

Effect of Photoperiodic Regime and Egg Laying Disturbance on the Oviposition of the Dog Ticks: *Rhipicephalus sanguineus* and *Haemaphysalis leachi leachi* in Nigeria

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ABSTRACT

Oviposition patterns of engorged adult females of dog ticks: *Rhipicephalus sanguineus* and *Haemaphysalis leachi leachi* were studied at various photoperiods under constant temperature and relative humidity in the laboratory. The ticks were subjected to six different photoperiods throughout the period of oviposition and eggs were removed six hours. There were no significant differences in the mean pre-oviposition period, duration of oviposition and the number of eggs laid by *R. sanguineus* and *H. leachi leachi* in all the photoperiodic conditions. The pre-oviposition periods of *R. sanguineus* and *H. leachi leachi* sampled every six hours (disturbed group) were significantly lower ($p < 0.05$) than those sampled every 24 hours (undisturbed group). No significant differences existed in the duration of oviposition of the disturbed (8.0 ± 1.78 and 8.0 ± 1.11) and undisturbed (8.6 ± 1.79 and 8.9 ± 0.23) groups. Also no significant difference occurred in the number of eggs laid per mg body weight of the disturbed and undisturbed groups for *R. sanguineus* and *H. leachi leachi* respectively. Oviposition started during the scotophase in all the experimental ticks and egg collection started at 24 hours. The oviposition patterns under continuous darkness for 24 hours and continuous light for 24 hours were characterised by fluctuating peaks of egg-laying which did not follow any obvious pattern or rhythm while in other photoperiodic conditions there were peaks of oviposition with more obvious frequency patterns for both species. No significant differences were observed in the mean total number of eggs laid at the photophase and scotophase in less than 12 hours of light and 12 hours of darkness and vice versa. However, more eggs were laid at the scotophase than at the photophase by *R. sanguineus* and *H. leachi leachi* respectively. Engorged adult females of *R. sanguineus* and *H. leachi leachi* laid more eggs during scotophase than during photophase respectively, suggesting that both species exhibited ovipositional rhythm.

Keywords: Dog tick, *Rhipicephalus sanguineus*, *Haemaphysalis leachi leachi*, photoperiod, oviposition, scotophase, photophase.

INTRODUCTION

The existence of a daily rhythm of oviposition in insects and mites has been established and light has been proved to be an important factor (1). Iwuala and Okpala (2) and Rechav (3) concluded that ticks exhibit behavioural rhythms which were controlled by environmental temperatures. The conclusion of these authors was based on the established facts of circadian rhythm in the dropping off of ticks from their hosts by Darling (4), Hadani and Rechav (5), Amin (6).

Some workers (Wright, (7, 8); Fujisaki *et al.*, (9); Belozero (10); Ouhelli *et al.*, (11) Amoo, (12); Dpeolu *et al.*, (13); Adeyeye and Philips, (14); Doube, (15) Lohmeyer *et al.* (16) have studied the effect of photoperiod on the oviposition pattern of engorged ticks with varying results. Wright (7, 8) reported a relationship between light and oviposition pattern of *Dermacentor nitens* and *Amblyomma maculatum*. Fujisaki *et al.* (9) observed that the frequency of oviposition in *Haemaphysalis longicornis* and *Ixodes persiculatus* was affect-

ed by alternating light and dark conditions, decreasing during photophase and increasing during scotophase. Ouhelli *et al.* (11) reported that light had no effect on oviposition of *Boophilus annulatus*. Amoo (12) also observed ovipositional rhythm of decrease and increased egg production during the photo- and scotophases, respectively with *B. decoloratus* and *B. geigy*. Dipeolu *et al.* (13) had also studied the oviposition pattern of engorged *Amblyomma variegatum*, *Boophilus decoloratus*, *Boophilus geigy* and *Hyalomma truncatum* under artificial and natural photoperiods. These authors reported that *A. variegatum* and *Boophilus* species kept in photoperiods other than continuous light for 24 hours exhibited ovipositional rhythm characterised by higher peaks during the scotophase while *H. truncatum* failed to oviposit. Adeyeye and Philips (14) in their study of photoperiodic response of the soft tick *Ornithodoros turicata* observed that ticks reared in continuous darkness developed more slowly than those reared under short-day or long-day conditions. These authors also noted that the pre-oviposition period was significantly longer for ticks reared in long-day conditions. Doube (15) observed that both engorged and unengorged females of Kangaroo tick *Ornithodoros gurneyi* were sensitive to short photoperiods (LD 8: 16).

