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Fly species on cows around the Tangkoko Nature Reserve, North Sulawesi, Indonesia and their role as zoonotic disease vectors

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Abstract. *Nangoy M, Sondakh E, Koneri R, Hadi UK.2022. Fly species on cows around the Tangkoko Nature Reserve, North Sulawesi, Indonesia and their role as disease vectors. Biodiversitas 23: 631-636.* Climate and land-use changes in additional to livestock rearing systems play a role in the distribution, species, and population of fly pests, which may pose a risk to humans and animal health. The rearing and grazing of cows at the forest edge and in coconut plantations provide an ideal medium for the growth and development of flies. This study aimed to identify the diversity and abundance of fly species found on cows grazing in two habitat types: forest edge habitat and coconut plantation,located at the Tangkoko Nature Reserve in North Sulawesi, Indonesia. The study was conducted from April 2021 to June 2021 using 24 Ongole Crossbred cows including three adult males and three adult females (250-300 kg) and three males and three female calves (60-75 kg) in each habitat. Flies were collected using two fly nets that were swung along the cows in each habitat type with ten swings. The flies collected were identified based on morphology. A total of 6,982 flies were collected and identified to the following taxonomic groups: Muscidae family (*Haematobia exigua* 91.21%, *Stomoxys calcitrans* 1.65%, *Musca crassirostris* 0.67%, *S. indicus* 0.54%, *S. sitiens* 0.42%, and *Musca domestica* 0.11%), and Tabanidae family (*Tabanus factiosus* 4.91%, *Tabanus striatus* 0.47%, and *Chrisops* sp. 0.01%). The abundance of *Haematobia exigua* in the coconut plantation habitat was higher than in the forest edges, while the number of *T. factiosus* at the forest edges was higher than in the coconut plantations. Our results confirm that the presence of a variety of fly species and their abundance depends part on the grazing habitat of the cows. Further study of fly species diversity and pathogen status is critical to provide epidemiological data needed to develop control strategies to prevent the emergence of zoonotic diseases.

Keywords: Flies**,** *Haematobia, Musca, Stomoxys, Tabanus*

INTRODUCTION

Flies and mosquitoes are ectoparasites that play an important role in humans and animals health. Apart from being a nuisance, sucking animal blood, they are also vectors of viral, bacterial infection, and parasitic diseases (Schorderet-Weber et al. 2017). For example, various economic losses in livestock have been reported due to disturbances caused by cage flies, such as loss of weight, milk production decrease, and parasitic blood diseases (Taylor et al. 2012; Phasuk et al. 2013; Adalberto et al. 2020). In humans, *Tabanus* sp. fly is a vector for parasitic blood diseases such as Trypanosomiasis and Leishmaniosis (Keita et al. 2020).

Land conversion, climate change, and deforestation affect the distribution of disease vectors (Gottwalt 2013; Loaiza et al. 2019). Moreover, the increase in human and livestock populations and their maintenance patterns have led to an increase in the fly population, disturbing humans and increasing the potential for the spread of disease (Novita 2019). In Indonesia, in some areas with limited land, the pattern of cows rearing is dominated by traditional rearing and aggregate (1-5 heads) during the day on loose land on the forest's edge or in the fields, such as coconut plantations, while at night, they are tied to trees. The moist environment under the trees and the presence of cow dung are suitable media for the development of flies; thereby, most cows are surrounded by flies and are thin (Nangoy et al. 2021). Furthermore, limited land leads to close contact between livestock, humans, and flies, promoting zoonotic diseases through fly vectors (Novita 2019).

Research on flies such as Tabanidae has been carried out in various countries, including Africa (Taioe et al. 2017), India (Banerjee et al. 2015; Maity et al. 2020), Korea (Choi et al. 2020), Thailand in Tabanidae (Changbunjong et al. 2018) and Muscidae (Low et al. 2017; Lorn et al. 2019; Paslarua et al. 2021). In addition, research on *Stomoxys* spp. flies have also been carried out on various dairy farms such as America (El Ashmawy et al. 2020), Israel (Kahana-Sutin et al. 2016), Indonesia (Afriyanda et al. 2019), and Australian beef cow to control fly populations and prevent the spread of disease (James et al. 2021).

