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BIOMORPHIC STUDY OF *RHIPICEPHALUS SANGUINEUS* (ACARI: IXODIDAE) UNDER LABORATORY CONDITIONS

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ABSTRACT

Rhipicephalus sanguineus can complete up to four generations per year, depending on factors such as climate and host availability. Biomorphic studies of *R. sanguineus* were conducted under laboratory conditions. The larval, nymphal and adult ticks were fed on sheep at 25^0C, 60 % relative humidity (RH) and exposed to daylight. The incubation period was lasted 35 days. The average mean of premoulting and moulting period of larvae was 70 ± 0.91 and 7.4 ± 0.6 days, respectively. The time durations of premoulting and moulting of nymphs were 62 ± 1.66 and 13 ± 6.4 days, respectively. The average developmental periods were 6.09, and 5.15 days for larval pre-feeding, and feeding, respectively. The average developmental periods were 3.54, and 6.11 days for nymphal pre-feeding, and feeding, respectively. The complete life cycle of *R. sanguineus* was 180 days with range of 120-286 days. The average weights of the unfed larvae, and nymphs were 0.04 ± 0.03, and 0.40 ± 0.09 mg, respectively while 0.60 ± 0.05, and 5.02 ± 0.20 mg for engorged larvae and nymphs, respectively. The index of reproduction efficiency (REI), egg production efficiency (EPE), and the index of reproductive fitness (RFI) averaged 14.99 ± 3.65, 0.31 ± 0.08, and 11.74 ± 3.01, respectively. Scutum length and width of male was 2.2 and 1.2mm, respectively while length and width of female was 1.04 and 0.99 mm, respectively. The current study findings could help the workers in adopting different management studies for ticks in the world.

Keywords: Ticks, brown dog ticks, sheep, biomorphic, laboratory conditions.

INTRODUCTION

Ticks (Acari: Ixodidae) are the highest important group of vectors of pathogens within the phylum Arthropoda after mosquitoes. Ticks spread various species of bacteria, viruses, protozoa and nematodes affecting reptiles, human, animals, amphibians, and poultry (Abdullah et al., 2016; Ola-Fadunsin et al., 2020,2021; Ramzan et al., 2020a; Jamil et al., 2021, 2022a,b,c,d). More than 800 tick species have reported throughout the world and out of 800 species, 53 species reported from Pakistan (Ramzan et al., 2020b; 2021; Rahman et al., 2022). Ixodidae, also known as hard ticks, are exclusively parasitic arthropods. *Rhipicephalus* is one of the 12 extant genera of Ixodidae and comprises 84 described species (William et al., 2019; Ernieenor et al., 2017). *Rhipicephalus* falls under the subfamily of Rhipicephalinae (Metastriata) with almost 4 species reported in Pakistan. *Rhipicephalus sanguineus* is major threating species in Pakistan after *Hyalomma anatolicum.* This species has been documented to transmit several pathogenic bacteria (*Rickettsia massiliae, R. rickettsia*) (Nava et al., 2018), and

protozoan diseases (*Ehrlichia canis*, *Hepatozoon canis*, *Babesia canis*) (Dantas-Torres et al., 2017; Senbill et al., 2018). There was need to reared it and check the biological and morphological parameters of this species in the country because before this study no such study was conducted. The rearing of tick was necessary, to fulfill the need, the current study was conducted to describe the biological parameters of *R. sanguineus* under laboratory conditions. The study results may provide basic background data for further inquiries.

MATERIAL AND METHODS

Collection and Rearing of Ticks

A survey was conducted to collect the unfed adults of *R. sanguineus* from vegetation in Dera Ismail Khan from April 2020 to April 2022 when ticks were searching hosts for blood feeding. Collected ticks were brought to Parasitology Laboratory to develop a colony of the ticks on sheep and maintained in glass tubes sealed with hydrophilic cotton in an incubator at 25 ^{0}C, 70 % RH with 14:10 D: L) (Ullah et al., 2022; Jamil et al., 2021, 2022e,f).. Rearing procedures of Sun et al. (2008) and Senbill et al. (2018) were adopted in the study.

Animals

Six Damani breed sheep of one year were divided into three groups and infested by adults, nymphs, and larvae, respectively. They were maintained at 25 ^{0}C, 60 % RH and exposed to daylight. Two-meter muslin cloth was purchased from nearby a market and bags of 25 x 25 cm were prepared and glued to the back of sheep. Ticks were released into the bags for rearing and opening end of the bags closed with rope to avoid the tick escape.

