2 gms on collection and was seen to deposit a total weight of 2.37 gms of eggs. Its weight at death was 0.722 gms. Therefore, this tick must have spent 108 mg (0.108 gms) on metabolic purposes other than egg production. *A. variegatum*, therefore, shows primitive features when compared with *B. microplus* and *R. e. evertsi*.

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Laboratorijska istraživanja životnog ciklusa i ponašanja krpelja *Amblyomma variegatum* (Fabricius, 1794) (*Acarina: Ixodidae*)

Sažetak

Amblyomma variegatum je trodomaćinski (3-host) krpelj koji parazitira na nigerijskoj stoci. Od velikog je ekonomskog značenja jer je poznati prenosilac patogenih mikroorganizama kao što su *Dermatophilus congolensis*, *Theileria mutans* i *Cowdria ruminantium*. Ima dugi životni ciklus koji je u laboratoriju na temperaturi 25 °C i pri relativnoj vlažnosti zraka od 85 % iznosio 160 — 200 dana. Zamijećeno je da je najduže razvojno razdoblje u stadiju jajeta (jajašca legu na zemlji), što čini ovaj stadij najranjivijim s obzirom da su jajašca najizloženija klimatskim opasnostima i opasnostima iz okoliša.