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# The Diversity and Activity of Flies (Diptera: Cyclorrhapha) as Forensic Indicators at Outdoor and Indoor Rat Carccasses in Dramaga, Bogor

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#### **Abstract**

This study aimed to identify the diversity of flies that were caught and interacted with carcasses at the day and night. Three rat carcasses (Rattus norvegicus) were placed at the outdoor and indoor area of IPB Dramaga campus, respectively. The carcasses were put into a screen cage. Observation of flies was begun at 06:00 am and continues every four hours until the skeletal stage of the carcasses was reached. The flies that come to the carcasses and into the screen cage were collected and identified. The decomposition of the indoor carcasses is slower than at outdoor, with the longer fresh period. Meanwhile, post-decay stage and skeletal stage were achieved at the same time at both locations. Nine species captured flies which are forensic indicator were Chrysomya megacephala (Fabricius), Chrysomya bezziana Villeneuve, Chrysomya rufifacies (Macquart), Chrysomya saffranea, Chrysomya spp, Lucilia spp, Sarcophaga spp, Calliphora spp and Musca domestica. C. bezziana Villeneuve and C. megacephala (Fabricius) were the dominant flies at outdoor carcasses. Meanwhile, the indoor carcasses were dominated by Lucilia spp.

Key words: Forensic Entomology, Chrysomya, Dramaga, Lucilia.

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#### Introduction

Measuring the decomposition and development of various insects on carcasses have the potential to be a useful and accurate tool for determining the duration of post mortality intervals (Post Mortem Interval = PMI). The lack of this method is the minimum of information on the flies species on carcasses in line with the decomposition process (O'Flynn 1983). Immediately after death, the decomposition process of the corpse will occur. The decomposition stages of the carcasses varies greatly but generally are begun with a fresh stage, bloated stage, decay stage, post decay stage, and skeletal stage (Joseph *et al.*, 2011). Corpse generally found in decaying condition (decay stage). This stage is known as the active decaying stage. The decomposition stage occurs due to the combination of bacterial activity and body tissue-eating larvae.

Involvement of insects on the process of corpses decomposition or carcasses can be used as evidence to determine PMI (Kristanto et al., 2009). There are many populations of insects in

nature, but not all insects can be an indicator of PMI. Several groups of insects belonging to the PMI indicator are a group of necrophagous, predatory, and omnivorous insects. Necrophagous insects of various species experience the population succession dynamics according to the stage of carcasses decomposition. Various insects will interact each other either as neutraly, competitionly, and predationly in the process of carcasses decomposition.

Several studies related to the forensic entomology studies in Indonesia have been reported. Supriyono (2013) reported that the speed of carcasses decomposition indoor is faster than the carcass outdoor. In addition, the existing of Diptera insects is more varied on the carcass outdoors. Insects of the Diptera order that dominate in process of carcasses decomposition are Calliphoridae, Tachinidae, Muscidae, and Sarcophagidae (Supriyono 2013; Wangko *et al.*, 2015). However, information on forensic insects in Indonesia has not been widely reported. In addition, insects that engage on the carcasses at night have not been reported. The obstacles of PMI determination is the behavior of flies that lay their eggs at night (Sharma *et al.*, 2015). This study aimed to identify the diversity of flies that caught and interacted to carcasses at the day and night.

# Materials and Methods

#### Study Site

This research was conducted from December 2017 to February 2018 in Dramaga district, Bogor Regency, Indonesia. The study was conducted at two different locations that are indoor and outdoor area. Preservation and identification of insects was conducted at the Health Entomology Laboratory, Department of Animal Diseases and Veterinary Public Health, Faculty of Veterinary Medicine Bogor Agricultural University (FKH IPB).

#### Modeling Animal

Animal models in this study used rat (*Rattus norvegicus*) derived from the Laboratory of Animal Management Unit FKH IPB. There are 6 white rat aged 1 to 2 months weighing 150-200 grams were killed by dislocation of the neck (SKEH Number: 080 / KEH / SKE / 1/2018).

#### Procedure of Research

The outdoor location was in the yard of the house which had no high vegetation characteristics (no shade) and the distance from the nearest house is approximately 15 meters. Meanwhile, indoor location is in a residential area of 5x5x5 m. There are two windows on one of the walls that were opened during the study with an opening clearance of  $\pm$  5 cm to provide the entrance of flies.

