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Seasonal Variation and Morphometric Differentiation of Egyptian Strain of *Rhipicephalus sanguineus* (Acari: Ixodidae)

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THE BROWN dog tick (*Rhipicephalus sanguineus*), which is thought to be the most common tick in the world and a carrier of numerous infections to both human and dogs, is currently the subject of debate regarding its taxonomic classification. The objective of this study was to evaluate the differences in biological parameters between the winter and summer and the morphological difference of *R. sanguineus* reared on different host (dogs and rabbits). Regarding the difference in biological parameters between different stages of ticks recorded in winter and summer, there were significant differences in the preoviposition, oviposition, incubation, hatching periods, longevity of larvae, nymphs and unfed adults in addition to feeding periods of larvae, nymphs and unfed adults. The total life cycle of ticks in winter took longer time (75±4.58 days) than in summer (61.56±4.79 days). Otherwise, there are some morphological differences recorded in the unfed and fed *R. sanguineus* reared on dogs versus those reared in rabbits. These differences were significant and noticed in the size of different stages of *R. sanguineus* tick as the shape of the dorsal tail of spiracular plate is longer in adult male tick reared on dog than those tick reared on rabbits. Also, the eggs were spherical in shape in ticks fed on dog, but were slightly oval in ticks fed on rabbits. In conclusion, there were some variations in the biological parameters of *R. sanguineus* ticks between different seasons in addition, the morphometric features of ticks were varied according to host.

Keywords: *Rhipicephalus sanguineus,* Dogs, Rabbits, morphology, summer, winter.

Introduction

Ticks are regarded as the second-most important arthropods for medicinal and veterinary purposes in tropical areas, second to mosquitoes [1]. *Rhipicephalus sanguineus* is one of the most significant canines ectoparasites, causing direct harm to the host, including blood loss, dermatitis, pain, and transmission several infectious agents such as *Ehrlichia canis* and *Babesia canis* to dogs and *Rickettsia conorii* to humans [2-4]. Tick borne pathogens (TBP) were found in 23.56% of the analyzed blood samples from Egyptian dogs [5]. Of these samples, 11.1% had *Anaplasma* and *Ehrlichia*, 8.2% had *Babesia canis*, and 4.33% of the samples had mixed infections with two pathogens. Additionally, 45.97% of hemolymph smears had TBP with *Hepatozoon canis*, *B. canis* and Anaplasmataceae accounting for 35.89%, 8.1%, and 2.01% respectively. The brown dog tick, *R. sanguineus*, is a widespread species with a characteristic reddish-brown coloring. (The species *R. camicasi* and *R. turanicus* which morphologically very similar and are frequently confused with it [6] but have different behavioral, ecological, and vector characteristics [3].

Ticks belonging to the *R. sanguineus* species have small and elongated body. They have short palps, festoons and eyes. Males have spiracular plates that are comma-shaped with a significant split

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in Coxa I in both males and females. The brown dog tick can be recognized by its hexagonal basis capituli. Although dogs are the most frequent host for *R. sanguineus* ticks **[7]**, it has also been studied on cats, rabbits, camels, cattle, goats, horses, sheep, bats, reptiles, ground-feeding birds, and human [3].

There were few studies attempted to determine the relationship between morphometric variation and host-related genetic differentiation [8 - 10]. According to Bickford *et al.* [11] genetic studies have exposed an increasing some of cryptic parasite lineages that are genetically different yet, but morphologically are indistinguishable. The adaptation of parasites on various hosts is an important process guiding the evolution of such cryptic variety [12]. There were morphological alterations such as tick sizes can differ significantly from population to population for parasite adaptation [13,14].

The level of host-parasite interaction determines the severity of the response that the tick evokes in its host. The immune response of the host is less severe when a tick species has well-conventional host-parasite relationship compared to a non-preferred host [15], where the signs of the host immunological response on the ticks were reduced engorgement weight, longer feeding intervals, lower egg production, decreased hatchability and survival of larvae beside molting inhibition [16]. The xerophilic species of ticks are more exposed to the pressures of continually altering climatic conditions that can directly affect their life cycle because they spend most of their time in the environment [17]. *Rhipicephalus sanguineus* is three-host tick and has four developmental stages (egg, larva, nymph, and adult and its life cycle might vary in length from nation to nation and region to region. The feeding periods of *R. sanguineus* are influenced by abiotic (e.g., ambient temperature and pre-feeding light cycles) and biotic (e.g., host species) factors [17, 18] so, the purpose of the present study was to record the biological variation of *R. sanguineus* in winter and summer as well as comparing the external morphology of all stages of the tick *R. sanguineus* reared on two different hosts (dogs and rabbits).

