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Prevalence of Ticks and Tick-Borne Diseases in Indonesian Dogs

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Abstract

The brown dog tick (*Rhipicephalus sanguineus*) is the most common ectoparasites of dog in the world. The study of ticks infestation and tick-borne diseases on the dog is necessary, because this information still limited in Indonesia. Ticks and dog blood test sampling were done at Animal Police Directorate Security Agency (Baharkam) and Atang Sendjaja Air Force (ATS). In addition, ticks and cases on ticks infestation data were obtained from 21 veterinary clinics in Bogor, Jakarta and Bandung. All ticks samples were preserved in bottles containing 70% alcohol for identification, and dog blood tests performed for Ehrlichiosis, Babesiosis and Anaplasmosis diagnosis. This research was done in March to December 2013. The results showed that all ticks samples collected from Baharkam dogs, ATS dogs and Veterinary Clinics in Bogor, Jakarta and Bandung were identified as one species, i.e., *Rhipicephalus sanguineus*. The prevalence of dogs infested by ticks were 67.90% in Baharkam 100% in ATS, and the prevalence in 21 Veterinary Clinic in Jakarta, Bogor, and Bandung from 2008-2013 tend to be increased every year. The tick predilections in Baharkam dogs distributed on the back regio (35.0%), the head, ears and neck (29.0%), the legs and the interdigital spaces (14.5%), the abdomen (10.9%) and the tail (10.9%). The tick larvae were found not only in the kennel but also on the grass field of Baharkam which often use for the training of police dogs. These places can be a source of transmission of tick infestation among dogs. The purebred and male dogs in Bogor, Jakarta and Bandung were more infested than the crossbred, local and female dogs. The prevalence of *Ehrlichiosis* on Baharkam dog was 12%, and 40% from ATS dogs. The results of blood smear examination on Baharkam dogs showed positive *Babesia* (8%) and *Anaplasma* (16%). There was no relationship between the degree of tick infestations to *E. canis*, *Babesia* and *Anaplasma* infections (p>0.05).

Keywords: *Anaplasma; Babesia; Ehrlichia canis; Rhipicephalus sanguineus*

Introduction

The brown dog tick (*R. sanguineus*) is the most common ectoparasites of dog in the world [1] and also in Indonesia [2]. This tick feeds on a wide variety of mammals including human and birds (by attaching to the skin and sucking blood. However, dogs are its preferred host and are where it is most commonly found. The brown dog tick can cause skin irritation in dogs where large numbers are present. It can infest houses and kennels [3]. More significantly, the tick can carry and spread a range of blood-borne diseases that can affect both animals and humans. These include Rocky Mountain spotted fever and Mediterranean spotted fever in humans and Ehrlichiosis, Babesiosis and *Anaplasmosis* in dogs [4]. Moreover, in the era of globalization and climate changes, the brown dog tick has becoming increasingly relevant from a public health perspective. This tick has also been implicated in the transmission of pathogens of zoonotic concern (e.g., *Ricktettisa rickettsii*) and recent studies have shown that *R. sanguineus* ticks exposed to high temperatures are more prone to bite humans [5].

Within the last few decades, newly identified tick-borne diseases or re-emergence of known tick borne diseases with new geographical patterns or prevalence have been described around the world. The important spreading pathogens by a greater mobility of human populations and their companion animals combined with changes in the ecosystems favorable to survival of ectoparasites have led to the recognition of tick borne diseases in areas usually considered as free of this infection [6]. *R. sanguineus* is a good example of parasite globalization to its ubiquitous distribution which has clearly been facilitated by dog movements with their owners or trough trade. As more attention is given to the care of the companion animals, especially in developed countries [7], and better detection tools (based on mollecular technique) allowing more sensitive and specific detection of tick-borne pathogens [8].

Tick-borne diseases are recognized as an emerging infectious threat not only to dogs but also to human. This information is still limited in Indonesia, whereas the epidemiological studies which support the emergence or re-emergence of tick-borne pathogen in dogs around the world is necessary. The present study will focus on the recent of ticks infestation and tick-borne diseases on the dog in several Cities of Indonesia based on direct observations, and medical record of Veterinary Clinics.

Materials and Methods

Location and time research: The ticks were collected in the Animal Police Directorate Security Agency (Baharkam) Kelapa Dua, Depok City and the Atang Sendjaja Air Force (ATS), Bogor City, and 21 Veterinary Clinics in Bogor, Jakarta and Bandung Cities. Tick identification and Ehrlichia rapid test analysis were carried out at the Laboratory of Entomology, while the identification of blood smear samples in Protozoology Laboratory, Department of Parasitology and Medical Entomology, Faculty of Veterinary Medicine, Bogor Agricultural University Indonesia. The research was conducted from March to December 2013.

