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Tick (Acari: Ixodidae) infestation in cattle from Sleman, Yogyakarta Province, Indonesia

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Abstract. *Sahara A, Budianto BH, Kunda RM, Firdausy LW. 2023. Tick (Acari: Ixodidae) infestation in cattle from Sleman, Yogyakarta Province, Indonesia. Biodiversitas 24: 4087-4094.* Massive attacks of were found *Haemaphysalis bispinosa* in beef cattle farms in Sleman Regency, Yogyakarta, Indonesia. Besides it makes blood loss, they also have the potential to become vectors of several diseases. The aim of this research is to evaluate the prevalence of infestation, describe the morphology, fecundity, and timing of oviposition, as well as the preferred attachment sites for ticks. The samples were collected manually, and the body of the cattle was divided into four regions to determine the preferred sites of attachment of ticks. The morphology and occurrence of tick attachment sites, egg production, and hatchability were examined macroscopically and microscopically, and the obtained data we assessed descriptively and qualitatively. The findings indicated that, in contrast to the body area and the front and back legs, the tick predilection locations were the head, ears, and neck region. An engorged female may generate an average of 731.5±106 eggs throughout her 10-day oviposition cycle. After 17-30 days, tick eggs hatch into larvae; on average, 626.3±56 eggs per tick female successfully hatch into larvae.

Keywords: Fecundicity, *Haemaphysalis*, morphology, oviposition, tick

INTRODUCTION

Tick infestation is thought to directly and indirectly impact 80% of the world's cattle population, particularly those in tropical and subtropical regions (Eskezia and Desta 2016; Rehman et al. 2017; Burrow et al. 2019). Ticks and tick-borne pathogens are persistent and increasingly challenging global public health threats due to expanding geographic ranges, the emergence of previously unrecognized and complex dynamic interactions among abiotic and biotic factors that influence the tick-host-pathogen triad (Wikel 2018). Ticks infestation is a major worldwide issue due to their ability to infect various hosts with several pathogens, such as viruses (tick-borne encephalitis and Powassan Virus), bacteria (*Rickettsia*, *Anaplasma*, and *Borrelia*), and blood parasites (*Babesia*) (Rochlin and Toledo 2020). In addition to this, the tick bite may predispose to many secondary infections, virus, bacterial infections, screw-worm myiasis, and dermatophytosis at the bite injury. Some species of ticks can cause red meat allergy due to the presence of galactose-α-1,3-galactose (α-gal) in their saliva (Young et al. 2021).

The climate impacts tick dispersal and illnesses transmitted by ticks. Farming global temperatures will influence the geographic range and population expansion of ticks, which, in turn, influences distribution patterns and incidences of tick-borne infections (Dantas-Torres 2015). The livestock sector suffers considerable economic losses due to tick infestation, reduced live weight, reduced milk production, and even death in young animals (Rodriguez-Vivas 2018; Singh et al. 2022). In recent years, more intensive efforts have been made to survey the diversity of ticks, along with their host associations, ecologies, and zoonotic potential (Kwak 2022).

South Asia houses several tick species, 97 species have been reported in this region, with over half of them belonging to the genus *Haemaphysalis* (Petney et al. 2019). However, the correlation between the resulting diseases and socioeconomic effects has not been established. Some in-field studies have shown that tick species are often found in livestock, particularly cattle. *Haemaphysalis* is the second largest genus of the family Ixodidae, distributed in Australia, China, Indonesia, Japan, Malaysia, Nepal, New Zealand, Pakistan, Sri Lanka, Thailand, Myanmar, and Vietnam. Statistic data in 2020 revealed that there were a total of 17,526,204 cattle in Indonesia; approximately 43% of the cattle population was distributed in Java, 25% in Timor, and 32% in various others. *Rhipicephalu*s (*Boophilus*) *microplus* was the dominant tick found in various areas, especially outside Java, while *Haemaphysalis bispinosa* ticks were more commonly reported in Java (Sahara et al. 2019).

