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Sizes and developmental viability of sequentially oviposited eggs of dog ticks: *Rhipicephalus sanguineus* and *Haemaphysalis leachi leachi*

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Summary
Sizes and developmental viability of sequentially oviposited eggs of up to 12th day oviposition by dog ticks *Rhipicephalus sanguineus* and *Haemaphysalis leachi leachi* were studied. The mean length and breadth of eggs oviposited on days 1 and 2 were greater (0.45 ± 0.111 and 0.44 ± 0.042) than those of subsequent ovipositions. The eclosion periods of eggs laid from 7th to 12th day of oviposition were progressively shorter (17, 16, 14 and 19, 17, 0 days) than those of eggs laid on days 1 and 2 (20 and 22 days) by *R. sanguineus* and *H. leachi leachi*, respectively. The percentage mortality of eggs of days 11 and 12 oviposition (70.3% and 100%) was significantly higher than that of earlier ovipositions (9.6% and 31.7%) for *R. sanguineus* and *H. leachi leachi*, respectively. The numbers of larvae which engorged on rabbits after hatching from eggs of 11th and 12th day oviposition (43 and 0) were significantly fewer than those which hatched from eggs of days 1 and 2 (79 and 63) for *R. sanguineus* and *H. leachi leachi*, respectively.A total of 383 larvae of *R. sanguineus* engorged on rabbits compared to 225 larvae of *H. leachi leachi*. It is concluded that eggs of early ovipositions of *R. sanguineus* and *H. leachi leachi* are longer and wider and more viable than those laid later in the oviposition cycle. The significance of these findings in terms of hatchability of eggs is discussed.

Keywords: Dog, *Rhipicephalus sanguineus*, *Haemaphysalis leachi leachi*, egg size, oviposition, viability.

Résumé
On a étudié les tailles et la viabilité de développement des œufs séquentiellement pondus jusqu'au 12e jour de ponte par des tiques de chien *Rhipicephalus sanguineus* et *Haemaphysalis leachi leachi*. La longueur et largeur moyenne des œufs pondus le premier et deuxième jour étaient plus grandes (0,45 ± 0,111 et 0,44 ± 0,042) que ceux pondus ultérieurement. Les périodes d'éclosion des œufs pondus du 7e au 12e jour de ponte étaient progressivement plus courtes (17, 16, 14 et 19, 17, 0 jour) que celles des œufs pondus le premier et deuxième jour (20 et 22 jours) par *R. sanguineus* et *H. leachi leachi*, respectivement. Le pourcentage de mortalité des œufs des jours 11 et 12 de ponte (70,3% et 100%) était considérablement plus grand que celui des pontes antérieures (9,6% et 31,7%) pour *R. sanguineus* et *H. leachi leachi*, respectivement. Les nombres de larves qui se gonflent sur les lapins après l'éclosion d'œufs du 11e et 12e jour de ponte (43 et 0) étaient considérablement moins nombreux que ceux de l'éclosion d'œufs des jours 1 et 2 (79 et 63) pour *R. sanguineus* et *H. leachi leachi*, respectivement. Un total de 383 larves de *R. sanguineus* se gonflent sur les lapins par rapport à 225 larves de *H. leachi leachi*. On conclut que les œufs pondus dans les premiers jours de ponte de *R. sanguineus* et *H. leachi leachi* sont plus longs et plus larges et plus viables que ceux qui sont pondus plus tard dans le cycle de ponte. Nous avons discuté de l'importance de ces résultats en termes d'éclosion des œufs.

Introduction

Rhipicephalus sanguineus Latrielle, 1806 and *Haemaphysalis leachi leachi* Audouin, 1826 are the only ticks found on dogs in Nigeria [1, 2] and the most wide spread ticks of dogs worldwide [3]. These ticks have been reported to be the vectors of *Babesia canis, B. gibsoni* and *Ehrlichia canis*, the causative agents of canine babesiosis and ehrlichiosis [4, 5].