Material and Methods

Ethical approval

The Faculty of Veterinary Medicine at Cairo University, Egypt, undertook rabbit handling procedures in compliance with the regulations established by the Institutional Animal Care and Use Committee (IACUC) Cairo University.

Ticks sampling

Rhipicephalus sanguineus males and fully engorged females were collected from infested canines at a private clinic in Giza, Egypt. Ticks were gathered in a unique glass tube that held filter paper strips. The morphological identification of ticks was determined using Walker key [19].

Tick colony

The study was performed at the Faculty of Veterinary Medicine Cairo University in the Parasitology laboratory in the winter (Jan and Feb) and summer (May and June). Each full-fed female was collected in a glass tube and kept in an incubator at 25 ± 1 °C and 75–80% RH to provide optimum conditions for oviposition. The hatched larvae were reared on rabbits for feeding using the back capsule technique **[20, 21]**. The detached larvae were collected after feeding and kept in the incubator as the same previous conditions till molting to unfed nymphs, and completed the cycle of feeding on rabbits until obtained full-fed adults.

Morphometric identification

All stages of ticks (eggs, unfed-larvae, fed nymphs, unfed and fed adults) after collection from two hosts (dogs and rabbits) were examined using a dissecting stereo-microscope (Olympus Japan SZ40) and Photographed by a digital camera (Canon 12 megapixel). The morphological differences between the stages of ticks reared on the two different hosts were performed. The characteristic features were measured in unfed adult males: basis capituli (width); idiosoma (length from scapular apices to posterior idiosomal margin and width); spiracular plates (width); All measurements of morphological features are expressed as millimeter (mean ± standard deviation).

Seasonal tick Biology

There were three groups of fully - engorged females (six females in each group) reared and followed in winter and summer. In the first group, the engorged females were the engorged females were weighed after detachment and each one placed in a separate glass tube to lay eggs. The laid eggs were weighed and counted daily as well as preoviposition, oviposition, egg incubation period and longevity of hatched larvae were recorded. In the second group, the egg mass of each engorged female was weighed and counted to determine the hatching percentage. In, the third group, two hundred eggs were weighed from each female and incubated till hatching. The hatched larvae were fed on rabbits (capsule technique) to record their feeding period, percent of fed larvae, incubation period after engorgement and, molting percentage. The molted nymphs were fed on other rabbits to monitor the

same parameters recorded in the larval stage. The adults molted from engorged nymphs were followed to record the same parameters recorded in both larva and nymphal stages, in addition to its reproductive index. Furthermore, the longevity of unfed larvae, nymphs and adults, was calculated [22].

Statistical analyses

Results were statistically analyzed using the SPSS program version 20 by student's *t*-test at $P<0.05$.

Results

Seasonal variation

There were differences in the biological parameters of *R. sanguineus* tick which estimated in the winter and summer seasons. The biological parameters of adults including longevity of unfed adults and feeding period of females showed significant difference ($P<0.05$) in winter versus the summer (65.43±1.93 days and 9.74±0.28 days) versus (25.71±0.89 days and 7.05±0.18 days), respectively. Also, engorged females of *R. sanguineus* showed significance ($P<0.05$) in the pre-oviposition and incubation period of eggs until hatching during the winter (5.53±0.12 day, 17.74±0.35 day) and summer (3.47±0.12 day, 15.95±0.32 day), respectively, despite no significant difference (P>0.05) between the oviposition period (10.33±1.20 day, 9.67±0.33 day) and hatching percentage (93.47±2.87, 97.31±0.92) in winter and summer, respectively. Furthermore, a significant difference ($P<0.05$) was recorded between egg weight, number and reproductive index in winter (0.12±0.01, 2546.67±249.83, 0.513±0.01) and summer (0.15±0.01, 3082±249.84, 0.585±0.02), respectively (TABLE 1).