Hard ticks have different host-seeking behaviors and typically feed for extended periods, ranging from 3-12 days, depending on the species and tick stage (Sonenshine and Roe 2014). Prolonged contact between ticks and their hosts facilitates the transmission of pathogens, especially bacterial pathogens that generally require over 24 h to be transmitted efficiently (Eisen 2018). *Bartonella bovis* was detected from eight of 200 *H. bispinosa* ticks and none from the *R. microplus* ticks (Kho et al. 2015). The presence of *Anaplasma phagocytophilum* and *Rickettsia* spp. was confirmed in eggs and unfed larvae *H. bispinosa* (Hembram et al. 2022). Some tick-transmitted blood parasites have spread across regions in Indonesia (Guswanto et al. 2017).

Conventionally, tick systematics rely mostly on the description of morphological features in integration with their geographical distribution, biology, ecology, host associations, and behavioral criteria. Although tick infestation in cattle is common in almost all the regions in Indonesia, information on the characteristics of *H. bispinosa*, including the morphology, infestation preferences, fecundity, and hatchability, is still limited. Therefore, we tried to explore the biological characteristics, including the morphology of each life stage, infestation preference, fecundity, and hatchability.

MATERIALS AND METHODS

Tick collection

Tick retrieval is carried out on Ongole crossbreed cattle in three sub-districts in Sleman Regency, the Special Region of Yogyakarta, Indonesia (Figure 1). The Special Region of Yogyakarta Province consists of four regencies and one city. Geographically, Sleman Regency is located between 110° 33′ 00″ and 110° 13′ 00″ East Longitude, 7° 34′ 51″ and 7° 47′ 30″ South Latitude. Administratively, Sleman Regency consists of 17 subdistricts and 86 villages. The ticks were collected in several subdistricts, namely Berbah, Sayegan, and Ngaglik, due to reports of tick infestations. The level of the distribution of ticks on the surface of the cattle's bodies was measured, and the ticks were carefully removed from the host using tweezers or thumb forceps. The ticks were then stored in glass bottles comprising 70% ethanol and labeled with the location of collection, host signalmen, and date of sampling. The level of tick distribution in each cattle was measured to obtain the attachment site preference of the ticks by dividing the cattle body surface into four parts (Figure 2), namely the head and ears (region 1), the neck and wattle (region 2), the body (region 3), and the forelegs and hind legs (region 4). The obtained data were then presented in the form of a percentage.

To determine the number of eggs and hatched larvae, observations were performed on nine engorged female ticks from the field that were reared individually. They were placed in a tube filled with wet cotton to observe the process of egg laying and hatching into larva. Of them, five ticks were left to lay eggs, and the eggs collected were counted after putting in alcohol. The rest, four ticks (A, B, C, and D), were allowed to lay eggs and the eggs were left to hatch into larvae; the number of the larvae was then counted after being put in 70% alcohol.

Tick identification

All collected ticks were fixed in 70 % ethanol for later identification. The tick samples stored in 70% ethanol were rinsed with distilled water, then soaked in 10% KOH for 24 hours. The blood was taken from the ticks and cleaned with distilled water. The samples were dehydrated in serial ethanol, namely 30%, 50%, 70%, 80%, and absolute ethanol. The samples were then soaked in clove oil for 24 hours, followed by mounting the glass slide using Canada balsam. The nymphal and larval preparations were made by soaking in lactic acid for 48 hours and then dehydrating with serial ethanol (Famadas et al. 1996). The morphological examination was performed using a microscope (Olympus BX 51, Japan) and captured with Dp12 microscope digital camera (Olympus, Japan). The images were then measured at tick basis capitulum, scutum, and body using ImageJ. Tick identification was carried out with the keys of Anastos (1950), Hoogstraal and Trapido (1966), Geevarghese and Mishra (2011), and Tanskul et al. (1989). The morphological measurements at each life stage, starting from larvae, nymphs, and adult ticks, were performed by averaging the measurement results on the five individuals.