Three carcasses were put in the morning (06.00 am) at outdoor and in indoor location, respectively. The rat carcasses were put in a trap of gauze-shaped of 60x60x60 m with holes on the bottom to prevent the flies of getting out. In addition, the trap cage was useful to avoid interference from other animals.

The observation of the decomposition stage and the collection of flies that enter in the trap were conducted until the carcasses reached the skeletal stage. Measuring of carcass

decomposition was conducted by observing the changes that occurred on the carcass every 4 hours.

Adult flies collection was begun in four hours after the carcass was placed and continues every 4 h until the skeletal stage of the carcass was reached. Flies that are around the cage were collected using sweeping net. The flies that are caught then were killed using chloroform and processed as pinning specimens (Hadi and Soviana, 2010). Identification of flies was conducted using Szpila identification keys (2009), Amendt *et al.*, (2010) Marshall *et al.*, (2011), and Spradbery (2002). Measurement of temperature and humidity, temperature and pH of carcasses are also conducted out at the same time and frequency with the collection of flies.

#### Data Analysis

The data of decomposition velocity, type, amount, and arrival time of flies are shown in table and graph. In addition, data analysis of flies species diversity was conducted by calculating relative abundance (KN), frequency (FS) and species dominance (DS), as well as the species diversity index (H ') Shannon-Wiener (Odum 1993). Criteria for high categorical diversity index if H > 3, medium  $1 \le H \le 3$ , and low H < 1 (Krebs, 1987).

# Results and Discussion

#### Decomposition of Carcasses

Fresh stage of decomposition of the carcass at indoors with environmental temperature range 25.03-28.87 °C was achieved in a longer time (24 hours) than outdoors in a temperature range 24.67-38.40 °C. While the bloated stage is the shortest in the room with a temperature range 26.77-29.00 °C than outdoor in the environmental temperature range 24.03-32.76 °C. Decay stage occurs longer on the carcass outdoor in a temperature range 22.03-40.47 °C and indoor in a temperature range 27.50-29.93 °C. Post delay to the skeletal is generally achieved at the same time by the carcass either indoor or outdoor in environmental temperature range 23.00-29.63 °C (outdoors) and 21.30-28.57 °C (indoors). The decomposition time length of the carcass outdoors was 100 hours and indoors was 112 hours. The temperature of the carcass at outdoor was 25.44-31.64 °C and at indoor was 24.47-27.89 °C, the degree of acidity (pH) of carcasses at outdoor was 4.8-6.7 and at indoor was 5.2-6.5 and outdoor humidity was 72.77-78.1% and indoor humidity was 72.38-81.03%.

The rate of carcasses decomposition outdoors was faster than the indoors carcass. Based on the results of previous research, Anderson (2011) reported that pig carcasses placed on outdoor decomposed faster than indoors. Observations of Mabika *et al.*, (2014) carcasses of rabbit placed on outdoor without shade were faster than carcasses with shade. Similarly, Zeariya *et al.*, (2015) carcasses of dogs and rabbits in Cairo. The rate of carcass decomposition is due to the sunlight exposure that increase the metabolic process. This was because of the optimum temperature of much-decaying microorganisms on meat was 28-30 °C (Prihharsanti, 2009). The metabolic process produced an odor that attracted many flies to come. The scent resulted from metabolic process can be captured by the flies antenna, which act as a sensory system. Types of small hairs located on the antenna act as a catcher of physical stimuli (tactile), scents, temperature, humidity, and sound recipient (Hadi and Soviana 2010). The activity of large flies attracted to carcasses also resulted in rapid decomposition (Ekanem and Dike 2010).

Meanwhile, Ahmad et al., (2011) and Supriyono (2013) report delay in the arrival of flies on the carcass located at indoor. The present of flies in the carcass at indoor was prevented by

the wall of the room and the small gaps to pass. Anderson (2011) reported that there was a postponement of flies arrival for 5 days in pig carcasses placed in closed rooms. The smell of carcass at indoor took a long time to spread to the environment. This factor affects the speed of flies finding carcasses.

The Relative Abundance, Frequency, Dominance Species, and Flies Diversity Index, and Fluctuation of Flies Presence

Five hundred and sixty-three flies were found at outdoors and 83 were at indoors. Flies species were *C. bezziana* Villeneuve, *C. megacephala* (Fabricius), *C. rufifacies* (Macquart), *C. saffranea*, *Chrysomya spp*, *Lucilia spp*, *Sarcophaga spp*, *Calliphora spp*, and *M. domestica*. Generally, the peak activity of the caught flies occurred towards and at night.