For adequate control measures which will be effective, cheap, acceptable and practicable, there is need for information on many aspects of the biology of these ticks. Several workers have provided information on many aspects of the biology of ixodid ticks of cattle [6, 7, 4]. Dipeolu [8] provided information on the oviposition capacity of engorged females of *Boophilus decoloratus* Koch, 1844 and *Boophilus geigyi* Aeschliman and Morel, 1965 (cattle ticks). Dipeolu [8] reported that the eclosion period of eggs of later ovipositions were shorter than those of earlier oviposition, meaning that some form of development had taken place in eggs of later

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oviposition *in vivo*. Amoo *et al.* [9] had also provided information on the developmental viability and sizes of sequentially oviposited eggs of *B. decoloratus* and *B.geigyi*. There is a dearth of information on the dimensions (lengths and breadths) and developmental viability of sequentially oviposited eggs of dog ticks *R. sanguineus* and *H. leachi leachi*. This study was therefore carried out to determine the dimensions of eggs of different ovipositions, their hatching pattern and mortality rates in relation to their development viability on suitable hosts.

Materials and methods

Engorged adult female ticks were collected individually by detachment with pairs of forceps from household dogs brought to Veterinary clinics in Ibadan into glass bottles and conveyed in a multipurpose kilner jar to the Parasitology laboratory section of the Department of Veterinary Microbiology and Parasitology University of Ibadan and identified into species as described by Soulsby [10].

Sixty fully engorged adult female ticks each of *R. sanguineus* and *H. leachi leachi* were used for this study and each tick was kept in a Bijou bottle plugged tightly with cotton wool. The ticks were maintained in an insectary at 25°C and 85% relative humidity (RH).

After the pre-oviposition periods, the eggs laid by each tick were collected at 08.00 hours every day during oviposition. The collection of eggs was carried out by carefully taking out the ovipositing tick and putting it into another dry and clean Bijou bottle as described by Dipeolu and Ogunji [7]. This procedure was carried out daily throughout the oviposition period. Eggs produced were characterized and labeled by the day they were laid, such that eggs laid by the ticks on the first day were referred to as day 1 oviposition, while those laid 12 days were referred to as day 12 oviposition.

Eggs of day 1 and day 2 ovipositions from each of five *R. sanguineus* and *H. leachi leachi* were pooled separately and 10 eggs from each pool (making a total of 50 eggs) each for *R. sanguineus* and *H. leachi leachi* were randomly picked using fine dissecting pins. The length and breadth of each egg were measured with an ocular micrometer. Measurement was facilitated by addition of drop(s) of xylene which dissolved the wax which had hitherto made the eggs stick together.

The remaining eggs of day 1 and 2 ovipositions from the five each of engorged *R. sanguineus* and *H. leachi leachi* were pooled together and 0.02gram eggs of each tick species were weighed and incubated at 25°C and 85% R.H. as described by Amoo *et al.* [9]. The eclosion period, hatching pattern and mortality rate of the eggs were determined and recorded [9].

After four days of hatching the larvae were separated from the eggs by putting the Bijou bottle containing the hatched larvae and eggs in a refridgerator for 5 minutes so as to immobilize the active larvae. Thereafter 100 larvae each of *Rhipicephalus sanguineus* and *Haemaphysalis leachi leachi* were counted out and used to infest one rabbit each by the ear bag method [11]. The number of ticks which got engorged on each rabbit was counted 25 days after infestation and recorded. The same procedure was repeated for paired groups of eggs of days 3 and 4, 5 and 6, 7 and 8, 9 and 10, 11 and 12 ovipositions.

All the data collected were subjected to statistical analysis using students t test (SPSS version 1.1 2002).

Table 1: Dimensions of eggs of *Rhipicephalus sanguineus* and *Heamaphysalis leachi leachi* at different oviposition days

