Is the cattle tick *Rhipicephalus annulatus* Say, 1821 reared on the rabbit?

Sobhy Abdel-Shafy¹

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Abstract The cattle tick *Rhipicephalus annulatus* Say, 1821 (Acari: Ixodidae) is the main tick species on cattle in Egypt. This study was designed to know the possibility of rearing *R. annulatus* on rabbits in order to obtaining an adequate tick number and maintaining this tick species in lab to the next generation. Additionally, a comparison was performed between some biological parameters in *R. annulatus* fed on rabbits with that fed on cows. Six New Zealand white rabbits were used as a lab animal for rearing *R. annulatus*. The animals were divided into two groups. The first (G1) included four rabbits and the second (G2) included two rabbits. In G1, larvae fed until to reach unfed adults those were detached, cleaned their mouth parts carefully and re-fed on another rabbit. In G2, larvae were maintained on rabbits until to the fully fed females dropped. Oviposition, hatchability and life cycle of *R. annulatus* fed on rabbits were recorded and compared with those fed on cows. Results showed that although the cattle tick *R. annulatus* is highly specific to cattle, it is possible reared on rabbit in limited scale. The larvae well developed on the same rabbit to nymphs and adults. In G2, a very few adults completed their feeding and laid very small egg mass, some of eggs failed to hatch and a few recorded hatchability percentage not more than 9.1% in comparing 98.3% in females fed on cow. In G1, the rearing technique led to slightly increase the egg mass and their hatchability that reached to 23.6% in comparing with the hatchability recorded in G2.

Keywords Biology · Egg mass · Hatchability · Ixodidae · Life cycle · Oviposition

Introduction

Rhipicephalus annulatus Say, 1821 (Acari: Ixodidae) infests mainly cattle. Some domestic animals such as horses, sheep goats can be infested by this tick species (Hoogstraal et al. 1981). It feeds as one-host tick. It takes 3 weeks to 2 months on cattle according to the environmental conditions. In the Mediterranean region the tick's activity starts in late summer with a peak in autumn. It was commonly recorded in North Africa, in addition to other countries as Portugal, Spain, Italy, Greece, Turkey, Saudia Arabia, Mexico, USA, Benin, Sudan and Iran. It causes direct damage to animals especially in case of heavy infestation that lead to destroy hides and reduce the rate of growth (Estrada-Peña et al. 2004). It is the main vector of *Babesia bigemina* and *Babesia bovis* that cause bovine babesiosis in Egypt (Adham et al. 2009). It also transmits bacterial diseases as *Borrelia theileri* and *Anaplasma marginale* (Trees 1978; Scoles et al. 2007) and viral diseases as Congo haemorrhagic fever virus and lumpy skin disease virus (Gunes et al. 2011; Rouby et al. 2017).

Tick colonization is an important procedure in the lab to obtain the developmental stages that used in the laboratory experiments. The developmental stages are either active or inactive phase. The active phase includes larva, nymph and adult in unfed or semifed statement, while inactive phase includes all these stages in engorgement statement in addition to the egg stage. The rabbit is a lab animal that used in establishing tick colonies in our lab. For example, the two host-ticks as *Hyalomma excavatum*, *Hyalomma*

✉ Sobhy Abdel-Shafy
aasobhy@yahoo.com

¹ Department of Parasitology and Animal Diseases, Veterinary Research Division, National Research Centre, Dokki, Giza, Egypt

dromedarii and *Hyalomma marginatum* and three-host ticks as *Rhipicephalus sanguineus* could be easily reared on rabbits to obtain an active phase of immature stages for scanning electron microscopy (Abdel-Shafy 2008; Abdel-Shafy et al. 2015). By using rabbit as a lab animal, these tick species could be maintained to the next generations in the lab to obtain a reasonable number that might be used in other experiments. However, the larvae and nymphs of one-host tick *R. annulatus* fed on rabbit until reached to unfed adults but the most of adults did not complete to engorgement females at the same animal (Abdel-Shafy et al. 2013). This means that *R. annulatus* failed to complete its life cycle to the next generation on the rabbit.

Therefore, this study was designed to know the possibility of rearing *R. annulatus* on rabbits in order to obtaining an adequate tick number and maintaining this tick species in lab to the next generation. Additionally, a comparison was performed between some biological parameters in *R. annulatus* fed on rabbits with that fed on cows.