Furthermore, research on flies has been carried out on bats in the Tangkoko conservation area (Nangoy et al. 2021).

Nowadays, conservation area experiences a lot of pressure due to the increase in human needs and population growth, such as land conversion and climate change, affecting the distribution, population, and types of flies as disease vectors affects both livestock and wildlife, carrying a significant risk of spillover and cross-transmission of species and strains between populations (Kasozi et al. 2021). The Tangkoko Nature Reserve (renamed "Tangkoko Conservation Forest Management Unit" in 2016) is one of the conservation areas in Indonesia (O'Brien and Kinnaird 1996; Kyes et al.2013). The peripheral/boundary area around the reserve has experienced land conversion due to the camping ground, tourism park, resort, and gold mines. In this area, there is Batuputih village, which is suspected of having the potential as an epicenter for the spread of zoonotic diseases in terms of geographical aspects (villages bordering natural tourism areas and conservation areas that receive visits from various foreign tourists), social and cultural aspects (breeders carry out traditional lifestyles) that rely on natural resources and not a touch technology, and the economic aspects (most of the population has a non-permanent livelihood, farmers, breeders, and fishermen) which changes depending on the weather. Furthermore, residents generally keep Ongole crossbred cows with 1 to 5 heads by indulging in the forest's edge or under coconut trees. Therefore, this study was conducted to identify the fly species and calculate their abundance in the forest edge habitat and in a coconut plantations on the edge of the area. Data and information on flies around conservation areas are then needed to control disease vectors to prevent potential zoonotic diseases from spreading.

MATERIALS AND METHODS

Study area

This research was conducted in the peripheral area of the Tangkoko Conservation Area (Figure 1). The average rainfall is 2500 - 3000 mm/year (Pasetha et al. 2019). The average temperature is 20-25°C at night and 28-32°C during the day and humidity ranges from 80 to 85% were measured using a thermo-hygrometer (Deko 637 Thermo-hygrometer).

Fly sampling location

The fly sampling locations were carried out in two habitat types, namely forest edge with dense vegetation levels and coconut plantations. The forest edge habitat is shrubs composed of vegetation including Jejarongan (*Eupatorium odoratum*), Tembelekan (*Lantana camara*), Gadung vines (*Dioscorea hispida*), forest betel saplings (*Piper aduncum*), flower wood (*Spathodea campanulata*), Bugis wood (*Melia azedarach*), Glagah (*Saccharum spontaneum*) surrounded by trees including Rao (*Dracontomelon dao*), Nantu (*Palaquium amboinense*), forest Bugis (*Koordersiodendron pinnatum*), *Arenga pinnata*, forest betel (*Piper aduncum*), flower wood (*Spathodea campanulata*), and Binunga (Macaranga). The vegetation habitat under coconut plantations are *Brachiaria mutica*, *Digitaria decumbens*, *Mimosa pudica*, *Brachiaria humidicola*, *Imperata cylindrica*, *Ageratum conyzoides*, *Setaria sphacelata*, *Eleusine indica*, *Synedrella nodiflora*, *Gliricidia sepium*, and *Pueraria phaseoloides*.