Dimensions	Days of Oviposition					
	1 & 2	3 & 4	5 & 6	7 & 8	9 &10	11 & 12
R. sanguineus						
Length (mm)	0.48 ± c	0.47 ± c	0.46 ± a	0.46 ± a	0.46 ± a.	0.45 ± b
	0.042	0.015	0140	0.12	0.012	0.013
Breadth (mm)	0.38 ± d	0.39 ± d	0..38 ±	34 ±	0.30 ±	0.32 ± c
	0.05	0.012	0.015	0.095	0.014	0.052
Area	0.182	0.183	0.174	0.156	0.147	0.135
H. leachi leachi						
	0.451 ± c	0.46 ± c	0.45 ± c	0.42 ± a	0.42 ± a	0.40 ± b
Length (mm)	0.011	0.024	0.021	0.016	0.019	0.13
Breadth (mm)	0.34 ± a	0.33 ± c	0.33 ± b	0.34 ± a	0.33 ± b	0.32 ± c
	0.113	0.017	0.017	0.014	0.021	0.017
Area (mm^2)	0.153	0.152	0.153	0.142	0.138	0.128

All values within row having the same superscript are not significantly different ($p > 0.05$).

Results

Table 1 shows the lengths and breadths of eggs of various ovipositions of both *R. sanguineus* and *H. leachi leachi*. For *R. sanguineus*, significant differences ($p < 0.05$) were found in the lengths and breadths of eggs laid during oviposition days 7 and 8, 9 and 10 and 11 and 12 when compared with eggs of the day 1 and 2 oviposition. There were no significant variations ($p > 0.05$) in the lengths of the eggs of *H. leachi leachi* in all the oviposition days. The results also show that eggs of *H. leachi leachi* were longer and narrower than the eggs of *R. sanguineus*.

shorter than those laid earlier in the ovipositon cycle. The eclosion periods decreased as days of oviposition increased (Tables 2B and 3B). Duration of hatching of larvae was 2 days in day 1 and 2 oviposition for *R. sanguineus* (Table 2B), this was extended to 5 days in the corresponding period of oviposition for *H. leachi leachi* (Table 3B). There were variations in the percentage mortality of eggs of the days 1 and 2 to 11 and 12 ovipositions in the two species of ticks. The days 9 and 10 and 11 and 12 ovipositions showed higher percentage mortality than eggs of earlier days oviposition. For *H. leachi leachi*, there was 100% mortality of the eggs laid on days 11 and 12 (Table 3B).

Table 2 (a &b): The hatching pattern and characteristics of eggs of *Rhipicephalus sabguineus* at differentOviposition.

A. Days of Oviposition	No. and % larvae hatched					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
1 & 2	320 (88.2)	8 (2.2)				
3 & 4	336 (92.5)	7 (1.9)	4 (1.1)	3 (0.8)		
5 & 6	50 (13.8)	85 (23.4)	56 (15.4)	21 (5.8)	10 (2.8)	2 (0.6)
7 & 8	36 (9.9)	87 (24.0)	56 (15.4)	21 (5.8)	10 (2.8)	2 (0.6)
9 & 10	10 (2.8)	39 (10.7)	41 (11.3)	30 (8.3)	8 (2.2)	
11 & 12	6 (1.7)	43 (11.9)	42 (11.6)	11 (3.0)	6 (1.7)	

B. Days of oviposition	Eclosion Period (days)	Duration of hatching	Total No. (% of Hathched)	Total Unhatched	% Mortality
1 & 2	20	2	328% (90.4)	35	9.6
3 & 4	19	4	350 (96.4)	13	3.6
5 & 6	18	3	224 (61.7)	139	38.3
7 & 8	17	6	212 (58.4)	151	41.4
9 & 10	16	5	123 (33.9)	240	66.1
11 & 12	14	5	108 (29.8)	255	70.3

Tables 2A and 3A show the hatching pattern of the eggs of *R. sanguineus* and *H. leachi leachi* respectively. The hatching patterns of eggs of both species appear similar. For *R. sanguineus*, the eclosion period of eggs laid on days 11 and 12 was

The data on the ticks that engorged on rabbits with larvae hatched from eggs of different ovipositions are shown in Table 4. In both species only the larvae hatched from the 7 and 8 and 9 and 10 oviposition days showed appreciable variation from the 1 and 2

Table 3(A & B): The hatching pattern and characteristics of eggs of *Heamaphysalis leachi leachi* at different oviposition.

A.

