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Research Article

Ectoparasite Infestation among Stray Cats around Surabaya Traditional Market, Indonesia

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ABSTRACT

This study was conducted to determine the prevalence of ectoparasite infestation among stray cats around Surabaya traditional markets. A total of 305 stray cats were collected around 17 traditional markets in Surabaya City and were examined for the presence of fleas with a fine-toothed flea comb. Surveys were conducted during May-June 2019. 228 of 305 stray cats (74.75%) were infested with one species of ectoparasite. The average number of *C. felis* in every cat was 2.54, while the number of *F. subrostratus* in every cat was 0.33. Additional data about the gender, pregnancy/ maternity, and bodyweight of every cat were recorded. The result of chi-square test shows that there is a significant difference between gender, pregnancy status, and bodyweight by the occurrence of ectoparasites (p=0.008; p=0.00; p=0.00). A total of 878 ectoparasites consisting of flea and lices, namely *Ctenocephalides felis* (88.27%) as the dominant ectoparasite, followed by *Felicola subrostratus* (11.73%). The highest infection rate (prevalence) of ectoparasite was found in Pucang Market (16.81%), while the lowest prevalence was found in Mulyorejo Market (0.8%). Coinfection was observed in only a few cats (1.63%). Multiple Regression showed that pregnancy is the most influential factor in the occurrence of fleas (p=0.000). These results should be taken into account among health workers to prevent a possible outbreak of zoonotic diseases caused by fleas.

Keywords: *Ctenocephalides felis*, ectoparasite*, Felicola subrostratus*, market

INTRODUCTION

Zoonotic infectious diseases caused by bacteria, viruses, and parasites that are transmitted from animals to humans are still some of the major public health problems. Tick fever, mange, leishmaniasis, and ascariasis are the diseases that often infect domestic animals, such as cats and dogs, and have the potential to spread to humans (Colombo *et al.,* 2011). Ectoparasites, as a group of animals in the Arthropoda phylum, cause the manifestation of skin diseases in dogs and cats (Akucewich *et al.,* 2002).

The common cause of skin disorders and anemia is blood-sucking, and the main consultations in small animal practice are ectoparasite infestations, especially flea infestations (Dyrden & Rust, 1994). *Ctenocephalides felis* is a flea that can transmit a tapeworm *Dyplidium caninum* (Pugh, 1987). Epidemiological surveys were already reported worldwide, but Indonesia is still limited. Only one study that reported ectoparasite distribution in the dogs from Indonesia, showing that *Rhipicephalus sanguineus* was the most manifested tick (Hadi & Soviana, 2015). Study in the USA also reported *Rhipicephalus sanguineus* as a predominant tick in dogs with the prevalence of 94.3%, and *Amblyomma americanum* as a predominant tick in cats with the prevalence of 74% (Burroughs *et al.,* 2016). In addition, studies in the USA showed a high prevalence of ectoparasite in cats caused by fleas (*Ctenocephalides felis*, *Pulex* spp., *Cediopsylla simplex*, and

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Nosopyllus fasciatus) and ticks (*Amblyomma americanum*, *Ixodes scapularis*, *Dermacentor variabilis*, *Rhipicephalus sanguineus*, and half of them were still in immature stages (Thomas *et al.,* 2016).

Some studies reported that *C. felis* is the most common external parasites found on dogs and cats, such as a report from New Zealand that showed mostly cat and dog infected by *C.felis* (Chandra *et al.,* 2017). This is also supported by studies in Nigeria (Omonijo & Sowemimo, 2017) and Ethiopia (Kumsa *et al.,* 2019). *Ctenocephalides felis* is known as a blood-feeder and an important vector of various pathogens, most of which are zoonotic such as *Yersinia pestis*, *Rickettsia typhi, R. felis, R. conori, Bartonella clarridgeiae*, and *B. henselae* (Beugnet & Marié, 2009; Boudebouch *et al.,* 2011; Chandra *et al.,* 2017; Lappin & Hawley, 2009; Shaw *et al.,* 2004). Frequent flea species reported from some countries such as in Germany were *C. felis*, *C. canis*, and *Archaeopsylla erinacei* (Visser *et al.,* 2001). A study in Mexico showed both *C. felis* and *C. canis* were manifested on dogs and cats (Cruz-Vazquez *et al.,* 2001). Four subspecies were already identified such as *C. felis damarensis*, *C. felis strongylus* that were mostly found in East Africa, *C. felis orientis* that was found in Australia and India, *C. felis felis* that spread in all continents except Antarctica (Shakya *et al.,* 2019).