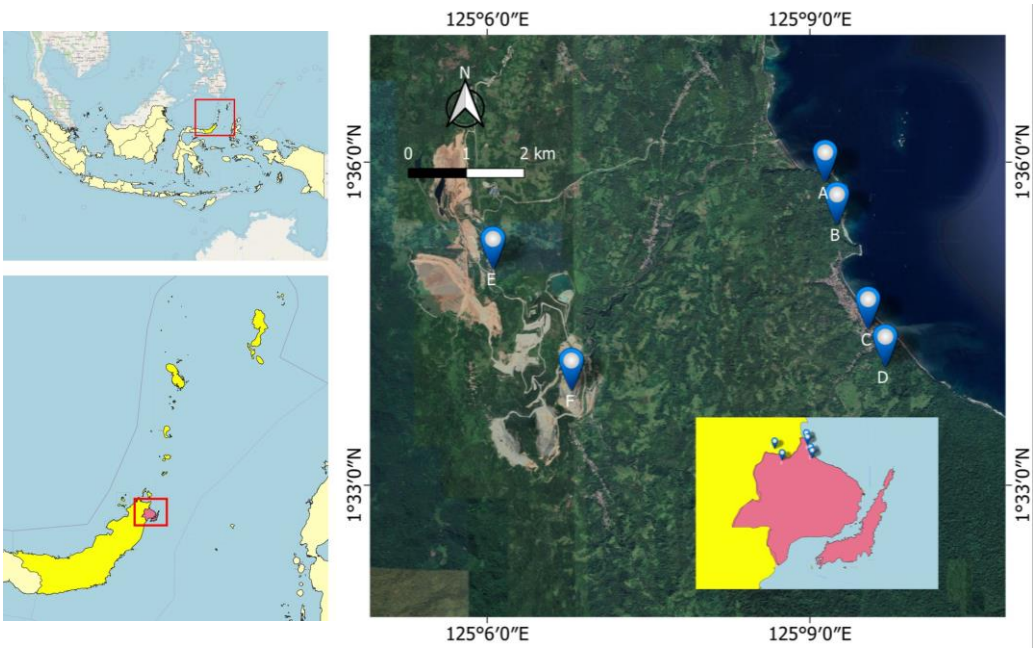

Figure 1. Map showing location of the Tangkoko Nature Reserve system (Tangkoko Conservation Forest Management Unit, outlined in white). The two sampling locations are indicated by the numbers white dots: 1. Forest edge; 2. Coconut Plantation; Yellow dot/letters identify the various locations mentioned in the Introduction: A. Canada Beach, B. Benteng Resort, C. Batuputih Village, D. Camping Ground, E. Toka Tindung Gold Minning F. Tondano Nusajaya Gold Minning (Courtesy: Google Earth 2020; Maxar Technologies 2020)

Sampling technique

A total 24 cows were sampled in this study. The fly collection was carried out using insect nets with a diameter of 23 cm and 35 cm on twelve cows (three males and three adult females (250-300 kg) and three males and three female calves (60-75 kg) in each habitat type. Meteorological factors effect on the activity of flies (Phasuk et al. 2011, Baldacchino et al. 2013, 2014; Tamas et al. 2015), therefore time collection of flies from 9:00 AM to 2:00 PM (high light intensity) from April to June in 2021 (last rainy and first dry season) in the study site, each month in the same cow. The cow used in this research was Ongole Crossbred with a grayish-white body-color weighing 250-300 kg for adult (18-30 months) males and females, while calves (6-9 months) weighed 60 to 75 kg. The sampling of flies on each cow was carried out using two insect nets which were swung alternately for 10 swings. The flies collected were killed using a killing jar and were immediately placed in labeled sample bottles for each animal. Furthermore, the fly samples were sorted identified based on the shape and size of the flies, and population calculations were carried out based on the species of flies. The identification of the Muscidae family flies was carried out using a key identification by Tumrasvin and Shinonaga (1978) and the Tabanidae family using the key identification by Stekhoven (1926). Moreover, the author (M.N.) took photographs under a binocular microscope using a digital Samsung Galaxi Note 3 camera and processed with Adobe Photoshop CS4. Each fly species was preserved as a dried insect in an insect box and deposited for further study in the Animal Wildlife Laboratory, Faculty of Animal Science, Sam Ratulangi University.

Data analysis

The collected data were analyzed based on quantitative descriptive methods, and parasite-host relationship analysis was carried out by examining the pattern of infestation based on abundance. Statistical analysis was performed to see preferences for sex, age, and habitat using the Bootstrap Method test (SPSS version 23) because the data was not normal distribution and limited sample (Reiczigel et al. 2019). The differences between gender, age, and habitat were not examined for flies with a small sample size (< 4%).

