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Research Article

Fecundity, Oviposition and Egg Incubation Period of Female *Rhipicephalus Sanguineus* Latreille (Acari: Ixodidae) Ticks in Indonesia

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Abstract

The brown dog tick, Rhipicephalus sanguineus (Latreille) is one of the most important ectoparasites of dogs and is often found in various countries in the world and is a blood-sucking ectoparasite and acts a vector of various diseases. This study aims to examine the fecundity and oviposition of engorged female brown dog ticks collected from Bogor, Indonesia, including the number of egg production, the pre-oviposition period, the oviposition period, the pre-hatching egg period, and the rate of eggs hatchability. Thirty engorged female ticks were collected from dogs then they were weighed, reared individually in plastic jars, and kept for 30 days in the laboratory at room temperature. The results showed that the average body weight of the engorged ticks collected from dogs was 68.3 mg (30-130 mg) and the number of eggs produced was 805.4 eggs/tick (135-1707 eggs). The eggs of R. sanguineus were oval, dark brown, shiny, measuring 0.5 mm in length and 0.4 mm in width, and the average egg mass weight was 32.68 mg/tick (5-70 mg). The egg hatchability was 53.95 % (25-82.3%) at a room temperature of 25-27 °C and a relative humidity of 80-90%. The average pre-oviposition period of this tick was 4.9 days (3-7 days), and the average oviposition period was a 14.3 days (9-19 days). The pre-hatching period, the period from the end of oviposition until the eggs begin to hatch into larvae was 6.9 days (1-17 days). Therefore, the egg incubation period of this tick is the oviposition period plus pre-hatching period: 14.3 + 6.9 days = 21.2 days (17-29 days). This is the first record of the fecundity of the brown dog tick of Indonesia.

INTRODUCTION

The brown dog tick, *Rhipicephalus sanguineus* Latreille is cosmopolitan, found throughout the world, and is a specialist on dogs in all stages. It can be occasionally found on wildlife. In Indonesia, this tick is the most common species among dogs [1,2]. This tick belongs to the hard tick group that establishes populations indoors, in a house or a kennel. The ticks will seek out cracks and crevices and retreat deep into the walls between feeding, and are very difficult to control once a population has become established. This species is known to be a vector of Babesiosis, Ehrlichiosis, Anaplasmosis, and Hepatozoonosis, particularly on dogs, and has been implicated in the transmission of Rocky Mountain spotted fever [3,4]. This tick is a parasite of dogs that can occasionally parasitize other hosts, including humans [5]. Moreover, *Rh. sanguineus* is a vector of many disease agents, some of them (for example *Coxiella burnetii, Ehrlichia*)

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- Indonesia

canis, Rickettsia conorii, and *Rickettsia rickettsii*) being of zoonotic concern [6].

R. sanguineus is small, red-brown in color, lacking any ornamentation on its scutum, has an elongated body shape and a hexagonal basis capituli. Infestation of this tick on dogs and in houses or kennels can explode to very high levels quickly. When only a few individuals of this tick are present, the dog owners are often completely unaware of them. They notice the tick problem when the ticks start crawling up the walls or curtains [2]. The parasitic phase occurs when ticks attach to a host and suck blood. These ticks require three blood meals to complete development; once as a larva, once as a nymph and once as an adult. A fully blood-fed female brown dog tick can lay up to 5000 eggs, and the number of eggs laid depends on the size of the tick and the amount of blood ingested [7-10].

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The feeding period of *Rh. sanguineus* can vary from two days (in the larvae) to several weeks (in females), depending on the tick's developmental stage and host. The feeding period of nymphs is longer than that of the larvae [11], and the engorgement of females may take longer on rabbits than on dogs [11,12]. The feeding period on each stage and the time required for development and molting are very dependent on temperature. At low temperatures (10 $^{\circ}$ C), the engorged larvae and nymphs may undergo diapauses and at higher temperatures the molting period is shorter [13]. After feeding, the larvae and nymph ticks drop off to the ground and molt before they are able to attach to another host. An engorged female tick drops off from the host then lays eggs and dies.

The biology and ecology of *R. sanguineus* in Indonesia have not been studied well, especially in relation to the fecundity of the female tick in Indonesia which include the number of eggs produced, the pre-oviposition period, the oviposition period, the pre-hatching egg period, the eggs hatchability rate and the egg incubation period. This research was aimed to study the fecundity and oviposition period of *Rhipicephalus sanguineus* collected from dogs in Bogor, Indonesia.

MATERIAL AND METHODS

Tick Collection

Engorged female ticks were collected from infested dogs in April 2010 in Animal Hospital, Bogor Agricultural University, Darmaga, Bogor, Indonesia. Then, they were reared individually at the Laboratory of Entomology, Department of Parasitology and Medical Entomology, Department of Animal Diseases and Veterinary Public Health, Faculty of Veterinary Medicine, Bogor Agricultural University. Indonesia.