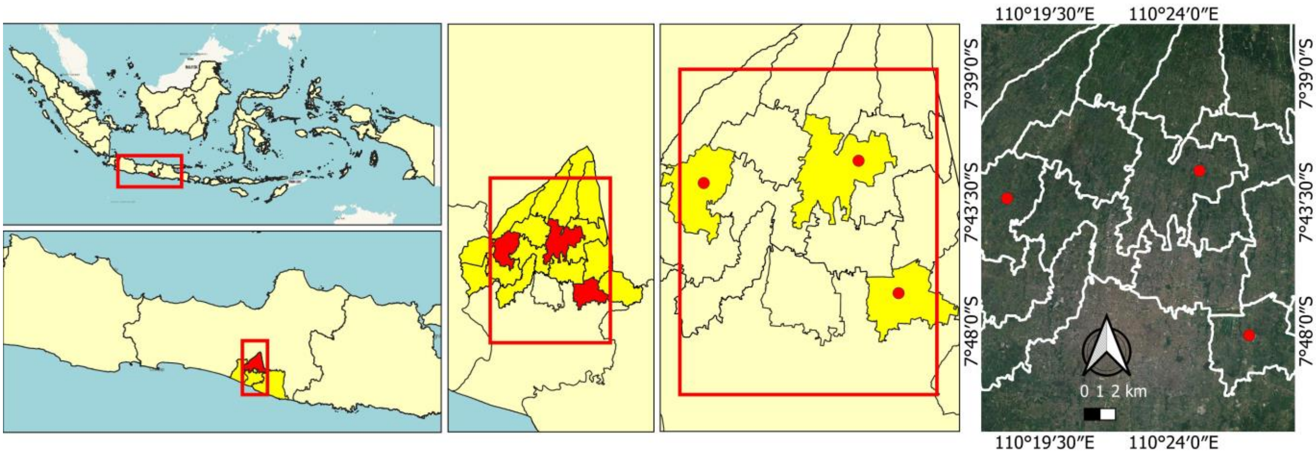

Figure 1. Location of tick collection in Sleman District, Yogyakarta Province, Indonesia. A. Seyegan, B. Ngaglik, C. Berbah

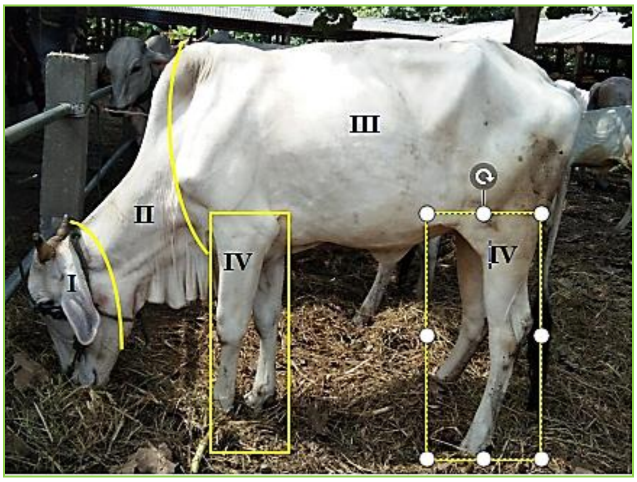

Figure 2. The division of the region of tick sampling on cattle

RESULTS AND DISCUSSION

Morphological characteristics of ticks

The cattle in this study were solely infested by *H. bispinosa*. In terms of its characteristics, the genus *Haemaphysalis* had an oval-shaped body, brown color, no ornate features, no eyes, and festoons; which provided a prominent dorsal spur on the posterior edge of pulp segment II for *H. bispinosa* species, as in Taylor et al. (2016) description. The length and width of the capitulum were almost the same, with a rectangular basis; the second segment of the palps was slightly wider with a short hypostome. The morphology of adult males, females, nymphs and larvae is presented in Figures 3 A-G. All the tick life stages found had a posterodorsal margin of palpal segment 3 with distinct spurs and a posteroventral margin of palpal segment 3 with broadly triangular spurs. The spurs on Coxa I were long and tapered, while the spurs on Coxae II-IV were short.