RESULTS AND DISCUSSION

Total flies collected

A total of 6982 fly individuals were collected in two research habitats. These flies belong to the families Muscidae (97.36%) and Tabanidae (2.64%). The Muscidae consists of *Haematobia exigua*, *Musca domestica*, *M. crassirostris*, *Stomoxys calcitrans*, *S. indicus*, and *S. sitiens* while the Tabanidae consists of *Tabanus factiosus*, *T. striatus*, and *Chrisops* sp. (Figure 2). *S. sitiens* was not in Figure 2 due to the low quality of the photo. Table 1 shows that the number of individual flies collected in the coconut plantation habitat (85.44%) was higher than at the forest edge (14.56%). The *Haematobia exigua* species dominated in both habitats 94.95%, while *Chrisops* sp. (1 individual only) was only found in forest edge habitats.

The total species found on the forest edge was higher (9 species) compared to under coconut trees (8 species) (Table 1). Subsequently, *Haematobia exigua* dominated both habitats by 97.95% under coconut trees and 77.33% at forest edges. Based on the total individuals, *H. exigua* under coconut trees was higher than at the forest edge, although the comparison test of two means using the Bootstraps Test showed no significant difference (P 0.071 > 0.05). The total number of *T. factiosus* was higher at the edge of the forest with 354 individuals (2.38%) compared to under coconut trees with 28 individuals (0.22%). Using the Bootstraps Test, the comparison test of two means showed a significant difference (P 0.001 < 0.05).

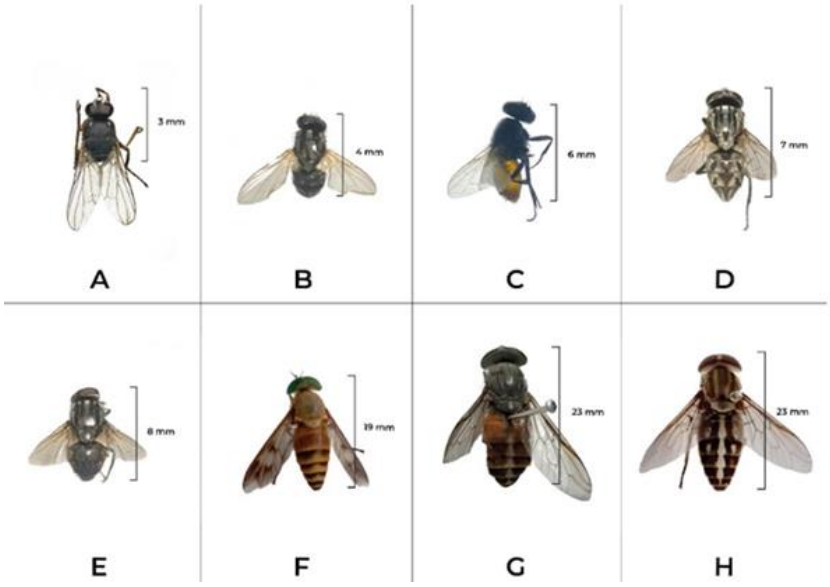

Figure 2. A. *Haematobia exigua*; B. *Stomoxys indicus*; C. *Musca domestica*; D. *Stomoxys calcitrans*; E. *Musca crassirostris*; F. *Chrisops* sp.; G. *Tabanus factiosus*; H. *Tabanus striatus*

Tabel 1. Total number of flies based on habitat, sex, and age

Family/species	Coconut plantation						Forest edge						Total	%
	Male		Female		Total	%	Male		Female		Total	%		
	A	C	A	C			A	C	A	C				
Muscidae														
Haematobia exigua	2703	409	1443	138	4693	97.45	928	493	134	120	1675	77.33	6368	91.21
Musca domestica	1	1	2	0	4	0.08	2	1	1	0	4	0.18	8	0.11
Musca crassirostris	12	3	5	4	24	0.50	4	11	4	4	23	1.06	47	0.67
Stomoxys calcitrans	20	3	18	1	42	0.87	15	27	13	18	73	3.37	115	1.65
Stomoxys indicus	8	3	7	1	19	0.39	6	6	3	4	19	0.88	38	0.54
Stomoxys sitiens	3	1	6	0	10	0.21	4	7	4	4	19	0.88	29	0.42
Tabanidae														
Tabanus factiosus	5	2	5	5	17	0.35	107	95	65	59	326	15.05	343	4.91
Tabanus striatus	1	2	1	3	7	0.15	2	14	4	6	26	1.20	33	0.47
Chrisops sp.	0	0	0	0	0	0	0	1	0	0	1	0.05	1	0.01
	2753	424	1487	152	4816	100.00	1068	655	228	215	2166	100.00	6982	100