Tick Rearing

Thirty engorged female ticks were collected from dogs then were weighed individually using digital scales. After that, each individual was put in a plastic jar (tube) with a perforated cover and coated with cotton fabric to prevent the hatching from crawling larvae out and placed in a plastic tray (Figure 1). Each individual tube with an engorged tick was labeled and then kept for 30 days in the laboratory at room temperature without any feeding to allow them to lay their eggs.

Fecundity Observations

The fecundity observations were done twice a day. In relation to the these studies, there were several observations including the measurement of the body weight of the engorged ticks collected and the weight of the eggs produced using an analytical scale. The number of eggs per tick and the egg hatchability rate were measured under a stereo microscope. The period of preoviposition, the period of egg-laying (the oviposition period) and the incubation period of eggs were calculated based on twice-aday daily observations. Finally, the results of the research were analyzed descriptively.

RESULTS

The number of eggs produced

The results showed that morphologically, all of the 30

individual engorged ticks collected from dogs belonged to one species, *Rhipicephalus sanguineus*. The body weight of collected engorged ticks was between 30 and 130 mg with an average of 68.33 mg/tick. In this research, all ticks (100%) were observed to lay their eggs in each individual tube (Figure 1) at laboratory room temperature. The average number of eggs produced by each tick was 805.4 eggs/tick (135-1707 eggs/tick). The eggs of *R. sanguineus* were oval, dark brown, shiny, 0.5 mm length and 0.4 mm width, and the average egg mass weight was 32.68 mg/ tick (5-70 mg/tick).

The egg hatching rate

The observations on the egg hatchability were conducted no later than 30 days starting from the time the first egg hatched. The eggs that failed to hatch after the 30-day observation period were considered non-fertile eggs. The result showed that the average hatchability of the tick eggs was 53.95 % (25-82.3%) (Table 1). This indicated a similar condition to what happens in nature; the hatchability is 50%.

Based on the data on Table 2, the engorged ticks that drop off to the ground need several days for egg maturation. The preoviposition (pre-laying) period of this tick varied between 3 and 7 days with an average of 4.9 days. The pre-oviposition period is the time required by the female engorged tick to fall from its host's body until the tick starts laying eggs.

The oviposition period is the time required by ticks from the moment they start laying eggs until they stop laying (time needed to lay eggs). Table 2 shows that *R. sanguineus'* mean oviposition period was 14.27 days (9-19 days).

The egg incubation period

There were two parts of the incubation periods, (1) the time required by ticks from the moment they start laying eggs until they stop laying (the oviposition period), and (2) the time form the moment they stop laying eggs until the eggs begin to hatch into larvae (pre-hatching period). Based on Table 1, the oviposition period of *R. sanguineus* was 14.27 days (9-19 days), and Table 2 showed the mean pre- hatching period of this tick was 6.9 days (1-17 days). When the data for oviposition period (14.27 days) and the pre-hatching period (6.9 days) were combined it was established that the egg incubation period of the tick was 21.16 days (17-29 days).



Figure 1 Individual tubes for tick rearing (left), female engorged *Rhipicephalus sanguineus*, collected from an infested dog (right).

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DISCUSSION

A number of studies on the biology and ecology of R. sanguineus have been carried out in many parts of the world; however, in Indonesia, this tick has not been studied well. R. sanguineus is the only species found in dogs in several urban areas in Indonesia such as Bogor [1], Jakarta [14], and Bandung [15]. In this study, the fecundity of R. sanguineus was studied and the results showed that all the engorged female detached from the hosts and successfully oviposited their eggs in the tubes in the laboratory. The engorged females with body weights varying between 30 and 130 mg (mean 68.3 mg) oviposited uninterruptedly with an average of 805 eggs/tick (135-1707 eggs). Because the weight of ticks collected in this research varied, it means there will be differences in the volume of blood that was ingested by the ticks. So, there was a variation in the number of eggs produced and the weight of the egg mass. The ticks used the blood for ovarian development and egg maturation. The female tick become bigger after sucking blood from the host and often reached four times the length of the original mature tick, and the body weight increased from 1mg to 450 mg [16]. The female ticks suck blood for 8-10 days and the body weight increases up to 100-fold [17].