Recent studies have certainly shown zoonoses in companion animal (Dantas-Torres & Otranto, 2014; ElSeify *et al.,* 2016; Kumsa *et al.,* 2019; Thomas *et al.,* 2016). However, it needs to elevate knowledge about the prevention and management of companion animals. Companion animals or pets could be a new potential health threat due to the frequent interaction with humans (Diakou *et al.,* 2017). Stray cats are almost found in many locations, including Surabaya as an urban area that is located in the East Java Province, Java Island. Surabaya has many traditional markets to support the daily needs of citizens. Markets were chosen as study areas regarding the possibilities of stray cats living in direct contact with human food. Markets and food courts are often visited by stray cats to support their survival. This different geographical area could lead to a different distribution of flea species. Thus, this study aimed to investigate the infestation of flea among stray cats around Surabaya traditional markets.

MATERIALS AND METHODS

Study Area

The survey was conducted from May to June 2019 in 17 traditional markets in Surabaya. Detail of coordinate locations can be seen in Table 1.

Table 1. Detail of sampling location.

No	Name of Market	Coordinate
1	Dinoyo	-7.937004, 112.608421
2	Gubeng	-7.264635, 112.752541
3	Pacar Keling	-7.259755, 112.759060
4	Karang Menjangan	-7.269295, 112.760920
5	Manyar	-7.280465, 112.762291
6	Pandegiling	-7.276136, 112.734760
7	Ngagel	-7.291142, 112.746650
8	Pucang	-7.283782, 112.753590
9	Banyu Urip	-7.274693, 112.720839
10	Simo	-7.267122, 112.713544
11	Jojoran	-7.272445, 112.766158
12	Menur	-7.280580. 112.762244
13	Keputih	-7.289643, 112.799469
14	Mulyorejo	-7.263779, 112.775044
15	Blauran	-7.256133, 112.733423
16	Asemrowo	-7.252092, 112.715279
17	Indrakila	-7.260296, 112.755938

Ectoparasite Collection

Random sampling was conducted in each market, in which samples were chosen by surrounding all market areas. Each cat was examined for the presence of ectoparasites by combing their fur using a fine-toothed flea comb for 5 min for each cat (Zakson *et al.,* 1995). Ear swabs were also conducted with an additional time of 5 min. Once the combing was completed, flea combs were placed in a white tray and ectoparasites fell into the tray covered by white paper. Each ectoparasite was then separately placed into a vial bottle filled with 70% ethanol for species identification. Afterward, the vial bottle was labelled with the number of cats, details of location, the name of the collector, and the time of collection. The collectors also recorded the gender, maternity, and bodyweight of each cat. When the cats had been checked, they were marked with a red rope around their neck to avoid double sampling.

Laboratory Examination

Samples were kept in 70% ethanol for identification. Samples were brought to the Laboratory of Animal Histology, Biology Department, Faculty of Science and Technology, Universitas Airlangga. Each sample was immersed in a slightly warm 5% potassium hydroxide (KOH) solution for 10-15 min. Then, samples were placed in 35% alcohol solution for 5 min to adjust pH, then moved to the series of 50, 70, 90, 95, and 100% ethyl alcohol solutions for dehydration for 5 min, respectively. After that, samples were cleared in xylene twice for 5 min to obtain transparency. The processed samples were mounted in Entellan® new 107961 Merck Millipore on microscope slides then they were identified to the species level under a stereomicroscope. Identification was made using the keys of the CDC flea identification key (2019) and the keys in the following references (Bowman *et al.,* 2002; Lewis, 1966; Soulsby, 1982; Wall *et al.,* 1997).