Methodology

Observations of tick infestations in dogs: Observations were done by counting the number of ticks in the fifth region of the body of a dog, i.e., (a) The head to neck including the ears, (b) the back, (c) the abdomen, (d) the legs and (e) the tail, then determination the degree of tick infestation. The processing tick specimens and the degree of tick infestations were based on Hadi and Rusli, as follows: (-)=no ticks, (+)=1-5 ticks (mild), (++)=6-10 ticks (moderate), (+++)=11-20 ticks (high) and (++++)=>20 ticks (very high).

Observations tick infestations in the environment: Larval tick densities were estimated using the blanket dragging method. The tools used in the form of white fabric measuring 40 x 40 cm surface is rough so the ticks will be easier to stick to the fabric. The fabric is then pulled over the field grass along the 5 to 10 m for 30 seconds [9]. Observations and sampling ticks also performed at the kennels manually.

Specimen processing and tick identification: The process of making preparations slide carried by ticks and Partosoejono and Ashadi method [10]. Identification of ticks using identification keys of Walker et al. [9].

Detecting ehrlichiosis, anaplasmosis and babesiosis: Blood and serum samples were obtained from 30% of examined dogs in Baharkam and ATS. Procedures of separation and storage of serum were based on Tsachev et al. [11]. The blood was collected by using a syringe and collected in vacutainer, then placed at room temperature until the process of coagulation. Vacutainer then centrifuged at 1700 g for 10 minutes to separate the serum. The serum was transferred into a microtube, then stored in a freezer temperature of -20°C. Dog serum samples examined using rapid test kits WITNESS Ehrlichia® (Synbiotics) for the detection of antibodies against *E. canis*. Identification of *Babesia* and *Anaplasma* based on the blood staining method in accordance with Tampubolon [12].

Cases of tick infestations in dogs: Secondary data was taken from the dog patient's medical record at the 21 Veterinary Clinics in Bogor, Jakarta and Bandung. Data were collected from 2008 to 2013 and grouped by the breed of dog, age and sex. Data on 2013 were obtained from tick sample collection of patients in veterinary clinics conducted by the veterinarian in charge of each clinic. Dog tick samples from patients were put into the collection tubes containing 70% alcohol, and then identified in the Entomology Laboratory.

Results and Discussion

Tick identification

Out of 950 samples of ticks obtained from 97 dogs in Animal Police Directorate Security Agency (Baharkam) Depok, Atang Sendjaja Air Force Bogor, and 21 Veterinary Clinics in Bogor, Jakarta and Bandung, showed that only one species of tick found, i.e., *Rhipicephalus sanguineus*. This tick was elongated body shaped, reddish brown in color, and lacking any ornamentation. The size of the adult ticks ± 1 cm, and with the curve of anal (anal groove) in the posterior anus. *R. sanguineus* has a slightly convex-shaped eyes located on the lateral scutum, hexagonal basis capituli, short hypostome, festoons in the posterior part of the body, and spiracles shaped like a comma. The ventral part of the male tick with narrowed adanal shaped pieces, and the size of the pieces of additional adanal quite large. The female tick does not have the shield and additional Adanal. Dorsal basis capituli

female ticks are a pair of porous area that secrete substances when ticks lay eggs. These substances serve to protect the eggs (waterproofing).

In Indonesia, Hadi and Rusli [13] reported from 28 ticks infested dogs in 6 districts of Bogor were identified as *R. sanguineus*. A similar result was reported in the Philippines, species of ticks that attacks the dog was *R. sanguineus* [14]. Dog ticks survey in four cities in India (Mumbai, Delhi, Sikkim and Ladakh) showed there were two genus of ticks i.e., *Rhipicephalus* and *Haemaphysalis* [15]. In Jeju island of South Korean, the ticks survey by using tick-drag showed *R. sanguineus* was never found, but *Haemaphysalis longicornis*, *H. flava*, *H. phasiana*, *Ixodes nipponensis*, *I. turdus* and *Amblyomma testudinarium* were collected well [16].