Table 1. The Relative Abundance (KN), Frequency (FS), Dominance Species (DS), and Flies Diversity Index (H') at the Outdoors

Flies	KN (%)	FS	DS (%)	H'
C. bezziana Villeneuve	26.82	0.63	16.89	1.94
C. megacephala (Fabricius)	21.31	0.59	12.63	
Chrysomya spp	13.50	0.37	5.00	
C. rufifacies (Macquart)	12.43	0.44	5.53	
Sarcophaga spp	8.17	0.48	3.93	
Lucilia spp	7.82	0.37	2.89	
C. saffranea	5.68	0.41	2.32	
M. domestica	2.66	0.11	0.30	
Calliphora spp	1.60	0.15	0.24	

Table 2. The Relative Abundance (KN), Frequency (FS), Dominance Species (DS), and Flies Diversity Index (H') at the Indoors

Flies	KN (%)	FS	DS (%)	H'
Lucilia spp	43.37	0.52	22.63	1.68
Calliphora spp	14.46	0.26	3.77	
Chrysomya spp	13.25	0.26	3.46	
C. megacephala (Fabricius)	9.64	0.26	2.51	
C. rufifacies (Macquart)	9.64	0.26	2.51	
C. bezziana Villeneuve	4.82	0.17	0.84	
Sarcophaga spp	3.61	0.09	0.31	
C. saffranea	1.20	0.04	0.05	

#### Outdoor

The existing of a forensic indicator flies at an outdoor carcass was begun 8 hours post-mortality (Figure 1). Furthermore, at 24-48 h post-mortality, vorious species of flies were present and were dominated by *C. bezziana* Villeneuve and *C. rufifacies* (Macquart). Flies *C. megacephala* (Fabricius) have been caught in the highest number at the 56 h post-mortality followed by *C. bezziana* Villeneuve at 52-68 h post-mortality. The highest number of flies have been caught inside and around the trap occurred at the 28-68 h post-mortality. Furthermore, 72-100 h post-mortality, the number of flies began to decline.

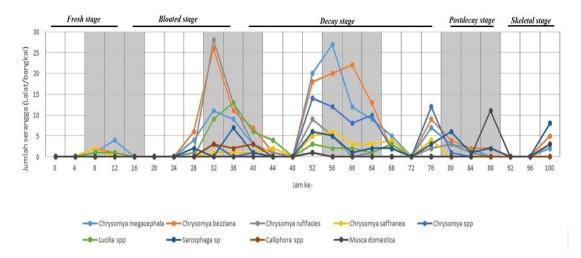


Figure 1. Fluctuations of flies captured per hour of outdoor observation. The bright part shows the time ante meridiem (am) and dark is post meridiem (pm).

C. bezziana Villeneuve and C. megacephala (Fabricius) were the flies that dominate the carcasses at outdoors (Table 1), belonging to the Calliphoridae group. Flies C. bezziana Villeneuve and C. megacephala (Fabricius) were always trapped from fresh stage to skeletal stage. The existing of both types of flies were high at the end of the bloated stage until the decay stage and tended to decrease at the end of the decay stage until the skeletal stage.

Previous research shows that flies from the family Calliphoridae dominate on carcasses placed outdoors (Supriyono 2013). However, flies which dominated in the carcasses at outdoors was *C. rufifacies* (Macquart) followed by *C. megacephala* (Fabricius). While *C. bezziana* Villeneuve flies were found in a little amount.

C. bezziana Villeneuve was a mandatory myiasis flies in livestock of Indonesia. Generally, this myiasis occurred post-partum was characterized by the presence of vaginal myiasis and followed by umbilical myiasis in calf. In addition, this flies was a major cause of traumatic myiasis in livestock. The biological properties of C. bezziana Villeneuve as the primary myiasis flies require a fresh wound to lay their eggs. In contrast the other types of myiasis flies (secondary) require odor stimulation as a signal to lay their eggs (Wardhana and Muharsini 2005; Wardhana et al., 2018).

Several studies that reported the existence of *C. bezziana* Villeneuve on carcasses were Lee *at al.*, (2004) in Malaysia, Sukontason *et al.*, (2007) in Thailand, and Supriyono (2013) in Indonesia, the findings in this study indicate the large of *C. bezziana* Villeneuve was present at the bloated stage until the decay stage. At the stage was a very stinging carcass smell. These findings suggest that *C. bezziana* Villeneuve flies that were known to oviposition on living tissues were also interested in carcasses. However, in this study, it is not known whether there are *C. bezziana* Villeneuve larvae on the carcass.