Statistical Analysis

Statistical analysis was done using SPSS IBM version 21. Chi-square test was used to analyze the difference between gender, bodyweight, and pregnancy status by the occurrence of ectoparasite. Significance levels were noted if p-value shows equal to or less than 0.05. Multiple Regression was applied to find out which factors were most influential on the occurrence of ectoparasite. The most influential factor is the factor that has the smallest p-value and the largest odds ratio among the other. Distribution of fleas was also figured out using ArcGIS 10.3 version.

RESULTS AND DISCUSSION

Distribution of ectoparasite that infected stray cats in Surabaya traditional markets

The infection rate of stray cats with ectoparasites from the study area was 74.75% of the 305 cats. A total of 878 ectoparasites were found, consisting of 775 *Ctenocephalides felis* (88.27%), 103 of *Felicola subrostratus* (11.73%) shown in Table 2. Almost all (99%) cats have a single infection and co-infection was seen in only five cats (1.63%). Coinfection was found in four study areas namely Pandegiling, Ngagel, Banyu Urip, and Jojoran (Table 3). The value of bodyweight was categorized by cut-off points. Cut-off points of bodyweight were determined by the roc curve. The optimal cut off is 2.87. If bodyweight > 2.87, bodyweight is classified as high. There was a significant relationship between bodyweight and the presence of ectoparasites (p = 0.00). There was a significant relationship between the gender of cats and the presence of ectoparasites (p = 0.008). Pregnancy also had a strong relationship with the presence of ectoparasites with a significance level (p = 0.00). All of them were proven by the Chi-square test (Table 4).

Multivariate tests showed that female cats were more highly infected than male cats (P=0.004; OR=2.896). Low Bodyweight was more highly infected than a high bodyweight cat (P=0.005; OR:2.988). A Pregnant cat was more highly infected than an unpregnant cat (P=0.000; OR:6.789). Among three variables, pregnancy factor was the most influential factor in the occurrence of ectoparasite because it had the smallest p-value and larger odds ratio than the other variables. All of