Tick infestations in animal police directorate security agency (Baharkam)

Baharkam is Security Maintenance Agency in charge of developing and conducting security management function that includes maintenance and improving the conditions of security and public order in order to realize security in the country (Indonesia). Here there are trained dogs and horses were assigned to assist the police to deal with various security problems. Tick infestation studies were carried out in the entire dog i.e., 81 dogs (53 males and 28 females). The prevalence of the tick infested dog was 67.90% (55 dogs). In Baharkam there were nine bred of dogs, i.e., Beagle, Doberman, Dutch Shepherd, German Pointer, German Shepherd, Golden Retriever, Labrador, Malinois and Rottweilers. Golden Retriever was the only breed of dog that was not infested with ticks compared to eight other breeds. Doberman and German shepherd were the highest tick infested dogs (100%), followed by German Pointer (75%), Malinois (70.83%), Dutch Shepherd (70%), Rottweiler (66.67%), Labrador (56%), and Beagle (50%) (Table 1). In Baharkam, it was known that the Golden Retriever was rarely active in the field so the opportunities for contact with *R. sanguineus* outdoors was limited, and the tick transmission becomes small. Based on Pearson Chi-Square test, there was no relationship between the breed of dog with a degree of infestation of ticks (p>0.05). The similar results were reported in the Ashton Court Estate-UK, that factor of dog races/breeds did not affect the dog tick infestations [17].

No	Dog breeds	Number of dog	Number of tick infested dog	
			Total	%
1	Beagle	2	1	50
2	Doberman	2	2	100
3	Dutch Shepherd	10	7	70
4	German Pointer	8	6	75
5	German Shepherd	6	6	100
6	Golden Retriver	1	0	0
7	Labrador	25	14	56
8	Malinois	24	17	70.83
9	Rottweiler	3	2	66.67
	Total	81	55	67.90

Table 1: Prevalence of dogs infested by *Rhipicepalus sanguineus* at Baharkam based on dog breds.

Generally in Baharkam, those dogs were infested by mild tick infestation category (41.98%), meanwhile in moderate category was 18.52%, and in high and very high were 2.47% and 4.94% respectively. The percentage of male dogs were infested by ticks higher (45.68%) than female dogs (22.22%), but based on Pearson Chi-Square, there was no relationship between the sexes dog and degree of infestation of ticks (p>0.05) (Table 2). This was because the training activity or guard duty did not distinguish between male and female dogs, so both dogs have the same time spent outdoors and opportunity to be infested by ticks. Research in Algeria and Mexico also showed a similar result with this result [18,19].

No	Degree of tick infestation catagory	Baharkam dogs (%) Total			ATS dogs (%)		
		Male	Female	Total	Male	Female	Total
1	None (0 tick)	19.75	12.35	32.10	0.00	0.00	0.00
2	mild (1-5 ticks)	27.16	14.81	41.98	0.00	0.00	0.00
3	moderate (6-10 ticks)	14.81	3.70	18.52	14.29	0.00	14.29
4	high (11-20 ticks)	1.23	1.23	2.47	21.43	0.00	21.43
5	very high (>20 ticks)	2.47	2.47	4.94	35.71	28.57	64.29
	Total	65.43	34.57	100	71.43	28.57	100

Table 2: The degree of infestation of *Rhipicepalus sanguineus* in Baharkam and ATS dogs based on sex.
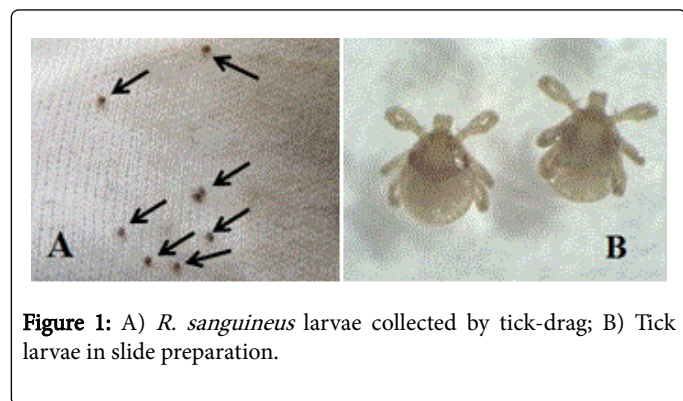
Based on information from veterinarians and the handler in Baharkam, not all the dogs were quite regularly bathed, however in some dogs were done. The statement indicated that the dogs without regularly bathed can be a source of tick infestations. The predilection sites of tick in the infested dogs at Baharkam varied. However the more preferred were in back regio 19 (35.0%), head, ears and neck 16 (29.0%), legs and interdigital spaces 8 (14.5%), abdomen 6 (10.9%) and tail 6 (10.9%) in total of 55 infested dogs (Table 3). The degree of infestation in the region of the back of the dog shows very high infestation. Ticks were found mainly in this area due to difficult for the dog to use his legs to get rid of a tick [20]. The behavior of an infested dog showed frequently scratching the body, especially the abdomen and head, ears, and neck in field training or in a kennel. This was because the effects of tick infestations caused such irritation. The tick larvae (Figure 1) were also found in Baharkam grass field which often use for the training of police dogs (about 71 larvae /100 m² area of the field). Grassy field for dog training was suspected for source infestation, because the infested dogs and the uninfested, practicing in the same field. When the infested training dogs entered their kennel, the transmission also move to the kennel area. This area will be suitable for the larvae and nymph for drop-offs and moulting. Drop-off also occurred in female tick to lay eggs and hide. Adult females will lay eggs in hidden places such as cracks and crevices walls, rocks and soil to protect themselves and their offspring from predators such as spiders, birds [21] and wasps [22]. *R. sanguineus* is an endophilic (adapted to indoor living), monotropic (all developmental stages feed on the same host species), and three-host (each life stage requires a new host to feed on) tick species. However, this tick is also able to survive in outdoor environments, and being able to adopt different strategies for survival [1].