Generally flies follow the trail of smell to find food sources. The experiment conducted by Bunchu *et al.*, (2008) shows the response of *C. megacephala* (Fabricius) that is more attracted to the smell of food products from the animal origin than plants.

C. megacephala (Fabricius) flies are scattered all over the world and are found throughout human settlements. This flies likes rotten household wastes, especially animal tissues. This is

what causes these flies to be found in many urban settlements (Badenhorst and Villet 2018, e Castro *et al.*, 2016). Flies *C. megacephala* (Fabricius) is also known as the main pest of fish products (e Castro *et al.*, 2016).

#### Indoor

Flies caught in the carcass at indoors start 20 hours post-mortality. At the 20-28 h, the flies Lucilia spp dominated the carcass indoors (Fig. 2). The flies still dominated the population of trapped flies up to 68 hour, but after 68 h post-mortem was dominated by Calliphora spp. Flies C. megacephala (Fabricius) were recorded high after 88 h and followed by Lucilia spp which also high at 92 h post-mortem. At the end of the decomposition stage, only C. rufifacies (Macquart) and Sarcophaga spp were still coming to the carcasses. Despite the number of fluctuating flies in the carcass during observation, but Lucilia spp dominated in its entirety.

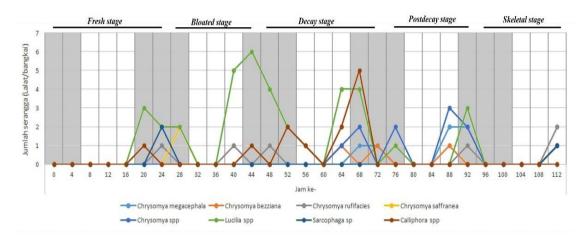


Figure 2. Distribution of insects per day per hour of indoor observation. The bright part shows the time ante meridiem (am) and dark is post meridiem (pm)

Lucilia spp flies was found coming to carcasses on fresh stage to post decay stage. Meanwhile, Calliphora spp from fresh stage to decay stage. Both of flies dominate on the carcass indoors (Table 2). Previous research in Southern Finland reported that flies that dominate human corpses in enclosed spaces are L. sericata Meigen and Calliphora vicina (C. vicina) Robineau-Desvoidy. Flies L. sericata Meigen is abundant during the summer (Pohjoisma ki et al., 2010). Martín-Vega et al., (2017), observing pig carcasses housed indoors in Spanish cities reported that L. sericata Meigen was colonized during summer, spring, and autumn. While C. vicina Robineau-Desvoidy is a type of fly that colonizes singly in winter and is present on the carcass after 24-48 h after death in all seasons.

Lucilia spp was faster coming to the indoor carcass. Previous research reports suggest that flies L. sericata Meigen in European countries are always found in corpses indoors. It is therefore estimated that the fly prefers an in-house rest (endophilic) than other types of Lucilia flies (Pohjoisma"ki et al., 2010; Martín-Vega et al., 2017). In addition, the size of the flies body is small to medium (<8 mm) so able to through a small gap.

#### Activity of Flies

Flies visited the carcass not only for oviposition or larvipara but also for copulation and eating (Zeariya et al., 2015). All species of trapped flies either outdoors or indoors did the activity at night. This fact suggests that the fly used as a forensic indicator can affect oviposition at night. This assumption is same as the findings of Smith et al., (2016) who reported that C. megacephala (Fabricius) came to the carcass at night or in the absence of light. The flies of Lucilia cuprina, L. sericata and C. megacephala (Fabricius) can do oviposition on the carcasses outdoor the night at Grahamstown and Durban, South Africa (Williams et al., 2017). Flies Calliphora vicina can do oviposition in total darkness condition (Bonacci et al., 2016).

#### Conclusion

The rate of carcass decomposition was faster at outdoor than indoor environment. The spesies of trapped flies were *C. bezziana* Villeneuve, *C. megacephala* (Fabricius), *C. rufifacies* (Macquart), *C. saffranea*, *Chrysomya spp*, *Lucilia spp*, *Sarcophaga spp*, *Calliphora spp*, and *M. domestica*. Generally, the peak activity of the trapped flies occurred afternoon and at night. *C. bezziana* Villeneuve and *C. megacephala* (Fabricius) dominated the carcass on outdoor. While indoor were dominated by *Lucilia spp* and *Calliphora spp*.

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