The biological parameters of *R. sanguineus* larvae: Longevity of unfed larvae (12.89±0.19 day), the larval feeding period (5.39±0.28 day), larval feeding percentage (49.50±5.41%) and molting period of fed larvae (7.88±0.21day) in winter showed significant difference ($P<0.05$) with the biological parameters of larvae in summer (11.16±0.23 day, 2.89±0.196 day, 76.78±6.80% and 6.24±0.11 day), respectively (TABLE 2).

The biological parameters of nymph (longevity of unfed nymphs, feeding period of nymphs, molting period of fed nymph showed significant difference (P<0.05) in winter versus the summer (14.53±0.33 days, 5.00±0.31 days, 14.29±0.29 days) versus (8.53±0.55 days, 3.43±0.20 days, 12.86±0.14 days), respectively, but no significance (P>0.05) between Molting nymphs percentage (65.72±6.16%, 79.71±2.30%) and feeding nymphs percentages (54.03±0.29%, 74.60±5.87%) in winter and summer, respectively (TABLE 3).

From the comparison of the biological parameters in the winter and summer, we recorded the duration of life cycle in the two seasons. The total life cycle of *R. sanguineus* fed on rabbit in winter was 75.9±4.58 days and 61.56±4.79 days in summer (Fig. 1). The laboratory conditions were constant in winter and summer (temperature of 25 ± 1 °C, a relative humidity of 75–80%) except the feeding period were under environmental condition.

Morphometric comparison

The stages of *R. sanguineus* ticks fed on two different hosts (dogs and rabbits) were noted that the unfed adult male tick fed on dog was larger than unfed adult male tick fed on rabbits in where the length was (2.87±0.12 mm vs 2.57±0.19mm), and width (1.51±0.05mm vs 1.37±0.07mm) of whole-body males' tick, and the basis capituli width (0.59±0.02mm vs 0.52±0.05mm), respectively with no significance ($P>0.05$). The shape of spiracular plate in unfed adult males of dog colony (wide comma shaped), where the dorsal tail appears longer than those of rabbit colony (comma shaped) and the broad area of spiracular plate was wider in ticks fed on rabbits than ticks fed on dogs. The postero-lateral and postero-median grooves are deeper in fed adult males reared on dog than those reared on rabbits (Fig. 2 and TABLE 4). The engorged males fed on dogs were larger than those fed on rabbits. The length and width of fed males on dogs were (3.45±0.18 mm, 1.83±0.08mm) and fed males on rabbits were (2.99±0.32 mm, 1.54±0.07mm), respectively (Fig. 2) with significance only on width ($P<0.05$). The adanal plate of fed adult males was nearly cleaver shaped in those reared-on dogs while nearly triangular in rabbit colony. Also, caudal protrusion appears trapezoid in dog colony while it is round in rabbit bred. Also, shape of eggs laid by females reared on dogs was mostly spherical while it was mostly oval on rabbits with no significance in length and width (Fig. 3 and 4). The length and width of larvae dog colony were (0.50±0.01mm, 0.42±0.01mm) but those of rabbit colony were (0.49±0.03 mm, 0.41±0.01mm), respectively with significant difference in length (Fig. 4). Also, comparing the length and width of nymph fed on dogs (2.85±0.04 mm, 1.89±0.08mm) noted that they larger than the nymph fed on rabbit (2.59±0.17 mm, 1.78±0.07 mm) respectively, with no significant difference (Fig. 5 & Table 5).

TABLE 1. Biological parameters of adult females and eggs of *R. sanguineus* (Mean ± SE).

Biological parameters	Winter	Summer	t	df	Sig.
Longevity of unfed adults (day)	65.43±1.93*	25.71±0.89	16.56	6	<0.001
Feeding period of females (day)	9.74±0.28*	7.05±0.18	17.44	18	<0.001
Female weight (g)	0.24±0.02	0.25±0.01	-1.63	5	0.165
Preoviposition period (day)	5.53±0.12*	3.47±0.12	12.69	18	<0.001
Oviposition period (day)	10.33±1.20	9.67±0.33	0.46	2	0.691
Incubation period (day)	17.74±0.35*	15.95±0.32	4.16	18	0.001
Egg weight (g)	0.12±0.01*	0.15±0.01	-7.92	5	0.001
Egg numbers (N)	2546.67±249.83*	3082±249.84	-8.04	5	<0.001
Hatching percentage (%)	93.47±2.87	97.31±0.92	-1.06	4	0.351
Reproductive index (RI)	0.51±0.01*	0.59±0.02	-8.79	4	0.001

SE= Standard error, * Different superscripts of biological parameter of ticks between winter and summer season indicates significant difference at $P< 0.05$.