Dog breed	No infested dog	No of dog and the degree of tick infestation by predilection sites				
		Head to neck region	Back region	Abdomen region	Legs and interdigitals	Tail region
Beagle	1	1 (++)	1 (++++)	1 (+)	1 (+)	(-)
Doberman	2	(-)	2 (++) ~ (++++)	(-)	(-)	(-)
Dutch Shepherd	7	3 (-) ~ (+)	2 (-) ~ (+++)	1 (-) ~ (+)	1 (-)~ (+)	1 (-)~ (+)
German Pointer	6	3 (-) ~ (+)	3 (+) ~ (++)	1 (-) ~ (+)	1 (-)~ (+)	1 (-)~ (+)
German Shepherd	6	2 (-) ~ (+)	2 (-) ~ (+)	1 (-) ~ (+)	1 (-)~ (+)	1 (-)~ (+)
Labrador	14	5 (-) ~ (++)	5 (-) ~ (++++)	1 (-) ~ (+)	1 (-)~ (+)	1 (-)~ (+)
Malinois	17	3 (-) ~ (+)	2 (-) ~ (++)	1 (-) ~ (+)	1 (-)~ (+)	1 (-)~ (+)
Rottweiller	2	(-)	2 (+) ~ (++)	(-)	2 (+)~ (+++)	1 (-)~ (+)

Total	55	16 (29.0%)	19 (35.0%)	6 (10.9%)	8 (14.5%)	6 (10.9%)

Table 3: The degree of infestation of *Rhipicepalus sanguineus* in Baharkam dogs based on predilection sites. (-)=No tick ; (+)=mild ; (++)=moderate ; (+++)=high ; (++++)=very high infestation.

Figure 1: A) *R. sanguineus* larvae collected by tick-drag; B) Tick larvae in slide preparation.

Tick infestations in Atang Sendjaja Air Force Bogor (ATS)

Senjaya Atang Air Force Bogor is a military air base where security is also equipped with guard dogs whose number 14 of the race Golden Retriever and Rottweiler. The prevalence of tick infestation in ATS dogs were 100% in total of 14 dogs (10 males and 4 females). In general, tick infestation were belong to category of very high infestation (64.29%), high (21.43%) and moderate (14.29%) (Table 2). Ticks predilection were found mostly on the dog's head to neck including ears (42.9%) (Table 4). The tick larva was not found in ATS grass field, but positively inside the kennels.

Dog breed	No infested dog	No of dog and the degree of tick infestation by predilection sites				
		Head to neck region	Back region	Abdomen region	Legs and interdigitals	Tail region
Golden Retriever	2	1 (++++)	2 (+)~ (++)	1 (+)	1 (+)	1 (+)
Rottweiller	12	5(+) ~ (++++)	2(+)~(++++)	(-)	1 (+)	(-)
Total	14	6 (42.9%)	4 (28.6%)	1 (7.1)	2 (14.3)	1 (7.1)

Table 4: The degree of infestation of *Rhipicepalus sanguineus* in ATS dogs based on predilection sites.