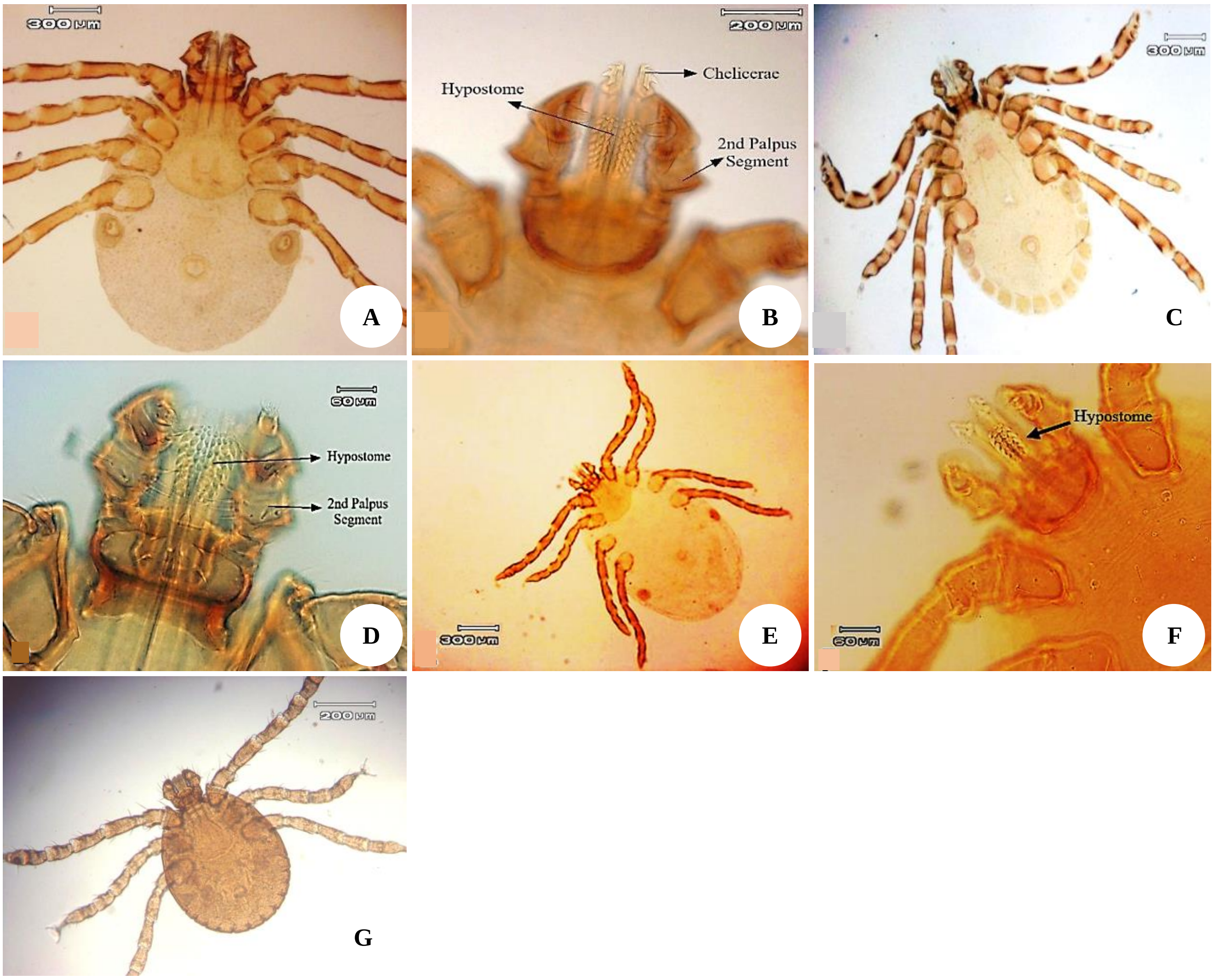

Figure 3. *Haemaphysalis bispinosa*: A. adult female; B. adult female gnatosome; C. adult male; D. adult male gnatosoma; E. nymph; F. nymphal gnatosoma section; G. larvae