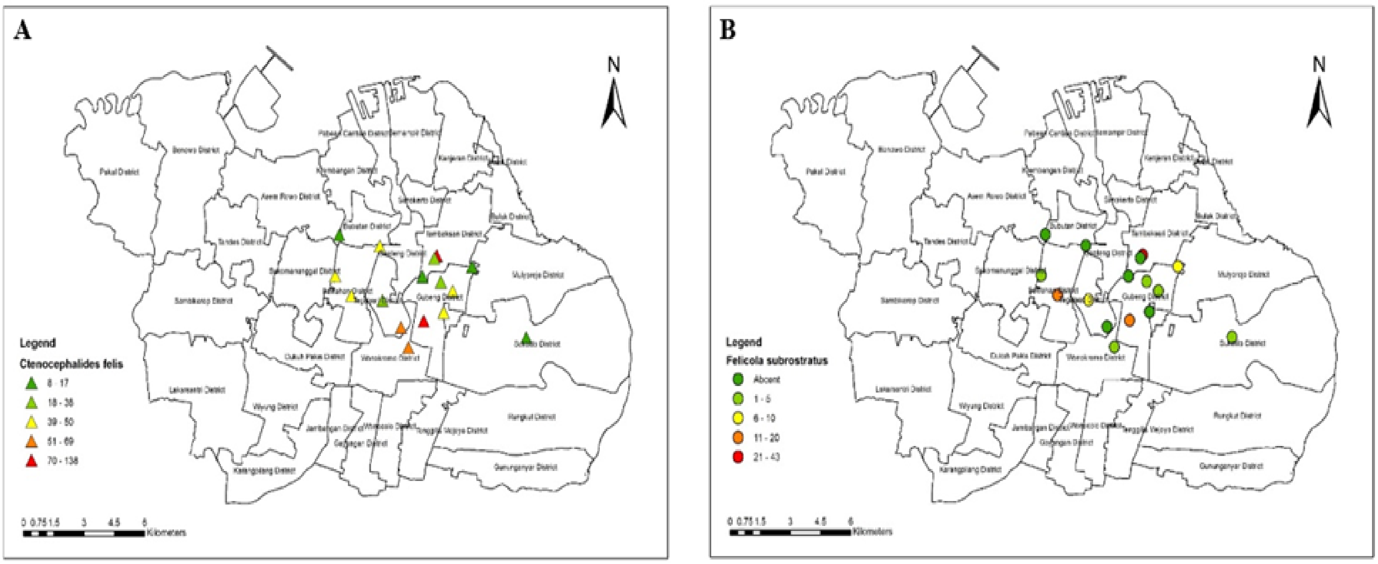

Figure 1. Mapping of *Felicola subrostratus* (A) and *Ctenocephalides felis* (B) distribution using ArcGIS 10.3 version: dark green dot indicates that *Felicola subrostratus* was not found; green dot indicates flea was found from one to five; yellow dot indicates flea was found from six to ten; orange dot indicates flea was found from eleven to twenty; red dot indicates flea was found from twenty-one to forty-three (Source: ArcGIS 10.3 version).

Table 2. Number of cats and ectoparasites collected in each study area.

Number	Name of Market	Number of Cats examined	Number of positive cats	Percentage of positive cats (%)	Number of Ectoparasites	Number of *C. felis*	Percentage of *C.felis*(%)	Number of *F. subrostratus*	Percentage of *F.subrostratus*
1.	Dinoyo	16	16	100	61	61	100	0	0
2.	Gubeng	12	4	33.3	15	15	100	0	0
3.	Pacar Keling	41	30	73.17	159	116	72.59	43	37.06
4.	Karang Menjangan	12	11	91.67	30	29	96.67	1	3.33
5.	Manyar	12	8	66.67	14	13	92.87	1	7.1
6.	Pandegiling	14	13	100	47	38	80.85	9	19.1
7.	Ngagel	31	18	58.06	70	69	98.57	1	1.43
8.	Pucang	48	39	81.25	155	138	89.02	17	10.96
9.	Banyu Urip	26	21	80.76	52	41	78.84	11	21,16
10.	Simo	12	8	66.67	51	46	90.19	5	9.8
11.	Jojoran	16	14	81.25	48	47	97.91	1	2.08
12.	Menur	10	9	100	46	46	100	0	0
13.	Keputih	10	6	70	13	8	61.5	5	38.46
14.	Mulyorejo	10	2	20	17	8	47.05	9	52.95
15.	Blauran	16	14	100	50	50	100	0	0
16.	Asemrowo	8	5	62.5	17	17	100	0	0
17.	Indrakila	10	10	100	33	33	100	0	0
Total	17 markets	305	228	-	878	775		103	-

them were proven by the Multiple binary regression test (Table 5). The distribution of *Felicola subrostratus* in the sampling site was shown in Figure 1A, whereas the distribution of *Ctenocephalides felis* was shown in Figure 1B.

This study was the first report from Surabaya, Indonesia, and it strongly indicated a high infection rate of flea *Ctenocephalides felis* (85.6%) and a low infection rate of *Felicola subrostratus* (14.4%) on the stray cats around traditional markets. Morphology of flea found among stray cat populations show in Figure 2. The average percentage of infection caused by findings are in line with recent worldwide studies that show *C. felis* as predominant species infected stray cats (Chandra *et al.,* 2017; Kumsa *et al.,* 2019; Omonijo & Sowemimo, 2017). Fleas can act as important vectors of diseases and can produce troublesome bites. *Ctenocephalides felis* is the most common nuisance fleas in cats distributed worldwide. This species can lay their eggs up to 25

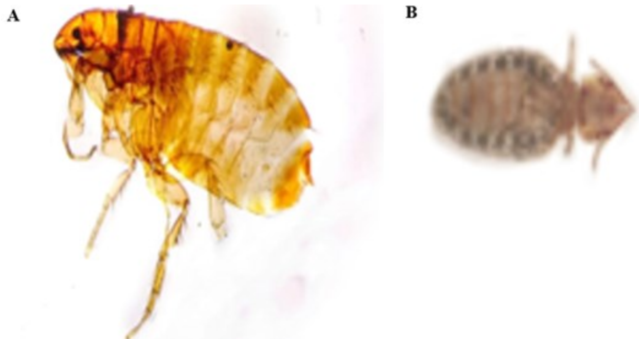

Figure 2. Flea species found among stray cat populations A. *Ctenocephalides felis* and B. *Felicola subrostratus* observed with a stereo microscope with magnification 10x.