Note: A: adult, C: calve

Flies by sex

Based on sex, the majority of fly species found was female, which is higher than the male with 8 species (Table 1). Subsequently, the total individual flies found in male cows was 4900 (70%), higher than in females with 2082 (30%) (Table 1). *Haematobia exigua* dominated both sexes (4533 (71%) in males and 1835 (29%) in female cows). The comparison test of the two means using the Bootstraps Test showed a significant difference (P 0.014 < 0.05). *T. factiosus* was dominantly found in male cows with 209 (61%) compared to female cows with 154 (39%). The comparison test of the two means using the Bootstraps Test showed a significant difference (P 0.001 < 0.05).

Flies by age

The total number of fly species found in adult cows was higher with nine compared to calves with 8 species (Table1). Based on age, 5536 flies were found on adult cows (79.29%) and 1446 on calves (20.71%). Using the Bootstraps Test, the two-mean comparison test between adult cows and calves showed a significant difference (P 0.001 < 0.05). Furthermore, about 28 individuals of *T. factiosus* were found in adult cows (0.22%).

Discussion

Generally, the flies found were blood-sucking with a significant role as mechanical vectors of bacterial, viral, parasitic diseases for animals and humans (Bawn et al. 2015). Flies species and their populations depend on their life cycle, host dependence (Foil and Hogsette 1994), and environment (Changbunjong et al. 2018). There are differences in the number of species and fly populations because of the distinctive environments with various vegetation. In the forest edge environment with a high level of vegetation, high humidity is a suitable habitat for Tabanus flies, while in the environment under coconut trees, the vegetation does not provide opportunities for *Haematobia* flies to lay eggs. Therefore, the fly population is strongly influenced by temperature, light intensity, and humidity (Baldacchino et al. 2013, 2014; Tamas et al. 2015). Furthermore, Schafaschek et al. (2021) reported that the population of *Haematobia irritans* fly is high in livestock rearing systems integrated with plants because this system provides a suitable environment for their development. The fly population by sex was higher in male cows due to hormonal influences because testosterone acts as a fly attractant (Dobson et al. 1970). Meanwhile, based on age, it was found that the population was higher in adult cows than in calves. This is because the body size affects the population, providing a place to perch, and the CO_2 produced by the adult cows is higher than that of calves, where CO_2 is an attractant for flies.

Haematobia exigua is the dominant species. It has gray-brown or black, small (3-4 mm) in size with sharp mouthparts capable of penetrating animal skin, found in hundreds to thousands in one livestock. Despite their small body shape, these flies have the ability to bite 40 times per day hence they are very disturbing and cause injuries (James et al. 2021). This species is obligate or always in livestock and only leaves livestock when laying eggs in fresh feces (Floate 2011). Without appropriate handling, it becomes pests because of the wounds caused by irritation and its role as mechanical vectors of the Stephanofilaria worm parasite. Climate change greatly affects the population (James et al. 2021). Controlling the population of *H. exigua* should be carried out if it is more than 200 individuals per head (Adalberto et al. 2020). This fly is found highly number in adult cows. In this study, more than 200 individuals per head was found in male adult cows at under coconut tree plantations with an average 300.33 individuals per head because cows are tied with ropes in coconut trees, hence their movement is limited and always close to fresh feces, which is a breeding ground for these flies. Moreover, the hormone testosterone in adult male cows is an attractant for these flies (Dobson et al. 1970).