The male of *H. bispinosa* had an average length of 1.96±0.21 mm and a width of 1.14±0.08 mm. The ticks had a short cervical groove and lateral grooves starting from the center of the idiosome to the posterior, evenly distributed and numerous punctations, the genital aperture parallel to coxa II, and the anal opening opposite to the oval-shaped spiracles. In addition, the ticks had long and thick legs as well as dentition (4/4) with 8 teeth per file.

The size of the female ticks had an average length of 2.33±0.17 mm, and width of 1.31±0.13 mm, while the length and width of engorged female ticks could reach 6.79 mm and 4.58 mm, respectively. The female ticks had an oval-shaped body, a capitulum of which the length and width were almost the same, a short hypostome, dentition 4/4 with 8 teeth per file, a deep cervical groove, evenly distributed and very numerous punctations, and a genital aperture located between the anterior border of coxa III, festoons of which the length and width were almost the same. However, the festoons in the engorged females were not visible because the bodies were enlarged.

The average length and width of the nymph body were 1.630.04 mm and 0.970.06 mm, respectively. The nymphs had an elongated capitulum, cornua not yet perfectly formed, dentition 2/2, a sub-pentagonal scutum on the posterior, and genitalia with an apertura. The average length of the larvae was 0.61±0.0007 mm, and the average width was 0.43±0.01 mm. The larvae had a round body, scutum on the anterior dorsal, no cornua, dentition 2/2, three pairs of legs, as well as short, blunt spurs on coxa I. The average body measurement of male adult, female adult, nymph, and larval ticks found in cattle in Sleman Regency, Yogyakarta, is presented in Table 1.

Infestation and attachment site preference

A total of 444 ticks were found on the body of the cattle from the two cattle farmer groups; 250 ticks were found on the cattle from the Berbah farmer group and the remaining 194 ticks were found on the cattle from the Seyegan farmer group (Table 2). There is a difference in the infestation percentage in that livestock farm.

Based on the results of the attachment site preference observations, the ticks preferred regions 1, 2, and 3, i.e., the ears, face, and abdomen. The percentage of the tick site preference is presented in Figure 4. There was one cattle with a severe infestation, in which there might be thousands of ticks on the body (the number of ticks on this beef cattle was not counted). Some ticks were found on the eyelids and face (Figure 5).

The observation results showed that the preoviposition period of the female ticks was seven days, the oviposition period was ten days, then they died two or four days later. The engorged female ticks could lay an average of 731.5 eggs (Table 3). On the seventh day after preoviposition, the number of eggs laid was higher than that on the following days. The eggs hatched into larvae on days 17-30. There was an average of 626.3±56 eggs that hatched into larvae.

Table 1. Average size of capitulum, scutum, and body of *Haemaphysalis bispinosa* at various life stages, size in (mm)

Stage	Capitulum		Scutum		Body	
	Length	**Witdh**	**Length**	**Witdh**	**Length**	**Witdh**
Adult male	0.39±0.07	0.37±0.06	-	-	1.96±0.21	1.14±0.08
Adult female	0.52±0.06	0.46±0.04	0.71±0.07	0.76±0.05	2.33±0.17	1.31±0.13
Nymph	0.23±0.01	0.21±0.01	0.36±0.01	0.41±0.02	1.63±0.04	0.97±0.06
Larva	0.125±0.007	0.134±0.008	0.27±0.02	0.20±0.01	0.61±0.0007	0.43±0.01

Table 2. Tick infestation in beef cattle in three sub-districts in Sleman, Yogyakarta