The eggs of *R. sanguineus* were oval, dark brown, shiny, 0.5 mm in length and 0.4 mm in width, and the average egg mass weight of 32.68 mg/tick (5-70 mg/tick). The average hatchability of the tick egg was 53.95 % (25-82.3%) (Table 1). This indicated similar conditions to what happens in nature, that the normal expectation was 50% hatchability. Although the tick egg mass was the same, the eggs do not necessarily hatch into larvae at the same time. This is because it is influenced by both intrinsic and extrinsic factors. The intrinsic factors are the ticks body weight, the amount of blood ingested, the tick species and the number of eggs, while the extrinsic factors are temperature, humidity, rainfall and some disturbances during the process of egg-laying [18,19].

The pre-oviposition (pre-laying) period is the time required by the engorged female tick to fall from its host's body until the tick start laying eggs. This research showed that the preoviposition period of this tick varies between 3-7 days with an average of 4.9 days (Table 1). The female ticks begin laying their eggs about 3 days [18], or 5-9 days [20], after they are engorged and fall to the ground.

The oviposition period is the time required by ticks from the moment they start laying eggs until they stop laying (time needed for laying eggs). Table 2 shows that the mean oviposition period of *R. sanguineus* was 14.27 days (9-19 days). There was a relationship between the length of egg-laying and the number of eggs produced. The longer egg-laying period allowed the ticks to produce eggs in larger numbers. This period is associated with the weight of the tick itself. Ticks that have a heavy body weight will have a long oviposition period and this directly affects the number of egg produced. [18], reported this tick's egglaying period to be ranging between 13 and 15 days, and [20], reported it to be between 12-21 days. The oviposition period can last for several weeks and the number of eggs laid by each female is directly correlated with the weight and the length of the oviposition period [11].

Our study demonstrated that the engorged females oviposited their eggs for several days until the process ended and they died, and the egg did not immediately hatch; they needed to complete the post embryonic process. The incubation period itself is the length of time between the moment the tick starts laying its eggs and the moment the eggs hatch. Therefore, this period is calculated from the moment the first egg mass is laid by the tick until the eggs hatch. The incubation period can be divided into two major parts, i.e. (1) the moment the ticks start laying eggs until they stop laying (oviposition period), and (2) the moment the ticks stop laying eggs until the eggs hatch into larvae (pre-hatching period). Based on Table 2, it can be seen that the

Table 1: The number of Rhipicephalus sanguineus eggs based on body weight and the egg hatching rate.										
No	Class (mg)	Median	Σ of ticks	Σ of eggs produced	$\boldsymbol{\Sigma}$ of unhatched eggs	Σ of hatched eggs (%)				
1	10 - 30	30	4	886	664	222 (25)				
2	40 - 70	45	12	5382	2834	2548 (47.3)				
3	80 - 110	97	13	17587	6822	10765 (61.2)				
4	120 - 150	130	1	1677	297	1380 (82.3)				
Average	of egg hatchability (%	53,95								

Table 2: Pre-oviposition, Oviposition, Pre-hatching Periods of Rhipicephalus sanguineus based on the body weight

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No	Class (mg)	Median	Σ of ticks	Mean Pre-oviposition period (days)	Mean Oviposition period (days)	Mean Pre-hatching period (days)				
1	10 - 30	30	4	5.75	12.25	10.25				
				(4-7)	(10-16)	(10 – 16)				
2	40 - 70	45	12	5.08	13.25	8.33				
				(4-6)	(9-17)	(2-17)				
3	80 - 110	97	13	4.46	15.85	4.77				
				(3-7)	(!2-19)	(1-2)				
4	120 - 150	130	1	5	14	4				
Total average				4.9	14.3	6.9				

mean egg incubation period was 21.16 days (17-29 days). [18], stated that the incubation period of this tick in Florida was 14-35 days and in Hawaii 19-60 days [21], and in the laboratory [22], reported the mean incubation period for tick eggs was 19-72 days.

CONCLUSION

The fecundity study on the brown dog tick, Rhipicephalus sanguineus, of Bogor, Indonesia, revealed several facts; the average of body weight of the engorged tick collected from dog was 68.3 mg (30-130 mg) and the number of eggs produced was 805.4 eggs/tick (135-1707 eggs). The eggs of R. sanguineus were oval, dark brown, shiny, 0.5 mm in length and 0.4 mm in width, and the average egg mass weight was 32.68 mg/tick (5-70 mg). The egg hatchability rate was 53.95 % (25-82.3%) at a room temperature of 25-27 °C and a relative humidity of 80-90%. The average pre-oviposition period of this tick was 4.9 days (3-7 days), and the average oviposition period was 14.3 days (9-19 days). The pre-hatching period, which is the period from the moment oviposition stops until the eggs begin to hatch into larvae, was 6.9 days (1-17 days). Therefore, the egg incubation period of this tick is the oviposition period plus pre-hatching period 14.3 + 6.9 days = 21.2 days (17-29 days). This is the first record on fecundity of the brown dog tick in Indonesia.

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