Materials and methods

Tick collection

The cattle tick *Rhipicephalus* (formerly *Boophilus*) *annulatus* Say, 1821 (Acari: Ixodidae) was collected from a private cattle farm at Giza, Egypt. The fully fed females were picked from ground of the cattle pens by a forceps and put in plastic tubes. These tubes were covered by a piece of clothes and secured by rubber band. The females were brought alive to the lab for the identification and the experiments. The identification was achieved by dissecting microscope according to the key of Hoogstraal (1956) and Estrada-Peña et al. (2004). The engorged females were incubated at 27 °C and 75% relative humidity to obtain eggs and larvae.

Experimental design

Six New Zealand white rabbits weighing 2.5 kg were used as a lab animal for rearing *R. annulatus*. The animals were divided into two groups, the first (G1) included four rabbits and the second (G2) included two rabbits. Larvae were introduced to all animals for feeding according the method described before by El-Kammah (1969). In G1, larvae fed until to reach unfed adults those were detached, cleaned their mouth parts carefully and re-fed on another rabbits. In G2, larvae were maintained on rabbits until to the fully fed females dropped.

Oviposition

Out of total 75 *R. annulatus* females, 25 fed on cows in the field, 25 fed on rabbits in G1 and 25 fed on rabbits in G2 were used to determine the female weight and total egg number per female. The daily egg number per female were counted and tabulated in females those fed on cows and rabbits in G2.

Hatchability

A total of 39 *R. annulatus* females, 13 fed on cow, 13 fed on rabbit in G1 and 13 fed on rabbit in G2) were utilized to compare the hatchability of their eggs. Eggs and larvae per female were counted and then the percentage of hatchability was calculated.

Life cycle

The duration of the life cycle of the *R. annulatus* females those fed on rabbits in G1 was determined. Whereas, the duration of the life cycle of *R. annulatus* females those fed on cows was shown according to Jagannath et al. (1982). The determined durations before host-attachment were pre-oviposition, oviposition, post-oviposition, while, the determined durations during host-attachment were larva, nymph and adult periods.

Statistical analysis

One way ANOVA was used by using SPSS software program to compare the means of some biological parameters of *R. annulatus* fed on cows and rabbits.

Results

Oviposition

The mean weight of *R. annulatus* female fed on cow, rabbit in G1 and rabbit in G2 was 128.1 ± 26 , 62 ± 31 and 30 ± 12.3 mg, respectively. The mean total number of eggs per female fed on cow, rabbit in G1 and rabbit in G2 was 1358 ± 334 , 502 ± 399 and 129 ± 81 eggs, respectively. These data show that the rabbit host was significantly lower preferable than cow (Table 1). Daily egg number per female fed on either cow or rabbit in G2 was shown in Table 2. On the first day, the mean number of egg per female fed on cow and rabbit was 264 ± 174 and 35 ± 18 eggs, respectively. On the second day, the mean number of eggs per female increased to be 418 ± 69 and 77 ± 61 eggs for cow and rabbit, respectively. On the third day, the number of eggs per female decreased gradually

Table 1 The female body weight and total egg number per female of *Rhipicephalus annulatus* fed on cows and rabbits

Host	Weight of female (mg)		Total egg number	
	Range	Mean $\pm$ SE	Range	Mean $\pm$ SE
Cow	83.1–207.4	128.1 $\pm$ 26 a	838–2298	1358 $\pm$ 334 a
Rabbit (G1)	15.9–137.5	62 $\pm$ 31 b	22–1624	502 $\pm$ 399 b
Rabbit (G2)	13–49	30 $\pm$ 12.3 c	19–280	129 $\pm$ 81 c
P value		< 0.001		< 0.001

Rabbit (G1) Larvae and nymphs of *R. annulatus* fed on rabbit while their adults fed on another rabbit; *Rabbit (G2)* All stages of *R. annulatus* fed on one rabbit

Table 2 Daily egg number per female of *R. annulatus* fed on cow and rabbit

Ovipositing (day)	Cow		Rabbit (G2)	
	Range	Mean $\pm$ SE	Range	Mean $\pm$ SE
1	26–528	264 $\pm$ 174	15–55	35 $\pm$ 18
2	280–551	418 $\pm$ 69	31–172	77 $\pm$ 61
3	150–478	298 $\pm$ 82	10–40	25 $\pm$ 13
4	54–365	227 $\pm$ 84	5–35	16 $\pm$ 13
5	48–292	117 $\pm$ 71	5–29	12 $\pm$ 10
6	18–200	57 $\pm$ 45	0–20	4 $\pm$ 9
7	6–95	26 $\pm$ 24	–	–
Total	582–2509	1407	66–351	169

Rabbit (G2) All stages of *R. annulatus* fed on one rabbit

until the end of oviposition in females fed on both cow and rabbit.