TABLE 2. Biological parameters of *R. sanguineus* larvae (Mean ± SE).

Biological Parameters	Winter	Summer	t	df	Sig.
Longevity of unfed larvae	12.89±0.19*	11.16±0.23	6.89	18	<0.001
Larval Feeding period	5.39±0.28*	2.89±0.196	13.49	17	<0.001
Feeding larvae percentage (%)	49.50±5.41*	76.78±6.80	-2.74	8	0.026
Incubation period of fed larvae	7.88±0.21*	6.24±0.11	7.29	16	<0.001

SE= Standard error, * Different superscripts of biological parameter of larvae between winter and summer season indicates significant difference at $P< 0.05$.

TABLE 3. The biological parameters of *R. sanguineus* nymphs (Mean ± SE).

Biological parameters	Winter	Summer	t	df	Sig.
Molting nymphs percentage	65.72±6.16	79.71±2.30	-1.78	8	0.112
Longevity of unfed nymphs	14.53*±0.33	8.53±0.55	11.50	16	<0.00
Feeding period of nymphs	5.00*±0.31	3.43±0.20	4.26	6	0.005
Feeding nymphs percentages	54.03±0.29	74.60±5.87	-3.39	2	0.077
Incubation period of engorged nymphs	14.29*±0.29	12.86±0.14	7.071	6	<0.001

SE= Standard error, * Different superscripts of biological parameter of nymph between winter and summer season indicates significant difference at $P< 0.05$.

TABLE 4. The measurements of unfed males of *R. sanguineus* (Mean ± SE) reared on different hosts.

Morphological Features	Unfed adult male ticks reared on		t	df	Sig.
	Dog	Rabbit			
Length (mm)	2.87±0.12	2.57±0.19	1.37	3.37	0.255
Width (mm)	1.51±0.05	1.37±0.07	1.65	3.56	0.17
Basis capitula width (mm)	0.59±0.01	0.52±0.03	2.359	2.69	0.078
Spiracular plate (mm)	0.20±0.0*	0.19±0.01	1	2	0.016

SE= Standard error, * Different superscripts of **measurements** between **dog and rabbit** indicates significant difference at $P< 0.05$.

TABLE 5. The measurements of different stages of *R. sanguineus* (Mean ± SE) fed on different hosts.

Tick stages	Host	Length (mm)				Width (mm)			
		(Mean±SE)	t	df	Sig.	(Mean±SE)	t	df	Sig.
Engorged males	Dog	3.45±0.18	1.25	3.21	0.27	1.83±0.08*	2.84	3.97	0.047
	Rabbit	2.99±0.32				1.54±0.07			
Eggs	Dog	0.44±0.02	1.0	2.78	0.11	0.41±0.01	2.12	4	0.1
	Rabbit	0.42±0.01				0.38±0.01			
larvae	Dog	0.50±0.01*	0.30	2.09	0.029	0.42±0.01	0.17	3.95	0.81
	Rabbit	0.49±0.03				0.41±0.01			
Engorged nymphs	Dog	2.85±0.04*	1.46	2.27	0.045	1.89±0.08	1.04	3.95	0.35
	Rabbit	2.59±0.17				1.78±0.07			

SE= Standard error. * Different superscripts of **measurements** between **dog and rabbit** indicates significant difference at $P< 0.05$.