Biological Parameters of Nymph, Larva and Adults

200 and 50 newly hatched larvae and emerged nymphs, respectively were released onto the back of two sheep daily to check their pre-feeding periods. Two hundred unfed larvae and fifty unfed nymphs were weighed and fed on sheep 7 days after hatching/moulting. Engorged ticks which naturally detached were collected into vials, and counted. Naturally detached engorged ticks weighed and kept into separate tubes. By using an analytical balance tick was weighed in pools. The group of ten larvae was considered a pool and five nymphs were also a pool. Sex was also checked after moulting. Biological parameters of adult ticks were also determined by placing newly emerged 25 males and 25 females on a sheep daily. Before placing on the back of sheep, both adults (males and females) were kept hungry for period of 4 days. After four days without food, adults weighted and put on the back of a sheep. The engorged females were collected, weighed and put into an incubator. All post developmental periods such as pre-oviposition, oviposition, and postoviposition periods and egg batches laid by females were collected, counted and observed or noted on daily basis. Number of eggs in a batch were counted, and kept into separate glass tubes. The incubation period was observed by using five hundred (500) eggs and percentage (%) of hatched larvae was calculated by using methods of early/previous adopted researchers (Chilton, 1992). Three hundred newly emerged larvae and fifty adults were collected from reared culture and kept into incubator to check their longevities. Morphological characters of larvae and adults were also checked.

Data Analysis

Data were statistically analyzed by using several Spearman's correlations and

Student's t tests, and Statistical Package for the Social Sciences (SPSS) (version 20).

RESULTS AND DISCUSSION

R. sanguineus, is the most significant contributor to the spread of tick-borne diseases in human, domestic and wild animals all over the world (Dantas-Torres et al., 2013). The prevalence percentage of this species is increasing now a days in the globe (Nava et al., 2015; Chitimia-Dobler et al., 2017).

R. sanguineus is an endophilic, monotropic, and three-host tick species. It can also feed on other hosts like humans and live in outdoor environments, despite being monotropic and endophilic in nature. It's clear that R. *sanguineus is catholic.*

All stages of *R. sanguineus* are pest of all ruminants (cattle, buffaloes, camel, goat, and sheep), and poultry (Benitez et al., 2012; do Amaral et al., 2012; Ma et al., 2016). Eggs were spherical in shape and dark brown in color. Larva has three pairs of legs while remaining stages (nymph and adult) with four pairs of legs. The length and width of body size of larva were 0.57 mm and 0.42 mm, respectively. Nymphs and adults were looked almost similar to each other but dissimilar in body size. Length and width of nymphs was 1.20-1.30, and 0.60-0.70 mm, respectively while 2.30-3.21, and 1.20-1.72 mm was the length and width of adult, respectively. Sonenshine and Roe (2014) had reported the similar findings about morphological characters of *R. sanguineus*. It has been recorded that body length increased during feeding. Sonenshine and Roe (2014) had reported that body size increased and reached the size of a raisin during blood feeding. The colour of adult and nymph was reddish-brown with an elongated body shape. Walker et al., (2005) had also reported the similar shape and colour of nymphs and adults. A hexagonal capituli was found on the back of adult ticks.

The incubation period was lasted 35 days. The average mean of premoulting and moulting period of larvae was 70 ± 0.91 and 7.4 ± 0.6 days, respectively. The time durations of premoulting and moulting of nymphs were 62 ± 1.66 and 13 ± 6.4 days, respectively. The average developmental periods were 6.09, and 5.15 days for larval pre-feeding, and feeding, respectively. The average developmental periods were 3.54, and 6.11 days for nymphal pre-feeding, and feeding, respectively. Developmental time and survival percentage of larvae and nymphs is given in Table 1. The complete life cycle of *R. sanguineus* was 180 days with range of 120-286 days. The average weights of the unfed larvae, and nymphs were 0.04 ± 0.03, and 0.40 ± 0.09mg, respectively while 0.60 ± 0.05, and 5.02 ± 0.20mg for engorged larvae and nymphs, respectively.

Average weights of engorged male and female adults are given in Table 2. The student's t test showed that the mean weight of engorged nymphs molting to females was significantly heavier ($P < 0.001$) than that of nymphs molting to males. It was observed that female laid least number of eggs at initial stage which increased with increased in time (on 7 days) and then gradually declined (after 10 days). The hatching percentage of eggs was 90 %. The average mean number of eggs laid by 25 females was 1898.09 ± 66.45 (range of 1509-2021) given in Table 3. Koch (1982) reported that a fully blood-fed female can lay over 7,000 eggs. He reported that egg lying capacity of female depends on the blood amount sucked by female and size of the tick. It was observed that female died after finishing her egg lying. Dantas-Torres (2008) and DantasTorres (2010) had investigated the similar findings about death of female ticks. The findings of oviposition pattern of *R. sanguineus* were in line to other species of *Rhipicephalus* and many other tick species like *Haemaphysalis* spp. (Chen et al. 2012).

