



OBSERVATIONS ON SOME BIOLOGICAL CHARACTERISTICS OF *RHIPICEPHALUS SANGUINEUS* SENSU LATO (ACARI: IXODIDAE) UNDER NATURAL AND LABORATORY CONDITIONS IN NIGERIA

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ABSTRACT

The brown dog tick, *Rhipicephalus sanguineus* sensu lato (s.l.) is a globally distributed tick of medical and veterinary significance. This study reports some observations on the biological characteristic of the brown dog ticks kept under natural and laboratory conditions in Nigeria. Variation in biological parameters was observed within the Nigerian *R. sanguineus* s.l. population kept under various conditions. A higher number of *R. sanguineus* exhibited the type 1 oviposition pattern than the type 2 pattern. The type 1 pattern was observed in 84.2 % and 80 % of *R. sanguineus* s.l. reared under laboratory and natural conditions, respectively. The pre-oviposition period was significantly shorter in ticks maintained under laboratory conditions than under natural conditions. Similarly, ticks kept under laboratory conditions laid significantly more eggs with higher egg mass weight than ticks kept under natural conditions. Furthermore, the incubation period and the total weight loss by female ticks throughout the oviposition period differed significantly between the

two groups ($P < 0.05$). The engorged *R. sanguineus* s.l. females kept at 4 °C survived for up to 21 days and remained viable producing fertile eggs. Egg of *R. sanguineus* s.l. kept at 4 °C for up to 63 days retain viability and were able to hatch to larvae after variable incubation periods when returned to ambient temperatures depending on the duration of storage at 4 °C. The results showed that the tropical lineage of the brown dog tick can survive and remain fecund at low temperatures, suggesting that it can be translocated and established in temperate regions.

Key words: fecundity; hatching; refrigerator; *R. sanguineus* s.l.; survival; zoonoses

INTRODUCTION

Rhipicephalus sanguineus sensu lato (s.l.) is reputed as the most widespread ticks of dogs and is a well-recognized putative vector of numerous pathogens to dogs and humans worldwide [7, 30]. Based on available bio-

logical, molecular, and genetic data, the *R. sanguineus* s.l. is a group of at least 17 morphologically closely related species [20, 21]. Therefore, a lot of studies have been conducted using morphological, biological, and genetic analyses to generate data that may assist in resolving the taxonomic ambiguity associated with this tick [3, 4, 12, 14, 20, 22, 23]. Specifically, variations in the biological characteristics of *R. sanguineus* s.l. strains obtained from different climes have been reported, attesting to the complexity within this taxon [10, 19, 27].

Although the origin of the brown dog tick has been traced to Africa [1, 9, 29], little has been reported on its biological characteristics in Nigeria. Therefore, the aim of this study was to highlight some observations on the biological characteristics of the brown dog tick under natural and laboratory conditions in Nigeria in order to update available data on this enigmatic tick from the continent.

MATERIALS AND METHODS

Study location and ticks sampling

Ticks were collected from owned dogs in Nigeria. The details of the tick sampling locations and climatic conditions are available in the study by K a m a n i [15]. Naturally infested dogs were properly restrained and the ticks were removed using forceps. The ticks were placed in labelled ventilated tubes and transported to the Entomology Research Laboratory, National Veterinary Research Institute (NVRI), Vom, Nigeria.

Ticks identification

The morphological and molecular identification of the ticks used in this study have been previously described [15]. Only ticks identified as *R. sanguineus* s.l. were used in this study.

Oviposition in *R. sanguineus* s.l. under natural and laboratory conditions

Partially or fully engorged female ticks were selected for this study. The selected female *R. sanguineus* s.l. ticks were weighed individually and placed in coded sterile ventilated tubes. Nineteen ventilated tubes each containing one female tick were placed in an incubator at 27°C, relative humidity of 85% with 12-hour photoperiods, while another 19 tubes were placed on a bench in the

laboratory at 22°C (range 18.3—25.1°C), and humidity 65% (range 42—76%). The ticks were monitored for the commencement of egg laying. Daily, at 7:00 hrs the ticks and the eggs laid were weighed and the number of eggs counted. Ten eggs were randomly picked from each clutch daily and measured (length and width) under a calibrated microscope (Nikon, Eclipse E100). This was continued until egg laying ceased evidenced by constant weight of the ticks for five consecutive days. Biological parameters; engorged female weight (FW), pre-oviposition period (POP), duration of oviposition (DO), total number of eggs produced (TNEP), egg mass weight (EMW), egg size (ES), tick final weight after oviposition (TFW), percent of initial weight lost during oviposition (WLO), incubation period (IP), efficiency of conversion of feed reserve to produce eggs (ERCE), oviposition efficiency (OPE), mass conversion rate (MCR), and mass conversion efficiency (MCE) were calculated according to D i p e o l u, et al. [6] and S z a b ó, et al. [28].