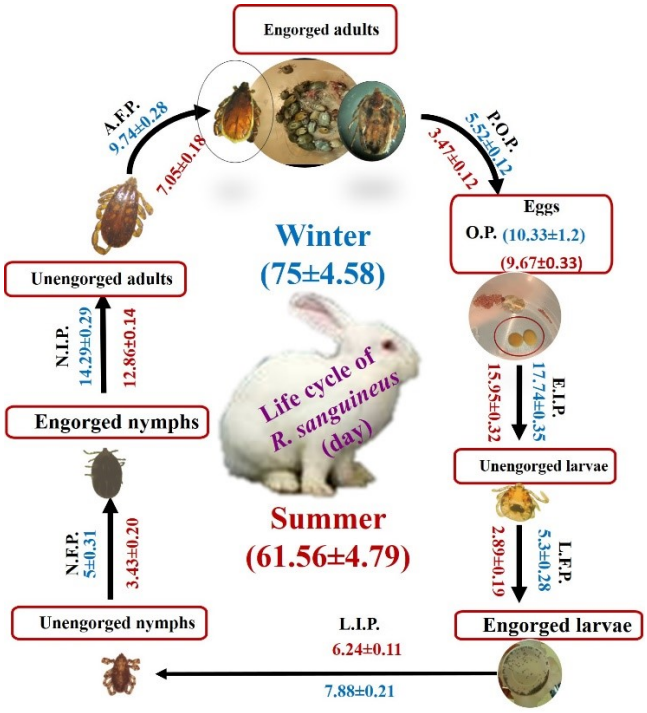

Fig. 1. Seasonal variation of life cycle of *R. sanguineus* fed on rabbit in summer and winter.
POP: Preoviposition period, **OP**: Oviposition period, **EIP**: Egg incubation period, **LFP**: Larval feeding period, **LIP:** Larval incubation period, **NFP:** Nymph feeding period, **NIP**: Nymph incubation period, **AFP**: Adult feeding period.
Blue values left of the arrow direction were calculated in winter while red values right of the arrow direction were calculated in summer.

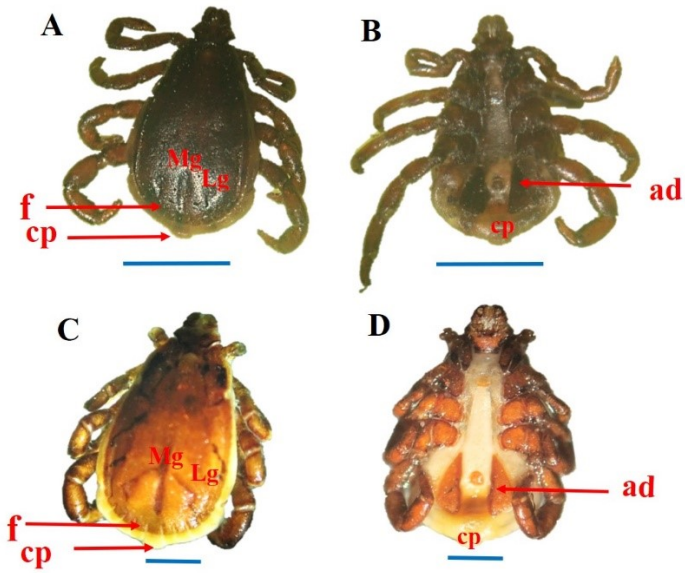

Fig. 2. Adult male of *R. sanguineus* fed on dog, A: dorsal view, **B**: ventral view, Scale bar = 1 mm, adult male of *R. sanguineus* fed on rabbit: **C:** dorsal view, **D**: ventral view. f (festoons); cp (caudal protrusion), ad (adanal plate), Scale bar = 0.5 mm.

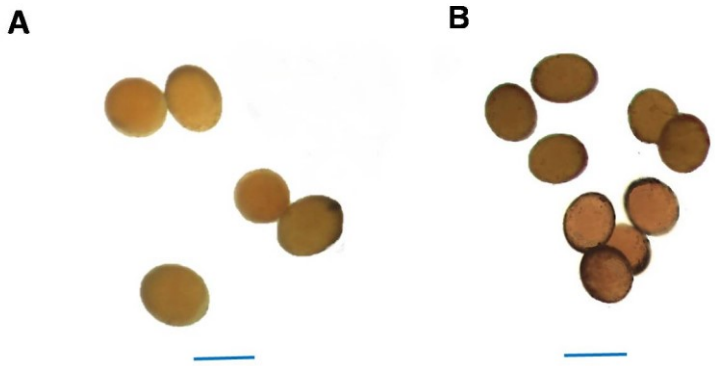

Fig.3. Eggs of *R. sanguineus*, A: Eggs laid from female ticks fed on dog, **B:** Eggs laid from female ticks fed on rabbit, Scale bar = 0.5 mm.