Table 3. Prevalence of coinfection in cats collected from every study site.

Number	Name of Market	Number of Cats examined	Number of positive cats	Infection rate within all study site (%)	Number of cats with single infection	Percentage of single infection (%)	Number of cats with double infection/ coinfectio	Percentage pf coinfection (%)
1.	Dinoyo	16	16	6.89	16	100	0	0
2.	Gubeng	12	4	1.7	4	100	0	0
3.	Pacar Keling	41	30	12.9	30	100	0	0
4.	Karang Menjangan	12	11	4.74	11	100	0	0
5.	Manyar	12	8	3.44	8	100	0	0
6.	Pandegiling	14	13	6.03	12	92.85	1	7.15
7.	Ngagel	31	18	7.75	17	94.44	1	5.56
8.	Pucang	48	39	**16.81**	39	100	0	0
9.	Banyu Urip	26	21	9.05	20	95.23	1	4.77
10.	Simo	12	8	3.44	8	100	0	0
11.	Jojoran	16	14	5.6	12	84.61	2	**15.39**
12.	Menur	10	9	4.3	9	100	0	0
13.	Keputih	10	6	3.01	6	100	0	0
14.	Mulyorejo	10	2	**0.8**	2	100	0	0
15.	Blauran	16	14	6.89	14	100	0	0
16.	Asemrowo	8	5	2.15	5	100	0	0
17.	Indrakila	10	10	4.3	10	100	0	0
Total		305	228		223	-	5	-

Table 4. Chi-square Test between independent variable and ectoparasite manifestation.

Variable			df	Total	Ectoparasites				p-value
					Negative (n=77)		Positive (n=228)		
					N	%	n	%	
Gender	Male		1	91	54	59.3	37	40.7	=0.000
	Female	Pregnant	1	134	5	3.7	129	96.3	
		Unpregnant	1	80	18	22.5	62	77.5	
Bodyweight	Low (< 2.85)		1	171	72	42.1	99	57.9	=0.000
	High (>= 2.85)			134	5	3.7	129	96.3	

Table 5. Multiple binary regression model of factors associated with ectoparasite manifestation.

Variable	Odds ratio	df	95% confidence interval		Standard error	P-value
			Lower	Upper		
Female vs Male	2.896	1	1.407	5.960	0.368	0.004
Low vs high weight body	2.988	1	1.403	6.354	0.386	0.005
Pregnant vs unpregnant	6.789	1	2.383	19.339	0.534	=0.000

eggs a day during a month so that the prevalence of ectoparasite still exist (Service, 2008).

The high infection rate of *Ctenocephalides felis* (85.6%) in this study is similar to other studies in Iran (ElSeify *et al.,* 2016); Israel (Salant *et al.,* 2014), and Nigeria (Omonijo & Sowemimo, 2017). The infection rate of this species was reported worldwide and varied, 25,6% in United Kingdom (Abdullah *et al.,* 2019), and 20.68% in Iraq borderline area (Bahrami *et al.,* 2012). In this study, we didn't check DNA samples of flea, while another survey in UK showed that most *C. felis* contained pathogens such as *Bartonella henselae*, *Bartonella clamidgeiae*, *Dipylidium caninum*, *Mycoplasma haemofelis*, and *Mycoplasma haemocanis* (Abdullah *et al.,* 2019). Urban area in Cuernavaca, Mexico, shows infection rate about 92.3% (Cruz-Vazquez *et al.,* 2001), United Kingdom during 2005 was 98.93% (Bond *et al.,* 2007), and Greece was 97.4% (Koutinas *et al.,* 1995). This survey was conducted during dry season, and the findings are in line with other studies showing *C. felis* as a predominant flea species during all seasons (Akucewich *et al.,* 2002; Chesney, 1995; Clark, 1999). This is also supported by the study result in urban areas in Germany (Liebich *et al.,* 1985; Visser *et al.,* 2001) and Denmark (Kristensen *et al.,* 1978).