Hatchability

To calculate the hatchability percentage, the number of larvae and the number of eggs for *R. annulatus* females fed on cows, rabbits in G1 and rabbits in G2 were counted. The mean hatchability (%) was 94.8 ± 2.5 , 14.3 ± 6.1 and $2.6 \pm 2.4\%$ for females fed on cows, rabbits in G1 and rabbits in G2, respectively. This result indicated to the

hatchability (%) for females fed on rabbits is significantly lower than that fed on cow (Table 3).

Life cycle

The life cycle of *R. annulatus* was determined on rabbits in G1 (Table 4). The life cycle from egg to egg attained an average 70 days (59–82 days). The duration before and during attachment of the rabbit host has an average 41 days (33–50 days) and 29 days (26–32 days), respectively. The period of pre-oviposition, oviposition and post-

Table 3 The hatchability of eggs laid by *R. annulatus* fed on cow and rabbit

Host	Number of egg per female		Number of larvae per female		Hatchability (%)	
	Range	Mean $\pm$ SE	Range	Mean $\pm$ SE	Range	Mean $\pm$ SE
Cow	838–1824	1341.2 $\pm$ 283.7 a	783–1724	1272.2 $\pm$ 277.1 a	91–98.3	94.8 a
Rabbit (G1)	281–1194	824 $\pm$ 301.2 b	19–282	121 $\pm$ 73.3 b	5.9–23.6	14.3 b
Rabbit (G2)	19–280	131.5 $\pm$ 77.8 c	0–19	4.3 $\pm$ 5.3 c	0–9.1	2.6 $\pm$ 2.4 c
P value		< 0.001		< 0.001		< 0.001

Rabbit (G1) Larvae and nymphs of *R. annulatus* fed on rabbit while their adults fed on another rabbit; *Rabbit (G2)* All stages of *R. annulatus* fed on one rabbit

Table 4 The durations of the life cycle of *R. annulatus* fed on cow and rabbit

Biological parameter	Duration (day)			
	Cow ^a		Rabbit (G1)	
	Range	Mean	Range	Mean
Pre-oviposition	6–7	6.5	2–5	3
Oviposition	11–16	13.5	5–9	7
Post-oviposition	23–25	24	26–36	31
Before host-attachment	40–48	44	33–50	41
Larva	12–16	14	8–10	9
Nymph	9–13	11	8–10	9
Adult	10–13	11.5	10–12	11
During host-attachment	31–42	36.5	26–32	29
Total life cycle	71–90	80.5	59–82	70

Rabbit (G1) Larvae and nymphs of *R. annulatus* fed on rabbit while their adults fed on another rabbit

^aJagannath et al. (1982)

oviposition was 3, 7 and 31 days, respectively. The duration of larva, nymph and adult has an average 9, 9 and 11 days, respectively.

Discussion

The cattle tick *R. annulatus* is the main tick species on cattle in Egypt. It is one-host tick and highly specific to cattle. This led to difficult to establish and maintain a large colony on lab animal. The rabbit is cheap and easy to control it during the experiment in comparing with the large cattle. It is important to know the possibility of rearing this tick species on rabbit as a lab animal model. Some experiments require a small number from different developmental stages of *R. annulatus* and did not need to maintain a tick colony for a long time. Therefore the purpose of this study was to try to rearing *R. annulatus* on rabbit and compare the obtained biological data from rabbit with that from cow.

In this study, the attempt of rearing *R. annulatus* on rabbit showed that larvae and nymphs success to well develop to adults. The obtained adults from ticks feeding on rabbits took a long time to complete their feeding and a very few females laid small mass of eggs. This finding agrees with that recorded before by Ammah-Attoh (1966) who reared the tick *Rhipicephalus decoloratus* on Euro-bean rabbit. He found that the larvae and nymphs of *R. decoloratus* well developed on rabbit but females fed slowly for 5 days without becoming even partially engorged.