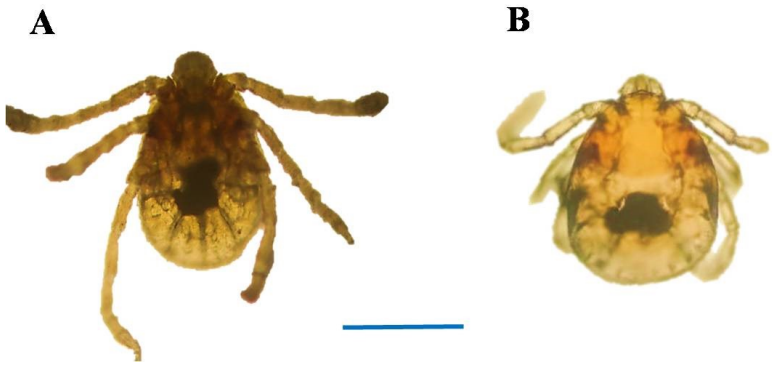

Fig. 4. Larva of *R. sanguineus*, A: larva fed on dog, **B**: larva fed on rabbit, Scale bar=0.25 mm.

Discussion

From the findings illustrated above, the ecosystem is the main factor affecting the biological parameters and morphological features of ticks so the present work showed that several variations were observed in reproductive and feeding parameters of *R. sanguineus* ticks between the winter and summer which agree with the finding of Rodrigues *et al.* [23] in Brazil, who reported that *R. sanguineus* infestations have a higher intensity during the rainy and hot seasons. Jacobs *et al.* [24] observed that the dogs were infested by the highest number of *R. sanguineus* during the warmer months (January to April) in South Africa.

Moreover, there was a significant difference between the duration of life cycle of *R. sanguineus* in winter and summer (p <0.05). These data are compatible with those obtained by many researchers [25- 27] who reported the duration of life cycle can be completed in 63–91 days in a favorable condition. In temperature of 27 C^{o}, Louly *et al.* [26] observed that, *R. sanguineus* stages fed on dog were developed their complete life cycle within 90 days, which came longer than in the present study. Furthermore, some studies [28- 32] showed the females *R. sanguineus* tick were fed on host for 5 to 21days with a mean temperature of 21-28°C and a mean relative humidity of 55-60%.

The preoviposition period of engorged females and incubation period of egg showed significant difference between winter and summer (P < 0.05). Furthermore, the eggs weight and numbers showed difference between winter and summer. These results agreed with many reports [28, 29, 32-35] as they recorded that, the pre-oviposition period ranged from 3 to 14 days and egg incubation period ranges from 6 to 23 days. Koch [33] noticed that, engorged females of *R. sanguineus* were laid 4,000 - 7,273 eggs. On the other hand, the oviposition period and hatching percent showed no significant difference between the winter and summer seasons, although some investigators [29, 33] recorded the mean duration of oviposition period to be 16–18 days.

In addition to many authors [28, 29, 31, 32] recorded that, the larvae feeding and molting periods ranged from (3 to 10 days) and (5 to 15 days), respectively. Moreover, the nymph feeding and molting periods ranged from 3 to 11 days and 9 to 47 days, respectively, that was compatible with the present results. Under constant laboratory conditions, the larvae, nymphs feeding and molting periods were prolonged in winter than in the summer. Also, the longevity of unfed larvae, nymphs and adults extended in winter than in summer, which inappropriate with Goddard [25] who found that the survival of unfed *R. sanguineus* larvae was approximately 8 months, while the survival of unfed nymphs and the adults can reach to 6 and 19 months, respectively.

The variation of feeding parameter of different stages of *R. sanguineus* between the winter and summer may be resulted from variation of ecological parameters such as temperature and humidity in the two seasons. Szabó *et al*.[36] observed that the biological parameters of different stages of *R. sanguineus* were varied even under constant temperature and humidity which these data also, compatible with our results. The engorgement and molting periods may be varied between populations that were influenced directly by factors such host availability and temperature [37]. Furthermore, attenuation of metabolic rate at low temperature may be led to increase the durations of preoviposition, oviposition, egg hatching and molting of *Haemaphysalis longicornis* under field condition [38, 39].