There is a dearth of information on the effect of light and darkness on the oviposition pattern of dog ticks: *R. sanguineus* and *H. leachi leachi* in Nigeria. The aim of this study therefore was to examine the effect of photoperiod regime and egg laying disturbance on the oviposition pattern of these key ticks of dog to determine if indeed an ovipositional rhythm existed or not and the possibility of employing it for the control of these ticks.

MATERIALS AND METHODS

The ticks used for this investigation were engorged female ticks collected individually by careful detachment with pairs of forceps from household dogs brought to Veterinary Clinics in Ibadan. The detached ticks were collected into universal bottles plugged tightly with cotton wool and conveyed in Kilner jars to the parasitological laboratory section of the Department of Veterinary Microbiology and Parasitology, University of Ibadan, Nigeria. They were identified into species: *Rhipicephalus sanguineus* and *Haemaphysalis leachi leachi* using morphological characters described by Soulsby (17) and Wall and Shearer (18).

Eighteen engorged female ticks each of *R. sanguineus* and *H. leachi leachi*, distributed into six sets of three ticks were used for this study. Each tick was weighed using a sensitive Sartorius balance (Type 2472) and put in individual labelled Bijou bottles plugged tightly with cotton wool. The bottles were then incubated in a desiccator maintained at 25°C and 85% relative humidity (R.H.). Each desiccator containing three ticks each of *R. sanguineus* and *H. leachi leachi* were subjected to the following photoperiodic conditions until the beginning and end of oviposition.

| | | |
|--|---|--|
| LL ₂₄ | — | continuous light for 24 hours |
| DD ₂₄ | — | continuous darkness for 24 hours |
| L ₁₂ D ₁₂ | — | 12 hours of light and 12 hours of darkness |
| D ₁₂ L ₁₂ | — | 12 hours of darkness and 12 hours of light |
| L ₁₈ D ₆ | — | 18 hours of light and 6 hours of darkness |
| D ₁₈ L ₆ | — | 18 hours of darkness and 6 hours of light |
| The D ₁₂ L ₁₂ and D ₁₈ L ₆ | | were reversals of L ₁₂ D ₁₂ and L ₁₈ D ₆ respectively. |

The pre-oviposition periods and duration of oviposition were noted for all the sets of ticks of both species, and eggs were removed from the ticks every 6 hours (0600, 1200, 1800 and 2400 hours) throughout the oviposition period as described by Amoo (12) and Dipeolu *et al.* (13). A fluorescent tube (60W; Phillip & Co., UK), with total available light intensity (lux) of 7.44W and 640nm wavelength was used as source of light (photophase). Darkness (scotophase) was provided by a dark room without any form of light. The collection of eggs at scotophase was carried out under a red dim light by means of an electric torch covered with a red filter. All the eggs collected were counted under the dissecting microscope and this was facilitated by the addition of drops of xylene to dissolve the wax holding them together (Dipeolu and Ogunji, (19)

To study the effect of disturbance (frequent collection of eggs) on oviposition by the ticks, another batch of 18 ticks each of *R. sanguineus* and *H. leachi leachi* were subjected to the same treatment as above but egg collection was carried out every 24 hours at 1800hr each day. The pre-oviposition, duration of oviposition and the total number of eggs laid by this second group were also observed and recorded. The experiment was carried out for a period of 10 days which was the maximum duration of oviposition by the ticks (20).

Statistical Analyses

All the data obtained were subjected to statistical analyses using the computer package SPSS (Statistical package for the Social Sciences, Chicago, SPSS, Inc.) version 1.1 2002. Chi-square test and the Student's *t* test were used to compare the means of values of the parameters obtained for two species of ticks at 95% confidence interval ($p < 0.05$).

The effect of light upon tick oviposition was determined on the basis of percent oviposition per hour. Percent oviposition is the percentage of the number of eggs laid by an individual tick for six hours to the total number of eggs laid since eggs were collected at six-hourly intervals. Oviposition per hour is expressed as the percentage of eggs laid for an hour calculated from the percent oviposition.

RESULTS

The mean pre-oviposition and oviposition periods and numbers of eggs laid per mg body weight of disturbed and undisturbed groups of *R. sanguineus* and *H. leachi leachi* under the six photoperiodic conditions are shown in Table 1.