Cattle farmer groups	Number of cattle	Infestation in male cattle (%)	Infestation in female cattle (%)	Total average (%)
Andini Mulya, Tegaltirto, Berbah	132	13/44 (92.8%)	19/88 (21.5%)	24.2%
Mergo Andini Bolu, Margokarton, Seyegan	62	15/16 (93.7%)	46/46 (100%)	98.38%
Tanimakmur Ngetiran, Sariharjo, Ngaglik	46	4/4 (100%)	37/42 (88.09%)	89.1%

Table 3. Number of eggs laid by engorged female ticks from the field per day, counted on the day after the engorged tick was taken from the field

Day	07	08	09	10	12	16	
A	406	213	92	47	56	13	827
B	215	156	64	39	54	23	551
C	293	210	73	73	81	46	776
D	323	196	100	45	75	33	772
Average							731.5±106

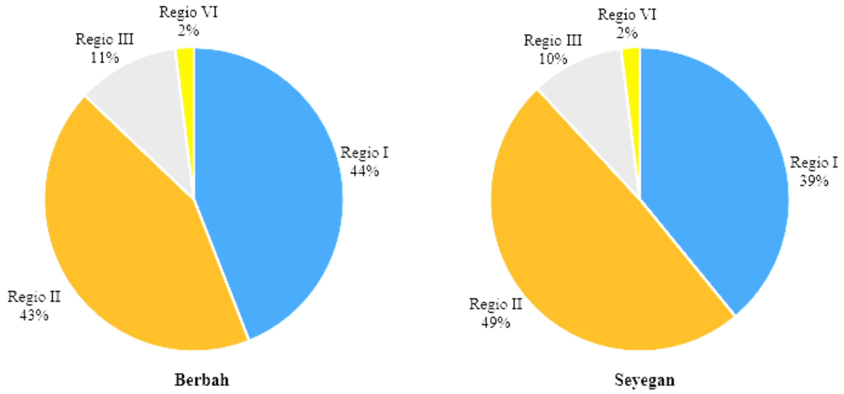

Figure 4. Percentage of tick attachment sites on host counted from the two cattle farmer groups in Sleman

Figure 5. Thousands of ticks were found on the face (small black dots in fascial), and some ticks were found on the eyelids (black arrows) in one cattle

Discussion

Owing to their ability to transmit a variety of pathogens, including viruses (such as tick-borne encephalitis and the Powassan Virus), bacteria (such as Rickettsia, Anaplasma, and Borrelia), and blood parasites (such as Babesia), ticks pose a serious threat to human and animal health worldwide (Rochlin and Toledo 2020). However, there is currently no evidence linking the ensuing disorders to socioeconomic consequences. According to the field survey, numerous tick species frequently infest animals, notably cattle. However, no link has been found between the ensuing diseases and socioeconomic consequences. *Haemaphysalis* is the second largest genus of the family Ixodidae, distributed in Australia, China, Indonesia, Japan, Malaysia, Nepal, New Zealand, Pakistan, Sri Lanka, Thailand, Myanmar, and Vietnam, whereas in Indonesia, it has become more prevalent to infest cattle on Java Island (Sahara et al. 2019; Hamid et al. 2022). *Haemaphysalis bispinosa* is also known for its ability to transmit *B. naoakii* to cattle in Central Java, Indonesia, which is characterized by fever, tachypnea and hemoglobinuria in the infected host (Hamid et al. 2022). It also takes a role as a competent vector for *Babesia gibsoni* (Shaw et al. 2001; Jongejan and Uilenberg 2004; Uilenberg 2006), Kyasanur forest disease virus (Geevarghese and Mishra 2011), *Anaplasma bovis* (Qiu et al. 2016), and *Theileria luwenshuni* (Lim et al. 2019).