Musca domestica, M. crassirostris, Stomoxys calcitrans, S. indicus, and *S. sitiens* were found in a low population (mean < 10 per head). *M. domestica* is the house fly and breeds in human waste and feces, acting as a mechanical vector for anthrax in animals and humans. It has an orange abdomen with a spongy mouthpart (Tumrasvin and Shinonaga 1978). Furthermore, it also acts as a mechanical vector of Salmonella and Escherichia bacteria that cause digestive disorders in humans. In this research, it was

relatively low compared to other flies due to the lack of food waste at the research site. However, it was found around the eyes and noses of cows by eating secretions of fluid in the eyes (Desquesnes et al. 2018) and plays an important role in human and animal health as disease vectors (Issa 2019).

Musca crassirostris, known as the cow fly, was found with a body size 5.5, up to 7.5 mm. The male has a black abdomen, and the female has a grayish-green color. Furthermore, it is categorized as biting flies even though they are believed to act as facultative blood-sucking flies and vector parasites of worms (Thelaziidae) in the eyes of animals and humans (Desquesnes et al. 2018).

The genus *Stomoxys* had a wide distribution, breeding in cow dung mixed with rotting grass (Rochon et al. 2021). It is synanthropic, persistent bloodsuckers and plays a role in anthrax disease (Baldacchino et al. 2013). Its body size of 5-7 mm is larger than *Haematobia*. Subsequently, males and females play a role in sucking the blood of cows with their mouthparts. The thorax has four black stripes, and the abdomen has patterned black spots (Tumrasvin and Shinonaga 1978). If there is no livestock in an area, these flies can attack humans to get food. In Florida, these flies disturb tourist areas. The threshold value for controlling its population is 10 flies per animal or 5 individuals per foot of livestock (Adalberto et al. 2020). The population of the *Stomoxys* genus in this research was low (0.42% to 1.65%) because data collection was carried out outside the range of time the flies were active. Furthermore, research by Soviana et al. (2019) showed that *S. indicus* and *S. sitiens* are active in the morning (06.00 to 08.00) and afternoon (16.00 to 17.00). Humidity and light intensity significantly affect population abundance (Phasuk et al. 2013; Semelbauer et al. 2018). *Stomoxys* sp. is found in the legs and lower abdomen. The same thing was also reported by Lendzele et al. 2019 who conducted research on the bionomics of this fly species in African Cameroon. This fly is very mobile has the ability to fly high (Rochon et al. 2021), hence it is not collected in this research because the collection was carried out using insect nets.

Tabanus has a large body size compared to other species. The females suck blood and inflict wounds on the bite marks to suck blood for smaller flies. Therefore, it acts as a vector for Surra disease in livestock (Maity et al. 2020) and humans (Novita 2019). According to Stekhoven (1926), there are four species of *Tabanus* occurred in Sulawesi, namely *T. factiosus, T. flexilis, T. reducens,* and *T. striatus*). In this research, only two species were successfully collected (*T. factiosus* dan *T. striatus*). This is presumably because the research area was limited time and only carried out in lowland areas. *Tabanus* flies live in moist, wet environments with high vegetation, especially in areas with small rivers or swamps (Baldacchino et al. 2014). Therefore, the population abundance was higher in adult cows at the edge of the forest because the vegetation in this location was dense. Moreover, the body parts that these flies like are the head and neck. *T. striatus* is known as the mechanic vector of *Trypanosoma evansi* in Asia (Wongthangsiri et al. 2019).

According to the breeders, the weight gain of the cows was very low, hard to breed, was pregnant but suffered a miscarriage. Therefore, it is suspected that cows suffer from Surra disease caused by the blood parasite *Trypanosoma* (Desquesnes et al. 2013). However, further examination is needed to confirm the diagnosis by examining the blood to find the parasite. Indonesia is an endemic area for Surra disease (Novita 2019). With the high of species and abundance of fly populations, the hot environment, and increasing human contact with animals and flies, the chances of the emergence of zoonotic diseases transmitted by flies need to be watched out for in the Tangkoko Nature Reserve.

In conclusion, this study provides data about species and their number of flies in forest edge and coconut plantation. Our results confirm the presence of species and number of flies depend on their habitat, environment. Further studies about species diversity and abundance of flies related to animal diseases in the areas can provide epidemiological data to develop control strategies to prevent the emergence of zoonotic diseases.

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