There were no significant differences ($p > 0.05$) in the pre-oviposition periods of both *R. sanguineus* and *H. leachi leachi* in all the photoperiodic conditions used in this study. The mean duration of oviposition of *R. sanguineus* ranged from 4.33 ± 0.58 days to 9.33 ± 0.58 days (mean = 6.83 ± 0.58). For *H. leachi leachi* it was from 7.33 ± 0.59 days to 10.33 ± 0.58 days (mean = 8.83 ± 0.58 days). The mean number of eggs

per mg body weight oviposited by *R. sanguineus* ranged from 11.35 ± 0.24 to 17.2 ± 0.06 (mean: 14.28 ± 0.15), while that of *H. leachi leachi* ranged from 8.84 ± 0.05 to 16.65 ± 0.07 (mean: 12.75 ± 0.06).

No significant difference occurred in the mean pre-oviposition periods of ticks reared in all the photoperiods. Also the mean duration of oviposition and the mean number of eggs laid by each tick species were not significantly different in all the photoperiods.

The pre-oviposition periods of ticks sampled at every 6 hours (the disturbed groups) for both *R. sanguineus* and *H. leachi leachi* were significantly lower ($P < 0.05$) than those sampled every 24 hours (undisturbed groups) (Table 1). No significant differences ($P < 0.05$) existed between the duration of oviposition of the disturbed (8.00 ± 1.78 vs. 8.22 ± 1.11 days) and the undisturbed (8.61 ± 1.79 vs. 8.89 ± 0.23 days) groups for *R. sanguineus* and *H. leachi leachi* respectively. Also, no significant difference ($p > 0.05$) occurred between the number of eggs laid per mg body weight of disturbed ticks (14.91 ± 2.07 vs. 12.61 ± 2.74) and the undisturbed ticks (14.68 ± 2.36 vs. 12.36 ± 2.67) for *R. sanguineus* and *H. leachi leachi*, respectively.

Figs. 1 to 4 show the oviposition patterns (the relationship between the numbers of eggs laid by experimental ticks at 6-hourly intervals and the different photoperiods) for both *R. sanguineus* and *H. leachi leachi* under different photoperiodic conditions. Oviposition started during the scotophase in all the experimental ticks except in those placed under con-

Table 1: Pre-oviposition, duration of oviposition and number of eggs laid per mg body weight of disturbed and undisturbed groups of *Rhipicephalus sanguineus* and *Haemaphysalis leachi leachi*

| <i>R. sanguineus</i> | Disturbed | | | Undisturbed | | | |
|---------------------------------|---------------------|-----------------------------|-------------------------------------|--------------------------------------|-----------------------------|-------------------------------------|--------------------------------------|
| | Photoperiod (hours) | Mean Pre-oviposition (days) | Mean Duration of oviposition (days) | Mean No. of eggs per mg. Body weight | Mean Pre-oviposition (days) | Mean Duration of oviposition (days) | Mean No. of eggs per mg. Body weight |
| DD | 4.0±0.0 | 9.0±0.0 | 14.70±0.549 | 4.0 ± 0 | 9.67 ± 0.584 | 14.68 ± 0.523 | |
| LL | 4.32±0.58 | 9.33±0.58 | 15.34±0.050 | 5.33 ± 0.58 | 9.33 ± 0.58 | 15.43 ± 0.040 | |
| L ₁₂ D ₁₂ | 4.0±0.0 | 9.0±0.0 | 17.12±0.075 | 5.0 ± 0 | 9.67 ± 0.58 | 17.10 ± 0.110 | |
| D ₁₂ L ₁₂ | 4.0±0.0 | 8.38±0.58 | 17.15±0.062 | 5.0 ± 0 | 8.67 ± 0.58 | 17.16 ± 0.072 | |
| L ₁₈ D ₆ | 4.0±0.0 | 8.0±0.0 | 13.82±0.040 | 4.0 ± 0 | 9.33 ± 0.58 | 12.94 ± 0.091 | |
| D ₁₈ L ₆ | 4.0±0.0 | 4.33±0.58 | 11.35±0.242 | 5.33 ± 0.58 | 5.0 ± 0.10 | 10.74 ± 0.478. | |
| <i>H. leachi leachi</i> | | | | | | | |
| DD | 4.0±0.0 | 7.33±0.58 | 9.75±0.643 | 4.67 ± 0.58 | 8.33 ± 0.58 | 9.33 ± 0.031 | |
| LL | 4.67±0.58 | 10.33 ± 0.58 | 8.84±0.050 | 5.0 ± 0 | 10.67 ± 0.58 | 9.03 ± 0.051 | |
| L ₁₂ D ₁₂ | 4.33±0.58 | 8.0 ± 0 | 13.77±0.029 | 4.67 ± 0.58 | 8.33 ± 0.58 | 12.52 ± 0.140 | |
| D ₁₂ L ₁₂ | 4.0±0.0 | 8.0 ± 0 | 12.43±0.520 | 4.33 ± 0.58 | 8.67 ± 0.58 | 12.53 ± 0.172 | |
| L ₁₈ D ₆ | 4.0±0.0 | 8.33 ± 0.58 | 16.55±0.070 | 4.33 ± 0.58 | 9.0 ± 0 | 16.31 ± 0.41 | |
| D ₁₈ L ₆ | 4.0±0.0 | 7.33 ± 0.58 | 14.29±0.060 | 4.0 ± 0 | 8.33 ± 0.58 | 14.44 ± 0.104 | |