According to Geevarghese and Mishra (2011), the distribution of *H. bispinosa* includes India, Sri Lanka, Nepal, Pakistan, Burma, China, Japan, Australia, New Zealand, Thailand, Malaysia, Vietnam, and Indonesia. However, based on the morphological and molecular studies, Chen et al. (2015) proved that *H. bispinosa* did not exist in China; the previously reported *H. bispinosa* in this country in fact is *H. longicornis*. In Indonesia, the distribution of *H. bispinosa* covers Sumatra, Java, Sumbawa, Flores, and Bali (Anastos 1950). The hosts of *H. bispinosa* are sheep, goats, wolves, civet cats, mules, antelope, buffalo, cows, cats, dogs, donkeys, horses, ponies, rabbits, rats, chitals, tigers, birds, wild mammals, and rodents (Geevarghese and Mishra 2011).

The cattle in Sleman Regency, Yogyakarta, were only infested by *H. bispinosa*. In the field, these ticks were also found attacking chickens, goats, and even cats. *Haemaphysalis bispinosa* was infested reared cattle in several regions in Indonesia, including Sumatera, Java, Sumbawa, Flores and Bali, both in and out of pens (Anastos 1950). In Bangladesh, *H. bispinosa* mostly parasitized goats rather than cattle, swine, or buffaloes (Islam et al. 2006; Gosh et al. 2007).

In every developmental stage, *H. bispinosa* has a different host to feed on. This pattern appears to be common in all other three-host tick species regardless of their genus or species. In this case, it seems in those case that all stages are only in cattle. There are no other types of livestock found in the area.

Anastos (1950) reported that *H. bispinosa*, *H. hystricis*, and *H. papuana* have similar morphological characteristics, i.e., blunt palps and dentition 4/4. However, the male of *H. bispinosa* has 7-8 teeth and the female has 8-9 teeth per filewhile *H. hystricis* has 10-12 teeth per file, and *H. papuana* has 9-10 teeth per file (Anastos 1950; the hypostome Geevarghese and Mishra 2011). The male *H. bispinosa* found in Yogyakarta had dentition 4/4 with 8 teeth per file on the hypostome. This is in line with a study by Anastos (1950), showing that the characteristics of male *H. bispinosa* include a short hypostome and dentition 4/4 with 7-8 teeth per file. The female *H. bispinosa* found in Yogyakarta had dentition 4/4 with 8 teeth per file in the hypostome and evenly distributed punctations. Geevarghese and Mishra (2011) revealed that the female *H. bispinosa* species in India has large and not dense punctations, but the lateral surface of the tick body has evenly distributed and numerous punctations.

The *H. bispinosa* nymphs found in Yogyakarta had an average scutum length of 0.36±0.01 mm and a scutum width of 0.41±0.02 mm. The average length of the larval tick was 0.61±0.0007 mm with a width of 0.43±0.01 mm. The larvae were observed to have a round shape, a scutum on the anterior dorsal but no cornua. The nymphs and larvae had dentition 2/2, but the larvae had three pairs of legs, short and blunt spurs on coxa I. The dentition of the nymphs and larvae of this species is in line with the findings of a study by Hoogstraal et al. (1968), distinguishing it from *H. longicornis* nymphs, which have a dentition of 3/3 with 6 teeth per file. According to Geevarghese and Mishra (2011), the scutum of the *H. bispinosa* nymphs found in India has an average length of 0.40 mm and a width of 0.49 mm, dentition 2/2, with an average of 6 teeth per file.

Generally, the life cycle of tick can be divided into two cycles, including the free-living cycle (pre-oviposition, oviposition, pre-hatching/incubation periods) and the parasitic cycle (larval feeding and pre-molting periods, nymphal feeding and pre-molting periods, and adult female engorgement period). *Haemaphysalis bispinosa* is a three-host tick, which has four life stages, eggs, larvae, nymphs, and bisexual adults, whereas each stage requires a different host for survival. The pre-oviposition and the oviposition period o *H. bispinosa* in this study is shorter (7 days) than previous study, 10±2.46 and 8.7±2.68 days, respectively (Senbill et al. 2022), while the incubation period needs longer time than Senbill et al. (2022), 14.6±2.39 days. According to Nagar (1968), the pre-oviposition periods of ticks are unfixed and rely on the nutrients, metabolic activity, or rate of individual ticks and the ability of females to transform nutrients to form eggs, while the oviposition period is mainly affected by the amount of blood embedded by each individual tick. In addition to the other two periods, the incubation period is primarily influenced by environmental factors, including the temperature and relative humidity to which the eggs were exposed, as well as the species-specific yolk capacities of the eggs.