Tick infestation in veterinary clinic

The cases of ticks infestation of dogs at Bogor, Jakarta and Bandung Veterinary Clinics (21 clinics in total) in 2008 to 2013 as many as 731 cases. In general, there was an increase of the ticks infestation prevalence at three cities in that range of year (Figure 2). This might illustrate the increasing of the awareness of the owner to check the dog to a veterinary clinic or because of the effect of climate change. In the years 2008-2011 and from January to June 2013, the average prevalence of tick infestations in Bogor higher than Jakarta and Bandung City.

The prevalence of tick infested dog based on bred, sex, and age in Bogor, Jakarta dan Bandung from 2008 to 2013 was presented in Table 5. Based on the dog breds, the purebred in three city showed the highest infestations (71.47%) followed by crossbred (18.98%) and local dog (9.55%). By sex, male dogs were more infested ticks (61.08%) than the female (38.81%). These data based on the patience coming to the veterinary clinic, so these results might be different in other countries.
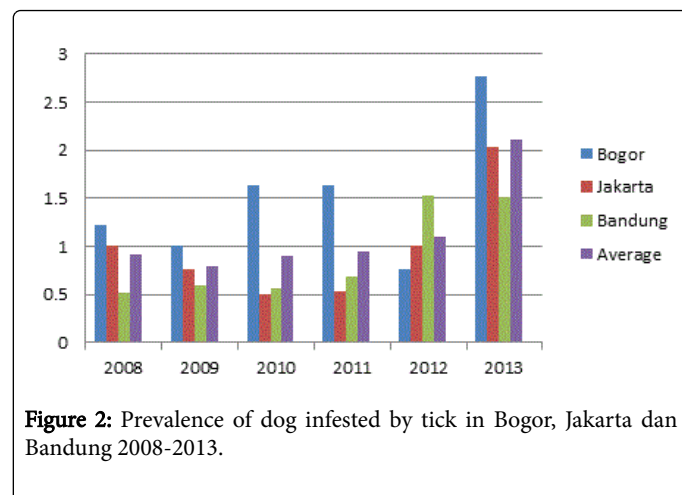

Figure 2: Prevalence of dog infested by tick in Bogor, Jakarta dan Bandung 2008-2013.

City area	Dog bred (%)			Sex (%)		Age (%)		
	Pure breed	Crossbreed	Local	Male	Female	<1 Year	1-5 year	>5 year
Bogor	84.91	6.92	8.18	60.13	39.87	53.33	34.29	12.38
Jakarta	70.00	16.97	13.03	60.43	39.57	21.29	62.74	15.97

| Bandung | 59.50 | 33.06 | 7.44 | 62.69 | 37.31 | 54.72 | 37.74 | 7.55 |
| Average | 71.47 | 18.98 | 9.55 | 61.08 | 38.91 | 43.11 | 44.92 | 11.96 |

Table 5: Prevalence of dog infested by tick based on bred, sex, and age in Bogor, Jakarta dan Bandung tahun 2008-2013.

In Indonesia, the most owners of purebred dogs belong to higher income persons, and they more aware to bring their dogs for the health checked. In addition, the most dog owner only has the male dog, and the most patience coming to the veterinary clinic in three cities were the male. Papazahariadou et al. [23] reported that the dog tick infestation varies between races or breds in Thessaloniki, northern Greece. He also noted that the purebred dogs group more infested by ticks (41.78%), while the crossbred was 18.9% and mongrel was 39.4%.

The prevalence of tick infested dogs based on three age groups as derscribe by Hadi and Rusli [2] and VCA [24] showed that the young dog (under 1 year old) and adult (between 1 and 5 years old) were more infested (43.11% and 44.92% respectively) than the older one (over 5 years old) with lower prevalence (11.96%). However, the significant prevalence of infested dogs aged 1-5 years old (62.74%) was shown at the veterinary clinics in Jakarta, and the prevalence of infested dog in Bogor and Bandung almost similar.

Ehrlichiosis, babesiosis and anaplasmosis detection

The detection results of Baharkam dog sera samples showed 12% positively *E. canis*, and 40% from ATS Samples. The positive result in Baharkam was originally come from the dogs with moderate tick infestation category (40%) and without ticks (12.5%). While ATS dog sera that *E. canis* positive were come from dogs with moderate and high degree of tick infestation. Based on Chi-Square test, there was no relationship between degree of ticks infestation to *E. canis* infection (P>0.05). In addition, the dogs with *E. canis* positive both from Baharkam and ATS showed no clinical symptoms and happened in ages ranging from 1 to 3 years olds. These subclinical stages of Ehrlichiosis were also reported in several countries in the world. Cardoso et al. [25] in Portugal reported that 4.1% of 557 healthy dogs found to have antibodies to *E. canis*. These antibodies can be persisted for a long time in the dog's body, and can be seen in subclinical infection [26]. However, as dogs in Baharkam and ATS, the incidence of Ehrlichiosis in dogs in Brazil did not show the clinical symptoms [27-29].