Effect of low temperature (4°C) on the survival and fecundity of *R. sanguineus* s.l.

The engorged female *R. sanguineus* s.l. (n = 30) collected from the dogs in Nigeria were weighed and individually placed in labelled ventilated tubes. The tubes were placed on a rack in a group of five and kept in a refrigerator (4°C). A group of five ticks were removed weekly and assessed for viability. Ticks that were viable were placed in an acaridium at conditions earlier mentioned and assessed for fecundity.

Effect of low temperature (4°C) on the survival and hatchability of *R. sanguineus* s.l. eggs

Eggs laid by seven *R. sanguineus* under natural conditions were pooled and gently mixed on a clean petri-dish. Thereafter, 100 eggs were counted and kept in ventilated tubes. The tubes containing the eggs were kept on a rack in a refrigerator (4°C). Two tubes containing the eggs were removed weekly and placed in an acaridium at conditions earlier mentioned and assessed for hatchability. Percent hatching was obtained by subtracting the number of unhatched eggs from 100.

Ethical statement

The study protocol was approved by the Animal Use and Care Committee (AUCC), National Veterinary Re-

search Institute (NVRI) Vom, Nigeria. Oral consent was obtained from dog owners before ticks were collected from the dogs.

RESULTS

Four of the ticks kept under natural condition escaped from the vials, hence data was only available for 15 ticks in this group. A higher number of *R. sanguineus* s. l. exhibited the type 1 oviposition pattern than the type 2 pattern. Type 1 pattern was observed in 16 out of 19 (84.2%) and 12 out of 15 (80%) of the ticks kept under laboratory and natural conditions, respectively. The type 1 oviposition pattern is characterized by initial low oviposition and attainment of peak after a few days, while the type 2 is characterized by early attainment of peak oviposition usually within the first set of eggs (Fig. 1). The pre-oviposition pe-

riod was significantly shorter in ticks maintained under laboratory condition than those kept under natural condition. Similarly, the ticks kept under laboratory conditions laid significantly more eggs and had higher egg mass weight than the ticks kept under natural conditions (Table 1). Furthermore, the incubation period and the total weight loss by female ticks throughout the oviposition period differed significantly between the two groups (Table 1). There was higher POP, DO, OPE and MCR for ticks kept under natural condition while those kept under laboratory condition had higher WLO and ERCE, although the differences were not significant ($P > 0.05$) (Table 1).

Effects of low temperature (4 °C) on adult *R. sanguineus* s. l. females

The engorged *R. sanguineus* females kept at 4 °C survived for up to 21 days and remained viable. There was an increase in the percent loss of the initial tick weight with

Table 1. Biological parameters of female *R. sanguineus* s. l. under natural and laboratory conditions in Nigeria

Parameters	Natural conditions (n = 15)		Laboratory conditions (n = 19)		F	P
	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range		
FW [mg]	170.3 $\pm$ 119.7	21–406	184.1 $\pm$ 154	34–533	0.6	0.15
POP [days]	6.9 $\pm$ 1.8	5–10	5.8 $\pm$ 0.9	4–7	3.8	0.003*
DO [days]	12.4 $\pm$ 5.4	1–21	10.5 $\pm$ 3.9	3–20	1.89	0.09
TNEP	1687 $\pm$ 1274	38–4041	2179 $\pm$ 1952	162–7084	0.43	0.04*
EMW [mg]	73.7 $\pm$ 57	2–185	95.8 $\pm$ 89.8	8–317	0.4	0.03*
ES [μ g]	L 454 $\pm$ 39.5	409–502	472 $\pm$ 28.2	418–525	0.98	0.5
	B 361 $\pm$ 18.1	334–399	364 $\pm$ 21	334–426	1.35	0.3
TFW [mg]	44.4 $\pm$ 25.3	12–112	34.9 $\pm$ 24	10–91	1.19	0.37
IP [days]	31.3 $\pm$ 3.7	24–36	26.7 $\pm$ 1.7	24–29	4.8	0.00*
ERCE [%]	39.7 $\pm$ 12.7	6.7–55.8	49.9 $\pm$ 13.7	19.7–67.4	0.9	0.37
WLO [%]	62.1 $\pm$ 19.8	17.6–80	75.1 $\pm$ 8.1	55.9–87.5	6.02	0.00*
OPE	0.13 $\pm$ 0.11	0.06–0.5	0.11 $\pm$ 0.07	0.05–0.3	0.85	0.37
MCR	0.14 $\pm$ 0.11	0.06–0.5	0.09 $\pm$ 0.05	0.05–0.25	1.82	0.12
MCE	0.07 $\pm$ 0.03	0.05–0.18	0.07 $\pm$ 0.01	0.05–0.1	0.95	0.46