Ticks have a certain attachment site preference (preferred feeding sites) on the body of their hosts (Sundstrom et al. 2021). Different tick species have different preference sites. The site preference of *Dermacentor variabilis* preferentially biting the head and neck and *Amblyomma americanum* preferring the thighs, groin, and abdomen. *Ixodes scapularis* was found across the body (Hart et al. 2022). The head and neck regions are parts of the body that most often come into contact with the feed trough and the wood poles of the pens, making it easier for ticks that live in these areas to reach these regions.

Several risk factors are associated with tick infestation in cattle, including traditional rural housing systems and grazing, the presence of wild animals, and farmers' knowledge of acaricide treatments (Rehman et al. 2017; Diaha-Kouane et al. 2020). Traditional housing is made up of almost entirely closed animal sheds without adequate ventilation, straightforward roof construction to prevent rain during the rainy season, and walls composed of firm bricks (Iqbal et al. 2013). An earlier study showed that animals are vulnerable to tick infestation in a closed-style habitat (Jouda et al. 2004). Less exposure to sunshine is thought to favor the retention of humidity in masonry and dung piles inside closed homes, creating ideal hiding spots for ticks all year. In addition, female ticks lay their eggs in the cracks and crevices of animal shed walls, which not only offers the best conditions for tick development but also serves as a preferred hiding place for nymphs and adults. Besides, grazing practices and the presence of wild animals increase the potency of tick infestation. The percentage of tick infestation varied in this study, with the Berbah Farmer Group having the lowest level. The difference was due to several Berbah Farmers has been sold their previous cattle, which had heavy infection either with ticks or blood parasite(s), and during this study, we collected the sample from their latest cattle. In addition, this may also have been brought on by two other farms' lack of information regarding the biology of ticks and diseases transmitted by ticks to cattle. However, due to the scarcity of our data, we were unable to identify the reasons for this disparity.

Male ticks are rarely found in experimental and natural infestations (Bremner 1959), but in this study, there was not any difficulty in finding male ticks, although the proportions of the male and female ticks had not been counted yet. The engorged female ticks went into the environment around the pens to lay eggs at night. The larvae and nymphs could be found in the pen structures, such as poles and feed troughs that are made of cement and bamboo, making them have many holes and gaps (Figure 6). The eggs laid by female ticks in the field were higher in number than the eggs laid by ticks reared in a laboratory, as reported by Diyes and Rajakaruna (2016) and Senbill et al. (2022).

Figure 6. The gaps in the building and posts in the cowshed are filled with ticks

According to the BPS - Statistic Indonesia in 2020, there were a total of 17,440,393 beef cattle in Indonesia, 309,259 of which were in the Special Region of Yogyakarta. Each of the cattle farmer groups had several pens, each of which could contain two to five cows reared by one farmer. This means that there were several rearing farmers in one farmer group. Some of the tick control measures that have been carried out are to manually remove ticks, bathe the cows, and sometimes give insecticides. However, these measures had not successfully resolved tick infestation. The pens were not cleaned simultaneously and thoroughly, so the ticks in the surrounding could re-infest the cattle. The hygiene level of each of the individual pens in the farmer groups varied. Not all farmers cleaned the pens regularly, so accumulated manure was found in some pens. In conclusion, *H. bispinosa* is the dominant species of tick found on cattle in Yogyakarta, according to our findings. In order to reduce the occurrence, local farmers as well as public health authorities must understand tick biology, epidemiology, pathogenicity, immunity, and their crucial role as potential vectors of new zoonotic infections, particularly in tropical settings.

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