The result of blood smear examination of Baharkam dogs showed positively *Babesia* sp (8%) and *Anaplasma* sp (16%), while samples of ATS were all negative (Table 6). *Babesia* sp infection in Baharkam dogs originated from the dog with moderate and high tick infestations. However, the analysis Pearson Chi-Square test explained that the degree of infestation of tick was not associated with *Babesia* sp and *Anaplasma* sp infections (p>0.05). *Babesia* sp density percentage was 0.4% in the dog with moderate tick infestations and 0.2% in the high category (Table 7). Mixed infections between *E. canis* and blood Protozoa such as *Babesia* sp and Theleria were reported in dogs of South Africa [30]. Although dogs are the main hosts of *R. sanguineus*, the finding of this tick on wild canids [31] indicates that free-ranging wild canids might be involved in its maintenance and dispersion through different regions. This could have implications in the control of ticks and in the epidemiology of tick-borne diseases, particularly in areas where dogs live in close contact with their wild counterparts.

Dogs category	No dog examined		Ehrlichia canis (%)		Babesia sp (%)		Anaplasma sp (%)	
	Baharkam	ATS	Baharkam	ATS	Baharkam	ATS	Baharkam	ATS
None (0 tick)	8	0	1 (12.5)	0	0	0	2 (25)	0
mild (1-5 ticks)	10	0	0	0	0	0	0	0
moderate (6-10 ticks)	5	1	2 (40)	1 (100)	1 (20)	0	1 (20)	0
high (11-20 ticks)	1	1	0	1 (100)	1 (100)	0	0	0
very high (>20 ticks)	1	3	0	0	0	0	1 (100)	0
Total	25	5	3 (12)	2 (40)	2 (8)	0	4 (16)	0

Table 6: Detection result of *E. canis*, *Babesia* sp, and *Anaplasma* sp infections among dogs of Baharkam and ATS in September 2013.

Dogs catagory	Density of Babesia sp (%)
None (0 tick)	0
Moderate (6-10 ticks)	0.4
High (11-20 ticks)	0.2

Very high (>20 ticks)	0

Table 7: Density of *Babesia* sp infected dogs of Baharkam in September 2013.

Conclusion

The tick infested dog in Depok, Bogor, Jakarta and Bandung based on the morphological characters was only one species i.e., *Rhipicephalus sanguineus*. The prevalence of dogs infested by ticks in Baharkam was 67.90%, 100% in ATS, and the prevalence in 21 Veterinary Clinic in Jakarta, Bogor, and Bandung 2008-2013 tend to be increased every year. The predilection *R. sanguineus* in Baharkam dogs were more in the region of the back (35%) and head, the ears and the dog's neck (29%), but also on the legs and interdigital spaces (14.5%), abdomen (10.9%) and tail (10.9%). The tick larvae were found not only in the kennel (indoor) in grass field (outdoor environment) especially in Baharkam which often use for the training of police dogs. These places can be a source of transmission of tick infestation among dogs. Based on the dog breds, the purebred in three city showed the highest infestations (71.47%) followed by crossbred (18.98%) and local dog (9.55%). By sex, male dogs were more infested ticks (61.08%) than the female (38.81%). The prevalence of tick infested dogs based on three age groups showed that the young dog (under 1 year old) and adult (between 1 and 5 years old) were more infested (43.11% and 44.92%, respectively) than the older one (over 5 years old) with lower prevalence (11.96%). The prevalence of *Ehrlichiosis* on Baharkam dog was 12%, and 40% on ATS dogs. Positive result in Baharkam were originally come from the dogs with moderate tick infestation category (40%) and without ticks (12.5%). The positive result in Baharkam were originally come from the dogs with moderate tick infestation category (40%) and without ticks (12.5%). The results of blood smear examination on Baharkam dogs showed positive *Babesia* (8%) and *Anaplasma* (16%). There were no correlation between the degree of infestation of ticks with infection of *E. canis*, *Babesia* and *Anaplasma* (p>0.05).

Acknowledgements

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