F—Fisher test; P—significance value; FW—engorged female weight; POP—pre-oviposition period; DO—duration of oviposition; TNEP—total number of eggs produced; EMW—egg mass weight; ES—egg size; TFW—tick final weight after oviposition; WLO—percent of initial weight lost during oviposition; IP—incubation period; ERCE—efficiency of conversion of feed reserve to produce eggs; OPE—oviposition efficiency; MCR—mass conversion rate; MCE—mass conversion efficiency; values with * are statistically significant

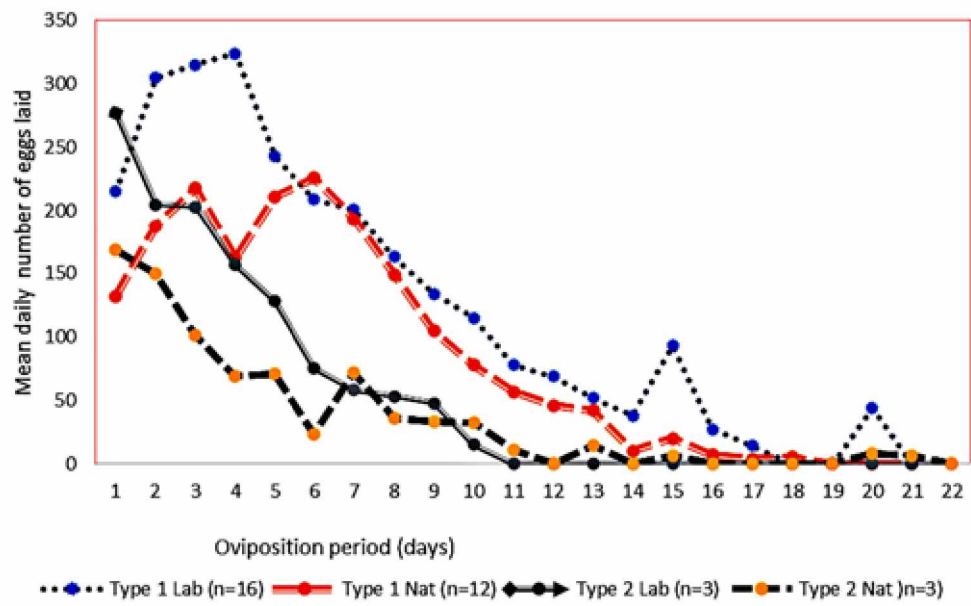


Fig. 1. Oviposition patterns exhibited by *R. sanguineus* s.l. under natural and laboratory conditions

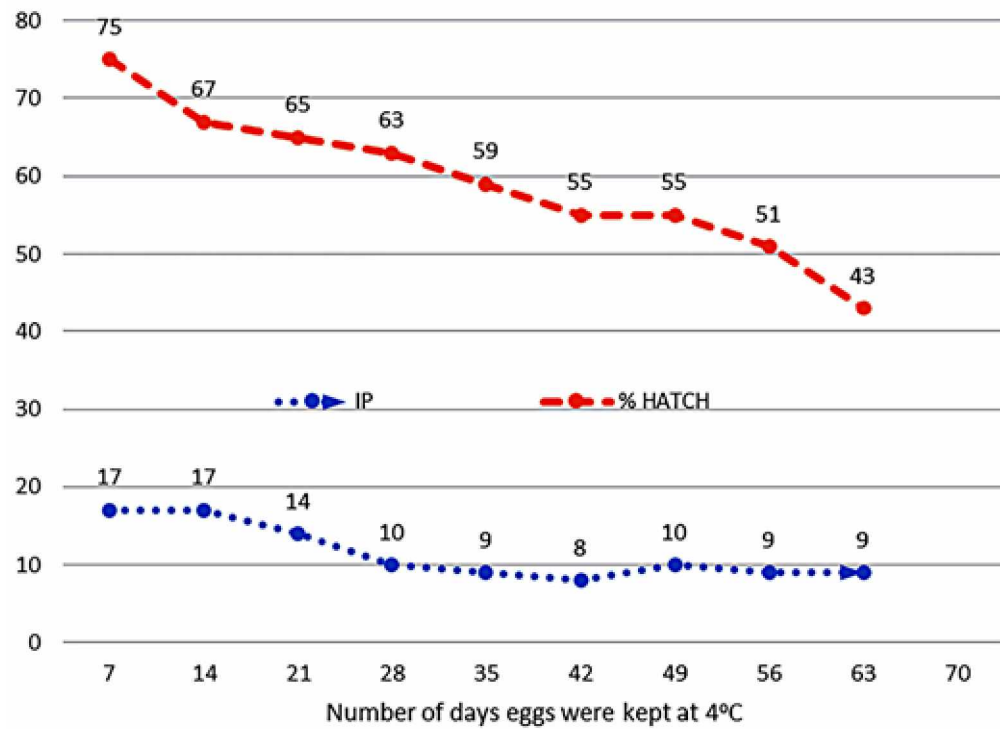


Fig. 2. Effect of low temperature (4 °C) on the survival and hatchability of *R. sanguineus* s.l. eggs