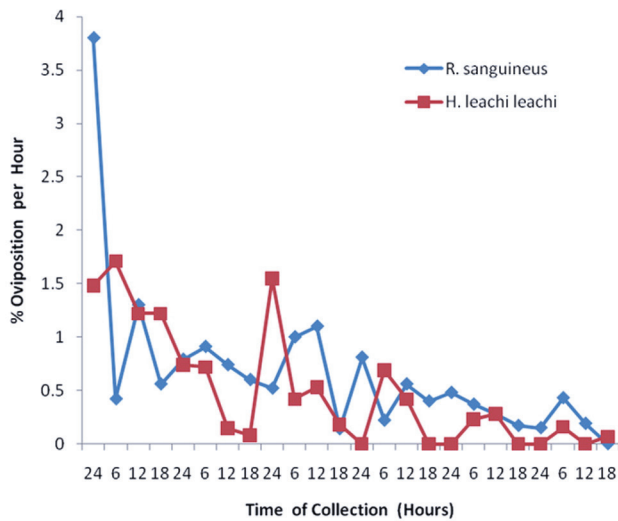


Figure 1: Oviposition pattern of ticks under continuous darkness photoperiod for 24 hours (DD_{24})

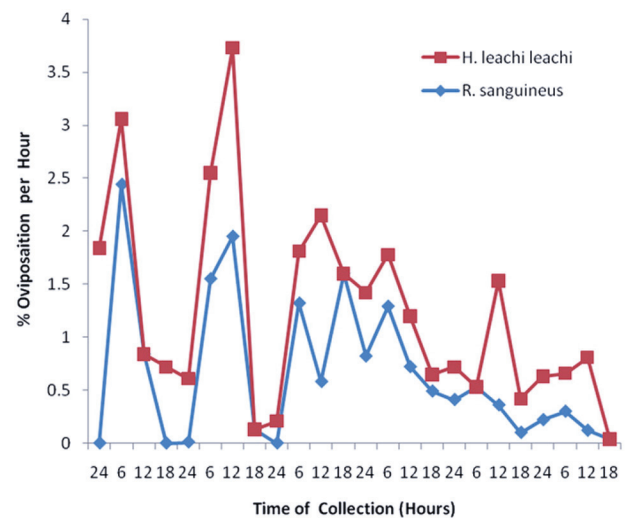


Figure 2: Oviposition pattern of ticks under continuous light photoperiod for 24 hours (LL_{24})

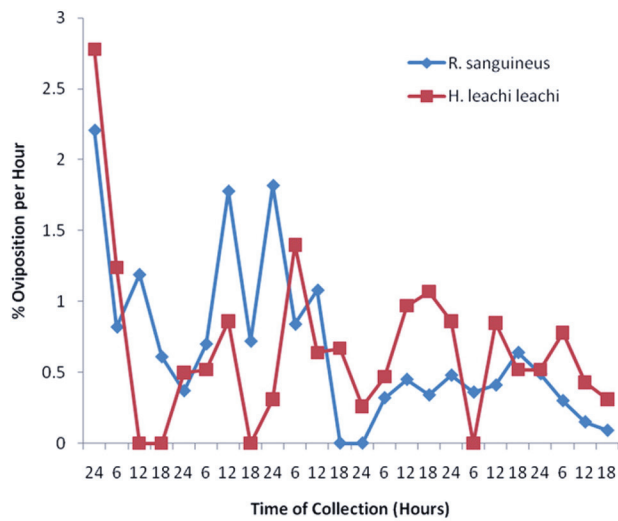


Figure 3: Oviposition pattern of ticks under 12 hours of light and 12 hours of darkness photoperiod ($L_{12}D_{12}$)