Days of oviposition	No. and % larvae hatched each day					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
1 & 2	37 (10.2)	77 (21.2)	72 (19.8)	55 (15.2)	7 (1.9)	6
3 & 4	57 (15.7)	86 (23.7)	93 (25.0)	20 (5.5)	0	0
5 & 6	21 (5.8)	46 (12.7)	90 (24.8)	40 (11.3)	15 (4.1)	4 (1.1)
7 & 8	2 (0.6)	59 (16.3)	71 (19.6)	41 (11.3)	30 (8.3)	0 (2.5)
9 & 10	5 (1.4)	70 (10.3)	20 (5.5)	12 (3.5)	9 (2.5)	1 (0.3)
11 & 12	0	0	0	0	0	0

B.

Days of Oviposition	Eclosion period (days)	Duration of hatching (days)	Total No. (% hatched)	Total Unhatched	% Mortality
1 & 2	22	5	248 (63.3)	115	31.7
3 & 4	21	11	256 (70.5)	107	29.5
5 & 6	20	6	216 (59.5)	147	40.5
7 & 8	19	7	204 (56.3)	159	43.3
9 & 10	17	6	117 (32.2)	246	67.8
11 & 12	0	0	0	363	100

oviposition days as regards the number of larvae engorging to adults on rabbits. A total of 383 larvae of *R. sanguineus* engorged on rabbits compared to 255 of *H. leachi leachi.*

Table 4: Number of *Rhipicephalus sanguineus* and *Heamaphysalis leachi leachi* ticks that engorged on rabbits after infestation with 100 Larvae.

Species	Days of oviposition					
	1&2	3&4	5&6	7&8	9&10	11&12
R. sanguineus	79*	87	69	57	48	43
H. leachi leachi	63*	68	47	41	36	0

Discussion

The reason(s) for the eggs of early oviposition of *R. sanguineus* being longer and wider than those of later ovipositions compared with those of *H. leachi leachi* of which only the length of the eggs of early ovipositions was longer than that of the later ones is not yet clear and is being studied in our laboratory. The present study has shown that the eggs of *R. sanguineus* are generally bigger than those of *H. leachi leachi.* Since big eggs have large surface area and volume, it is reasonable to suggest that the egg of *R. sanguineus* accumulates more food reserves such as carbohydrates, proteins, lipids and water. This will induce greater hatching success of the eggs, lower mortality and increased viability of larvae of *R. sanguineus* compared with those of *H. leachi leachi* as observed in this study. Also since small eggs have small surface area and volume, dessication would be reached quickly as a result of water loss leading to unhatchability of eggs Dipeolu [8]. This is in agreement with the observation of Williams [12] who reported that the amount of food reserves in various eggs may affect the development and viability of the embryo and the subsequent hatching process of eggs. This might partly be the explanation for the

higher preponderance of *R. sanguineus* compared to *H. leachi leachi* in Ibadan, South western Nigeria [1, 2].

It was observed in this study that eggs of later ovipositions of *R. sanguineus* and *H. leachi leachi* have shorter eclosion periods than those of later ovipositions. This observation is in agreement with the findings of Amoo *et al.* [9] in their study of the developmental viability and sizes of sequentially oviposited eggs of *Boophilus decoloratus* and *B. geigyi*. It also partly agrees with the findings of Dipeolu [8] who reported shorter eclosion period in eggs of later ovipositions than earlier ones in 70 – 75% of engorged *Amblyomma variegatum* and shorter eclosion period in earlier ovipositions than the later ones in 25 – 30% of engorged ticks. This was probably due to the fact that the process of embryogenesis in eggs of early ovipositions is gradual and it is quick and faster in eggs of later ovipositions resulting in shorter eclosion period in the latter as suggested by Amoo *et al.* [9]. This could be one of the factors responsible for the diverse viability of larvae which hatched from eggs of later ovipositions observed in this study, since the process of embryogenesis in eggs of later ovipositions started *in utero* [8], the resultant larvae are weak because most of the food reserves which provide energy were used up during oogenesis.