In the present study, the mean weight of *R. annulatus* female fed on cow, rabbit in G1 and rabbit in G2 was

128.1, 62 and 30 mg, respectively. This finding means that the weight of female fed on cow was 2.1 and 4.3 times more than that fed on rabbit in G1 and rabbit in G2, respectively. Meanwhile, Cooksey et al. (1989) found that the mean weight of *R. annulatus* female taken from deer was (243.5 mg) significantly less than of tick recovered from cattle (344.5 mg). The mean number of eggs per female fed on cow, rabbit G1 and rabbit G2 was 1358, 502 and 129 eggs, respectively. The female fed on cow laid eggs 2.7 and 10.5 times more than that fed on rabbit G1 and rabbit G2, respectively. Jagannath et al. (1982) found that the number of eggs per *R. annulatus* female was 1548–5837 eggs more than the obtained results in this study. This difference may attribute to the change in the environmental conditions. It was previously found that the females of *R. annulatus* had larger size and heavier weight in autumn season than another season (Abdel-Shafy 1994, 2000). Moreover, Davey (1988) stated that the number of eggs per female was 2700 at 25–27 °C. In the present study, the mean number of eggs per *R. annulatus* female was high on the first day of oviposition and reached to the apex on the second day recording 418 and 77 eggs for female fed on cow and rabbit, respectively and declined steadily until the end of oviposition for female fed on both cow and rabbit. The daily egg number per female fed on rabbit was greatly less than that fed on cow. These findings agree with that mentioned by Jagannath et al. (1982) who found that the peak of egg number for *R. annulatus* fed on cow recorded during 1–3 days after the start of oviposition, but the highest egg number per day was 990 eggs more than that recorded in this study. Moreover, Davey et al. (1980) recorded that an average daily oviposition peaked on the 4th day of egg laying 489 eggs/female/day. These

differences may attribute the changes in environmental conditions or strain of *R. annulatus* that differs from country to another. In the present study, the mean hatchability percentage was 94.8, 14.3 and 2.6% for *R. annulatus* female fed on cow, rabbit G1 and rabbit G2, respectively. This finding indicates to the hatchability % of eggs laid by female fed on cow was 6.6 and 36.5 times more than eggs laid by female fed on rabbit G1 and rabbit G2, respectively. In previous studies, the hatchability % of *R. annulatus* eggs was different from that recorded in this study. It was 80% at 20–35 °C (Davey et al. 1980). It was 63.1 and 76.7% for eggs laid by female fed on deer and cattle, respectively (Cooksey et al. 1989). This means that the rabbit and deer are lesser suitable for *R. annulatus* feeding than cow and rabbit is greatly lesser suitable than both deer and cow.

In this study, pre-oviposition period for *R. annulatus* female fed on rabbit was 3 days in agreement with the period recorded by Jagannath et al. (1982), Ouhelli et al. (1982) and Davey (1988). Oviposition period for female fed on rabbit was 7 days lesser than that for female (19.2 days) fed on cow that recorded by Davey et al. (1980). In the present study, the egg, larva, nymph and adult stage of *R. annulatus* on rabbit lasted 26–36, 8–10, 8–10 and 10–12 days, respectively, while Jagannath et al. (1982) stated that these stages lasted 23–25, 12–16, 9–13 and 10–13, respectively on cow. The life cycle from egg to egg for *R. annulatus* fed on rabbit attained 70 days (59–82 days) slightly lesser than that recorded on cow (71–90 days).

In conclusion, although the cattle tick *R. annulatus* is highly specific on cattle, it is possible reared on rabbit in limited scale. The larvae well developed on the same rabbit to nymphs and adults. A very few adults completed their feeding and laid very small egg mass, some of eggs failed to hatch and a few recorded hatchability percentage not more than 9.1%. A detachment procedure of unfed females that developed on a rabbit and carefully clean their mouth parts and were fed on another rabbit can increase the egg mass and their hatchability that reached up to 23.6%.

Author contributions This study was designed by the corresponding author (SA), who also collected the ticks, conducted the experiments, analyzed the obtained data and prepared the manuscript.

Compliance with ethical standards

Conflict of interest The author declares that they have no conflict of interest.

Ethical standard The study was conducted according to the ethical standards of the relevant national and institutional guides on the care and use of laboratory animals

Informed consent Informed consent was obtained from the owner of the animals included in the study.

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