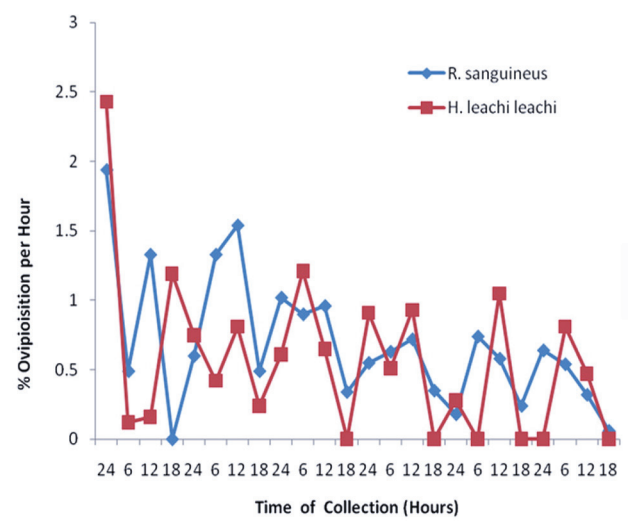


Figure 4: Oviposition pattern of ticks under 18 hours of light and 6 hours of darkness photoperiod ($L_{18}D_6$)

tinuous light for 24 hours. The first six hour egg collection occurred at 24 hours. For the convenience of presentation of results, data of five days within each oviposition phase is presented. The oviposition patterns under continuous darkness for 24 hours (DD_{24}) and continuous light for 24 hours (LL_{24}) for both species were characterized by fluctuating peaks of egg-laying which did not follow any obvious frequency pattern or rhythm (Figs. 1 and 2). In 12 hours of light and 12 hours of darkness ($L_{12}D_{12}$) and 18 hours of light and 6 hours of darkness ($L_{18}D_6$) photoperiodic conditions *R. sanguineus* and *H. leachi leachi* exhibited a rhythm in their oviposition

with more obvious frequency patterns (Figs. 3 and 4). This rhythm was characterised by higher peaks of oviposition in the dark (scotophase) than in the light (photophase) meaning that more eggs were produced by both *R. sanguineus* and *H. leachi leachi* during the dark than the light periods. The ovipositional rhythms of $L_{12}D_{12}$ and $L_{18}D_6$ (Figs. 3 and 4) were reversed at $D_{12}L_{12}$ and $D_{18}L_6$ photoperiods respectively.

Table 2 shows the mean total number of eggs produced by *R. sanguineus* and *H. leachi leachi* in photo- and scotophases every 6 hours. No significant differences ($p > 0.05$) were observed in the mean total number of eggs laid at the photo-

Table 2: Mean total number of eggs laid by *R. sanguineus* and *H. leachi leachi* in photo- and scotophases every 6 hours

| Light phase | Photoperiodic condition | <i>R. sanguineus</i> | <i>H. leachi leachi</i> |
|-------------|---------------------------------|----------------------|-------------------------|
| Photophase | LL ₂₄ | 1385.67±3.06 | 967.33±3.51 |
| | DD ₂₄ | - | - |
| | L ₁₂ D ₁₂ | 864.00±0 | 473.67±1.15 |
| | D ₁₂ L ₁₂ | 628.67±4.04 | 356.33±5.03 |
| | L ₁₈ D ₆ | 121.67±2.52 | 558.67±1.15 |
| | D ₁₈ L ₆ | 52.00±4.00 | 219.67±2.31 |
| Scotophase | LL ₂₄ | - | - |
| | DD ₂₄ | 1804.67±3.21 | 722.67±1.53 |
| | L ₁₂ D ₁₂ | 667.33±2.52 | 590.00±11.00 |
| | D ₁₂ L ₁₂ | 920.00±2.00 | 511.67±4.16 |
| | L ₁₈ D ₆ | 547.33±4.51 | 266.67±4.18 |
| | D ₁₈ L ₆ | 506.00±5.00 | 639.33±2.52 |

phase and scotophase under 12 hours of light and 12 hours of darkness (L₁₂D₁₂) and its reversal 12 hours of darkness and 12 hours of light (D₁₂L₁₂). However, more eggs were laid at the scotophase (1587 and 1103 eggs) than at the photophase, (1492 and 830 eggs) respectively by *R. sanguineus* and *H. leachi leachi*. There was a significant increase ($p < 0.05$) in the mean number of eggs laid at both the photo- (691.50 eggs) and scotophases (742.56 eggs) by *R. sanguineus* compared to *H. leachi leachi* at these phases (429.28 and 455.39 eggs). The results of this study also show that engorged adult females of *R. sanguineus* and *H. leachi leachi* laid more eggs during scotophase (742.56 and 455.39 eggs) than during photophase (691.50 and 429.28 eggs) respectively, suggesting that both species exhibited ovipositional rhythm.