The results of this study also show that eggs of early ovipositions of *R. sanguineus* and *H. leachi leachi* were more viable and survived longer than those of later ovipositions. This could be due to the biochemistry of the food reserves (inform of carbohydrate, proteins and lipids needed to support the development of the eggs). Iwuala *et al* [13] observed that the protein content of sequentially oviposited eggs of *B. deocloratus* was low in the eggs laid between 9th and 12th day of the oviposition cycle. These authors attributed the low protein to the phased breakdown and utilization of host blood proteins followed by gradual synthesis of specific egg proteins during oogenesis in the engorged female tick as earlier reported by Boctor and Kamal [14]. It is therefore possible that the high mortality rates of larvae and low number of larvae attachments resulting in the few larvae engorging on rabbits found in the later ovipositions in this study were due to the low protein content of the food reserves of the eggs. The results of mortality rate is in agreement with the earlier study of Iwuala and Okpala [15] who reported that the hatch rate of *Amblyomma variegatuum* and *Boophilus annulatus* showed significantly different mortalities ranging from less than 5% in the earliest egg batches to over 95% in the last batches.

References

1. Dipeolu O O and Akinboade O A. Scavenging dogs and the spread of tick infestation in Nigeria. Int. J. Zoonosis 1982. 9: 90 – 96
2. Adejinmi J O. Bionomics of the Dog ticks: *Rhipicephalus sanguineus* and *Haemaphysalis leachi leachi* – Ph.D Thesis, University of Ibadan, 2005: pp 1-117.
3. Craig T M. Infectious Diseases of the dog and Cat (Greene, C.E. Ed.) W.B. Saunders Co. Philadelphia 1990. pp. 778 – 785.
4. Kutler K L. In: Babesiosis of Domestic Animals and man (Ristic, M.Ed.), CRC Press Inc., Florida 1988. pp. 1 – 22.
5. Bobade P A. Oduye O O and Aghomo H O. Prevalence of antibiodies against Babesia canis in dogs in an endemic area. Rev. Elev. Med. Vet. Pays Trop., 1989. 42: 211 – 217.
6. Dipeolu O O. Survey on external parasites infestation of dogs in Nigeria. J. Small Anim. Pract. 1975. 16: 123 – 129.
7. Dipeolu O O and Ogunji F O. Studies on ticks of Veterinary importance in Nigeria I. On the development of the ixodid ticks *Amblyomma variegatum* (Fabricius, 1794) and *Hyalomma rufipes* (Koch, 1844) under quasi natural conditions in Nigeria. J. Pharm. Med. Sci. *1977a.* I, 243 – 248.
8. Dipeolu O O. Studies on ticks of Veterinary importance in Nigeria V. The size, hatching patterns and mortality rate of eggs of *Amblyomma variegatum* (Fabricius, 1794). Insec. Sci. Applic. 1982. 3: 227 - 231.
9. Amoo A O J. Dipeolu O O and Akinboade O A. The developmental viability and sizes of sequentially oviposited eggs of *Boophilus decoloratus* and *Boophilus geigyi.* Expt. Appl. Acarol. 1985: 1, 219 – 225.
10. Soulsby E J L. Helminths, Arthropod and Protozoa of Domesticated Animals. 7th ed. (London bailliere Tindall) Pg. 469 – 495.
11. Adejinmi J O and Akinboade O A. Resistance of dogs to *Rhipicephalus sanguineus* and *Haemaphysalis leachi leachi.* Vet. Arhiv. 2007, 77(4): 313 – 318.
12. Williams J. Yolk Utilization In: The Biochemistry of Animal Development Vol. II (Weber, R. Ed.) Academic press, New York and London: 1967. 341 – 382.

13. Iwuala M O E. Umezurike G M and Nwadiogbu N. Quantitative studies on the proteins, fatty acids, and glycogen contents of eggs of *Amblyomma variegates* and *Boophilus decoloratus.* Acarologia 1981. 22(2): 132 – 143.
14. Boctor I Z and Kamal K A. Biochemical and physiological studies with insects and ticks. Insect. Biochem. *1*976. 6: 233 – 240.
15. Iwuala M O E and Okpala I. Egg output in relation to weights and stages of engorgement of *Amblyomma variegatum* and *Boophilus annalatus*. Folia parasitologica (Proha) 1977 24: 162 – 172.