Ctenocephalides felis was also reported as an ectoparasite that infected many mammals other than cats and dogs, such as red foxes (*Vulpes vulpes*), black rats (*Rattus rattus*), European rabbits (*Oryctolagus cuniculus*), and brown rats (*Rattus novergicus*). Meanwhile, in native species, *C. felis* was known infecting American opossums (*Virginia opossum*, *Didelphis virginianam*, *Didelphis marsupialis*); North American gray foxes (*Urocyon cinereoargenteus*), and Australian brushtail possums (*Trichsuorus vulpecula*) (Clark *et al.,* 2018).

Coinfection in five cats that were examined shows the distribution of *Felicola subrostratus*. This is in line with the survey reported in Greece and UK (Bond *et al.,* 2007; Koutinas *et al.,* 1995). Common coinfection was reported in the studies in Mexico and Germany (Beck *et al.,* 2006; Bond *et al.,* 2007; Cruz-Vazquez *et al.,* 2001). The species infecting found from the investigations on England include *Pulex irritans* coinfected with *C. felis* (Bond *et al.,* 2007). The low prevalence of *Felicola subrostratus* was in line with the previous investigations in Brazil (De Castro & Rafael, 2006; Morales-Malacara & Guerrero, 2007). The prevalence of *Felicola subrostratus* was higher than in United States which was only 1% (Thomas *et al.,* 2016); Florida roughly 1% (Akucewich *et al.,* 2002), and Thailand (4.2%). On the contrary, this prevalence was less than the investigations conducted by Salant *et al.* (2014) in Israel (14.4%). Increased prevalence may be because of different habitats between two populations.

This study highlighted the average number of *C. felis* in every cat was 2.54, while the average number of *F. subrostratus* was 0.33. The prevalence of *F. subrostratus* is not common, supported by low prevalence that has been reported from all continents, from Asia (Amin-Babjee, 1978; Eduardo *et al.,* 1977; Mustaffa-Babjee, 1969; Shanta, 1982), Europe (Trotti *et al.,* 1990), and Australia (Coman *et al.,* 1981). Highly infection rate of ectoparasites was more common in female stray cats (62.62%) than male stray cats (37.3%); this finding is supported by the study results from Sahimin (2012) in Kuala Lumpur. Sahimin (2012) also found *Ctenocephalides felis, Felicola subrostratus*, *Heterodoxus spiniger*, *Haemophysalis bispinosa*, and *Lynxacarus radovskyi* in their survey; and found that female stray cats were more likely to be infested with ectoparasites than in male stray cats (OR 2.8; p<0.004) (Aldemir, 2007). The prevalence of ectoparasite infestation was higher in female than male stray cats (89.3 % and 40%, respectively). This finding is in line with the study in Ismailia city which reported a greater ectoparasitic infestation in female stray dogs (AbuZeid *et al.,* 2015). Although there was no significant association between ectoparasitic infestation with sex, females domestic dogs from Erzurum, Turkey, tended to be more frequently infected by ectoparasites, especially by *C. canis* (Aldemir, 2007). It is believed some female behavioral factors would be responsible for this tendency, such as confining of female pets during the reproductive period that could favor re-infections by fleas in domestic areas (Aldemir, 2007).