In the morphological comparison of *R. sanguineus* among two different hosts, the length and width of unfed adult males of dogs and rabbit's colony of the present study agreed with Dantas-Torres [17] in which adult males' length and width of *R. sanguineus* ranged from (2.28–3.18 mm long X 1.11–1.68 mm wide) but the width of basis capituli of them disagreed with Coimbra-Dores *et al.* [40]

that recorded the width of basis capituli is (0.75 ± 0.06 mm).

On the other hand, the width of broad part of spiracular plate is nearly equal in male tick of rabbit's colony and dog's colony where Sanches *et al.* [41] recorded that the spiracular plate width of *R. sanguineus* isolated from dogs in Brazil is 0.25 (0.22–0.31). In contrast the dorsal tail of spiracular plate is longer in adult male ticks reared on dogs than these reared on rabbits.

Eggs of *R. sanguineus* collected from dogs are spherical, small and dark brown in color, the hatched larvae are small (length, 0.54 mm; width, 0.39 mm) [17], while the present study observed that the eggs are mostly sphere in tick colony reared on dogs but mostly oval in tick colony reared on rabbits also, the length and width of larvae of tick colony reared on dogs and rabbits are incompatible with Dantas-Torres [17]. These measurements of different stages of ticks may be widely varied due to change in locality, season and type of hosts which similar to findings of De Oliveira *et al.* [14] who reported that, population-to-population *R. sanguineus* ticks' size may be differed widely.

Conclusion

Reproductive parameters of *R. sanguineus* ticks in two seasons were varied under the constant condition. In addition to, feeding of different tick stages on different hosts such as dog and rabbit led to morphometric variation in these stages of ticks. Finally, we recommend these seasonal and morphological variations need further genetic studies.

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التباين الموسمي والتمايز المورفومتري لسلالة ريبيسيفلس سانجوينس المصرية (اكاراي:إكزودودي)

ايمان احمد ابوطالب[1] ، مى ابو وردة[1] ، صبحى عبدالشافى[2] ، نسرين عزالدين[1] و مجدى مصطفى فهمى[1]

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يعتبر قراد الكلب البني (ريبيسيفلس سانجوينس) الأكثر شيوعًا في العالم وحامل للعديد من حالات العدوى لكل من البشر والكلاب، حاليًا الوضع التصنيفى لهذا القراد موضوعاً للنقاش. كان الهدف من هذه الدراسة هو تقييم الاختلافات في المعايير البيولوجية بين فصلي الشتاء والصيف والفرق المورفولوجي لقراد ريبيسيفلس سانجوينس المربى على عائلين مختلفين (الكلاب والأرانب). هناك فروق معنوية في معظم النتائج المتعلقة بالمتغيرات البيولوجية بين المراحل المختلفة للقراد التي تم تسجيلها في الشتاء والصيف، حيث وجد أن فترة ما قبل وضع البيض و فترة وضع البيض وفترة حضانة البيض وفترة الفقس وطول عمر اليرقات والحوريات و الأطوار البالغة الغير متغذية هذا بالاضافة الى فترات تغذية اليرقات والحوريات والأطوار البالغة كانت في الشتاء أطول من الصيف. استغرقت دورة الحياة الإجمالية للقراد في الشتاء وقتًا أطول (75 ± 4.58 يومًا) مقارنة بالصيف (61.56 ± 4.79 يومًا). هناك بعض الاختلافات المورفولوجية المسجلة للأطوار غير المتغذية والمتغذية لـ ريبيسيفلس سانجوينس التي تمت تربيتها على عائلين مختلفين (الكلاب و الأرانب)، مثل الصفيحة التنفسية للذكور ذات ذيل أطول في القراد المربي على الكلاب مقارنة بتلك التى تربى على الأرانب. كما أن البيض يتميز فى الغالب بالشكل الكروى في القراد الذي يتغذى على الكلاب ولكنه بيضاوي قليلاً في القراد الذي يتغذى على الأرانب. يستنتج من هذه الدراسة أن هناك اختلافات في المعايير البيولوجية لقراد ريبيسيفلس سانجوينس بسبب اختلاف الفصول و أن هناك تنوع في السمات المورفومترية لهذا القراد مع تغير العائل.

الكلمات الدالة: ريبيسيفلس سانجوينس, الكلاب، الأرانب، الشكل، الصيف، الشتاء.