the duration of stay at 4 °C before moving them to the ideal environmental conditions for tick breeding. However, they were able to produce fertile eggs when returned to the ideal conditions. The IP was 26, 28 and 30 days respectively for ticks kept for 1, 2 and 3 weeks at 4 °C before returning them to ambient conditions. The POP decreased with increased duration of stay at 4 °C; from six days for 7 and 14 days to 5 days for ticks kept for 21 days at 4 °C. Similarly, the EM and ERCE decreased with longer storage at 4 °C.

Effects of low temperature (4 °C) on survival and hatchability of *R. sanguineus* s.l. eggs

Egg of *R. sanguineus* kept at 4 °C for up to 63 days retained viability and were able to hatch to larvae when they were returned to the ideal environmental conditions after variable IP depending on the duration of storage at 4 °C. The IP and percent hatching were inversely related to the period of storage at 4 °C (Fig 2). Although no hatching was recorded during the period of egg storage at 4 °C for up to 63 days, there might be some biological activities taking place in the eggs, which may explain the shorter IP in eggs stored for longer period. Equally the low percent hatch in eggs kept for longer period at 4 °C could be due to exhaustion or depletion of nutrient reserve in the egg leading to embryonic death.

DISCUSSION

This study reports on the observations of some biological parameters of *R. sanguineus* s.l. removed from naturally infested dogs in Nigeria. The FW and EMW for *R. sanguineus* s.l. recorded in this study were higher than the values reported for the same tick species from Brazil and Indonesia but lower than that of Argentina [10, 27]. Most of the biological parameters assessed in this study varied from other studies in different climes [10, 12, 25, 27]. These differences can be attributed to variation in climatic, as well as, the intra species variation among the diverse *R. sanguineus* complex [24, 25, 27]. Interestingly, varying the environmental conditions among the same tick population in this study resulted in significant differences in some of the biological parameters like the POP, IP, TNEP, EMW and WLO, confirming the role of abiotic factors on tick fecundity. In addition, a wide variation in the sizes of

R. sanguineus s.l. was recorded in this study similar to reports from other countries [2, 10—13, 27, 28]. The female engorgement weight is a key determinant of the number of eggs laid and most of the other biological parameters of *R. sanguineus* s.l. in this study which is in agreement with reports from other studies [5, 16, 26]. Furthermore, the type 1 oviposition pattern was recorded in >80% of the *R. sanguineus* s.l. in this study similar to the earlier report of 78% in Nigeria [5]. It has been established that environmental temperature and humidity are critical factors in the survival and fecundity of ticks [7, 8]. Some studies have reported that temperatures of 20—35 °C and humidity of 35—95 % are ideal for the survival and optimum fecundity of *R. sanguineus* s.l. [18, 26]. Differences were observed in the fecundity of *R. sanguineus* s.l. reared under laboratory (T = 27 °C; RH = 85%) and natural condition (T = 22 °C; RH = 65%) in this study. This is in agreement with the results from other studies [18, 26]. Environmental temperature has been suggested as the key determinant for tick survival and establishment in a locality [30]. Indeed, it has been reported that an increment of 2—3 °C in the mean annual temperature favors the establishment of the tropical lineage of *R. sanguineus* s.l. in temperate climes [8]. In this study the ability of *R. sanguineus* s.l. tropical lineage to survive at low temperature (4 °C) was investigated. Interestingly, engorged females and eggs survived at 4 °C for over 21 and 63 days, respectively and maintained their viability. This finding agrees with the reported ability of *R. sanguineus* s.l. to overwinter in the environment and even infest dogs during winter in some regions of temperate climates [17]. Arguably, the ticks may not be able to perform full biological activities like: oviposition, egg hatching, or moulting at the low temperature [11, 18], but the ability to survive and remain viable for weeks at 4 °C have implication for its translocation, introduction and establishment into new climes, as well as, for research. Adult *R. sanguineus* and eggs can be stored in the fridge for several weeks for later use.

CONCLUSIONS

The variation in biological parameters of *R. sanguineus* s.l. in Nigeria is indicative of the existence of the diversity of haplotypes under this taxon. This has veterinary and public health implications taking into cognizance the var-

iations in vector capacity for different pathogens by the various haplotypes. Therefore, efforts should be geared towards elucidating the epidemiology and vector competence of this tick for various pathogens of veterinary and public health significance in Nigeria.

CONFLICT OF INTEREST

There is no conflict of interest.

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