Season and environmental factors affected the various prevalence manifestation of ectoparasite (Dyrden & Rust, 1994). The high prevalence of ectoparasite in this study may be affected by the dry season. Studies from Sahimin (2012) shows high prevalence of ectoparasite during dry season than in rainy season. Insemination and fertilization of flea can be affected by the host's body temperature and the occurrence of food around the host (Dean & Meola, 2002). The optimum temperature for the fertilization of fleas was 38°C, meaning the common temperature of cat and dog (Yue *et al.,* 2002). *C. felis* has a specific ability that supports it to move from one infected-host to each other with an average jumping speed of 3.6 m/s, jumping height of 13.2 cm, and jumping length of 19.9 cm (Cadiergues *et al.,* 2000). Association between bodyweight and the occurrence of ectoparasite in this study with p-value =0.000 shows the possibility of fleas jumping from one cat to another cat. This specific ability can also lead to the movement of fleas to humans regarding direct contact in traditional markets. The movement speed of each cat is different from each other and can be affected by some factors, such as pregnancy status. In this study, we found that pregnancy status shows a positive association with the occurrence of flea (p-value=0.000).

Market as a place that provides possible direct contact between flea-infected stray cats and humans must be considered regarding the occurrence of flea-borne diseases. Since *C. felis* found with high infection rate has been shown to transmit murine typhus and also has been implicated as a vector of plague, *Bartonella henselae*, which is the etiologic agent of cat scratch disease (Dyrden and Rust, 1994; Jameson *et al.,* 1995; Schrierfer *et al.,* 1994; Sorvillo *et al.,* 1993). This finding should be a baseline for flea management control. Flea allergic dermatitis is the most common nuisance caused by fleas in cats and dogs (Lee *et al.,* 1997). Those fleas can also bite humans and cause heavy inflammation (Youssefi and Rahimi, 2014). Six students from Malaysia were reported to be affected by flea allergic dermatitis (Chin *et al.,* 2010).

The high infection rate of stray cats with ectoparasites was affected by the high temperature and humidity of Surabaya which is 68%-84%, with a temperature of 27.8°C and 30.5°C. Reproductivity of flea will increase in humidity range of 80% and temperature of 27°C (Silverman *et al.,* 1981). The possibility of fleas to infect humans must be a consideration (O'Neal *et al.,* 2014). Serologic examination on stray cats in Yunani showed infection with some pathogens, such as *Bartonella henselae* (58,8%), *Rickettsia* spp. (43,2%), *Leishmania infatum* (6,1%), *Ditofilaria immitis* (4,7%), and *Ehrlichia canis* (2%) (Diakou *et al.,* 2017). However, the prevalence of flea-borne disease in stray cats still get limited consideration among health workers due to insufficient information about zoonotic diseases. The distribution of *C. felis* was mostly not affected by global warming (Roy *et al.,* 2009). This study was also supported by Maina *et al.* (2016) who have found 37.2% of squirrel and cats were infected with *C. felis*. Billeter and Metzger (2017) argued the possibility of fleas as a vector of *R. typhi*, but still not completed with the data distribution in humans so that additional study is important to reveal any association between murine typhus and flea. The lesion caused by cat's paws results in cat scratch disease (CSD) that is brought by *C. felis* (McElroy *et al.,* 2010), but the prevalence among cats is still unclear. Laboratory studies showed *C. felis* as the secondary vector of *Yersinia pestis*, though the efficiency was not as high as *Xenopsylla cheopis* (Eisen *et al.,* 2008). During the plague investigation in Uganda, Eisen *et al.* (2008) found that *C. felis* was the main fleas in rodents. In addition, *C. felis* is also known as a vector of a flea tapeworm, *Dipyridium caninum*. Humans can be infected if they ingest cysticercoids of *D. caninum*. High prevalence was associated with the occurrence of infected dogs or cats as their pet (Pan American Health Organization, 2003).

The importance of identifying flea in companion animals due to the role of the flea to transmit pathogens to humans with the historical note resulting in human plagues and black death (Bubonic Plague) (Gubler, 2009) and many impacts of the occurrence of flea in the environment such as nuisance, anemia, allergic reactions, and discomfort (Iannino *et al.,* 2017).

CONCLUSION

It can be concluded that the high prevalence of ectoparasites on the stray cats in Surabaya traditional markets must be a consideration among health workers as early mitigation and prevention of vector-borne diseases. Serologic and molecular test for the pathogens in stray cats should be conducted for the early detection of vector-borne zoonotic diseases.

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