DISCUSSION

The general pattern of oviposition under constant laboratory conditions common to many tick species have been reported for *Boophilus* and *A. variegatum* (2, 13, 19), *B. microplus* (20), *Hyalomma anatolicum anatolicum* (21), *R. sanguineus* (22) and *Anocentor nitens* (8). The oviposition curves of *R. sanguineus* and *H. leachi leachi* in the various photoperiodic conditions observed in this study followed essentially the same pattern reported by these previous workers with peaks of oviposition attained within 1-3 days after onset of egg laying and a progressive decline until the death of the ticks.

The results of this study show that continuous light (LL₂₄) and darkness (DD₂₄) for 24 hours compared with other photoperiodic conditions did not have a profound effect on pre-oviposition period for both *R. sanguineus* and *H. leachi leachi*. This agrees with the observations of Wright (9)

and Fujisaki *et al* (10) who reported that the length of pre-oviposition periods was not influenced by photoperiod in *Amblyomma maculatum*, *Haemaphysalis longicornis* and *Ixodes persiculatus*, but at variance with the findings of Wright (8) who reported that both pre-oviposition and oviposition periods were protracted with an extended photophase.

In this study no adverse effect was observed on disturbed ticks in terms of pre-oviposition and oviposition periods when compared with undisturbed ticks. This observation agrees with the findings of Drummond and Wheatstone (23), Fujisaki *et al.* (9) but disagrees with the report of Sonnenshine and Tinger (24).

Although it has been established that light is an important factor in the ovipositional rhythm of insects (1) and ovipositional rhythm has also been reported for other arthropods like mite (25), ticks (10). In this study no ovipositional rhythm was observed in ticks kept in continuous light (LL₂₄) and darkness (DD₂₄) for 24 hours meaning that they were affected by the diurnal rhythm of egg-laying as a response to the length of the photoperiod under laboratory conditions. Also, the increased egg output during the scotophase is an indication that light has an inhibitory effect on oviposition. Furthermore, the non-significant difference observed in the pre-oviposition periods, duration of oviposition and the number of eggs laid by each tick species in all the photoperiods implies that photoperiod is not the major determining factor in the production of eggs by these ticks.

The results of this study also showed that engorged adult females of *R. sanguineus* and *H. leachi leachi* laid more eggs during the scotophase than the photophase. It suggests that an ovipositional rhythm of increased and decreased egg production during the scoto- and photophases did occur. This observation has implication for the control of these ticks, which are vectors of canine babesiosis and ehrlichiosis in Nigeria, and which constitute about 99% of dog ticks (20, 26) Since these ticks lay higher number of eggs during darkness, it means higher number of eggs would be laid during the night under natural condition and higher number of eggs would be available in crevices, cracks, corners and other dark places in the dog kennel early in the morning. Also since brine have been observed to be cheap and have acaricidal properties, causing mortality of eggs and adult females (20) the crevices, cracks in kennels and their surrounding could be flooded with saturated sodium chloride solution very early in the mornings. This is because it is at this time that greatest kill of eggs and adults would be made.

Dipeolu and Akinboade (26) in their study of the spread of ticks by scavenging dogs reported that engorged female dog ticks: *R. sanguineus* and *H. leachi leachi* when dropped off from their host showed an initial phase of inactivity and then moved slowly into dark places in the kennels before laying their eggs. This behaviour by these ticks seems to be an adaptation to avoid the effect of direct light on them. This confirms our observation in this study that more eggs were laid at the scotophase than in the photophase.

Dipeolu and Akinboade (26) also reported that the completion of life cycle was faster in *R. sanguineus* than in *H. leachi leachi* under natural condition. The tolerance of a wider range of temperature (20) coupled with the production of higher number of eggs in all photoperiodic conditions as observed in this study and the fact that *R. sanguineus* completes its life cycle faster than *H. leachi leachi* (26) probably explains why *R. sanguineus* is more preponderant and abundant than *H. leachi leachi* in Nigeria. However, the two tick species would occur throughout the year.

Since *R. sanguineus* and *H. leachi leachi* occur throughout the year (20, 26), it is therefore suggested that an all-year round kennel floor brine wash may be efficient in controlling dog ticks and also reduce the over-dependence on imported acaricides.

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