The reproductive efficacy of female was assessed by using indices. The

index of reproduction efficiency (REI), egg production efficiency (EPE), and the index of reproductive fitness (RFI) averaged 14.99 ± 3.65, 0.31 ± 0.08, and 11.74 ± 3.01, respectively. The average tick recovery rate was 5.0 % (range= 4.0–6.0%). The biological parameters and indices of tick are given in Table 3. Pre-ovipositional, ovipositional, and post-ovipositional periods are given in Table 4 while morphological parameters are given in Table 5. Pre-ovipositional period was 17-22 days which is not in line with the previous studies. For example, 4 days were recorded by do Amaral et al. (2012), and 2.9–3 days by Benitez et al., (2012). This variation may be due to differences between tick populations, geographical regions, and different hosts.

Table 1: Developmental time and Survival percentage of engorged larvae and nymphs of *R. sanguineus.*

Parameters	Stages					
	Larvae			Nymphs		
	Time/days			Time/days		
	Mean ± SE	Range	Survival %	Mean ± SE	Range	Survival %
Premoulting	70±0.91	67-74	83	62±1.66	56-65	88
Moulting	7.4±0.6	8-May	93	13±6.4	12-15	100

Table 2: Variations in body weight of larva, nymph and adult of *R. sanguineus* before and after feeding.

Parameters	Larva		Nymph		Adult male		Adult female	
	Mean ± SE	Range	Mean±SE	Range	Mean ± SE	Range	Mean ± SE	Range
Unfed (mg)	0.04 ± 0.03	0.01-0.06	0.40 ± 0.09	0.35-0.51	1.53 ± 0.08	1.45-1.70	2.00 ± 0.07	1.33-3.01
Engorged (mg)	0.60 ± 0.05	0.50-0.67	5.02 ± 0.20	4.00-6.10	2.20 ± 0.21	2.00-2.40	95.89±3.88	190-100
Weight ratio	10.11	9-12	12.6	10-14	1.6	0.99-1.10	87.99	85-100

Table 3: Biological parameters and indices of females under laboratory conditions

Parameters	Mean±SE	Range
Engorged weight (mg)	289.89 ± 6.43	154.76–310.10
Egg mass deposited (mg)	201.54 ± 9.00	86.66–210.00
Oviposition (number of eggs)	1898.09 ± 66.45	1509–2021
Reproduction efficiency index (REI)	14.99 ± 3.65	6.6–25.50
Reproductive fitness index (RFI)	11.74 ± 3.01	5.6–21.30
Egg production efficiency (EPE)	0.31 ± 0.08	0.20–0.51
Hatchability (%)	86.8±3.10	80-90
Tick recovery rate (%)	5.0	4.0-6.0

Table 4: Developmental times for the three females engorged ticks.

Parameters	Mean ± SE	Range	Success rate (%)
Pre-oviposition period (days)	20.02±9.13	17-22	72
Oviposition period (days)	31.64±7.34	27-34	94
Post-oviposition (days)	5.3±3.29	2-7	100

Table 5: Morphological parameters of *R. sanguineus*

Parameters	Male		Female	
	Mean	Range	Mean	Range
Scutum length	2.2	2.91 –3.09	1.04	(0.72–0.92
Scutum width	1.2	1.10–1.34	0.99	0.45–1.05
Pedipalps length	0.51	0.40–0.59	0.17	0.09–0.23
Pedipalps width	0.21	0.10–0.36	0.15	0.13–0.20
Spiracular plate length	0.3	0.16–0.45	0.29	0.26–0.40
Spiracular plate width	0.1	0.06–0.20	0.08	0.06–0.13

CONCLUSION

The current study describes the biological parameters or life cycle of one of the most important cattle ticks, *R. sanguineus*. This species can successfully complete life cycles under controlled conditions like *Boophilus microplus.* The results of current study are very important for directing and implementing epidemiological experiments and control bioassays to ensure the health of livestock industry around the globe, especially in Pakistan.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

AUTHOR'S CONTRIBUTION

All authors have equal contribution in this article.

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