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The Number of *Escherichia coli* on Several Flies from Residential and Landfill Areas: *Drosophila* is The Least!

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Abstract. Flies are vectors of disease because flies can transfer bacteria from dirty places to food or the human body. This study aimed to determine the differences in the number of *Escherichia coli* carried by different flies from different locations (residential and landfill areas). This study involved three types of flies, i.e., *Musca domestica*, *Chrysomya megacephala*, and *Drosophila* sp. Catching flies was carried out at 02.00 to 05.00 pm for four times a week. Calculation of *E. coli* number using the colony counter and two-way ANOVA was chosen to analyze the data. As a result, the type of fly had a significant effect on *E. coli* number ($p < 0.001$), while differences in location did not give a significant effect ($p = 0.533$). The location and the type of fly also did not show a significant interaction ($p = 0.816$). Interestingly, Drosophila is the type of fly that carries the least amount of *E. coli* compared to the other two types of flies.

INTRODUCTION

Health problems caused by waste contamination have become a vital issue for humans. Waste is identical to the source of various diseases. Not surprisingly, the health problems of residents living around waste disposal sites are often reported, such as on Antilles Island of the Caribbean [1], South Africa [2], Nigeria [3], Brazil [4], and India [5]. Health problems become increasingly critical if waste processing is still carried out with the concept of collect-haul-dispose, a method that is still commonly found in various locations in Indonesia [6].

Various diseases are increasingly appearing in the area of landfills, from colds and eye irritation [2] allergies, asthma, and gastrointestinal disease [5], as well as various other infectious diseases [7]. One of the major causes of the disease is the presence of pathogen microbes [3,8]. Pathogenic microbes will be easy to find in dirty places because they naturally live in the trash [8–10]. The problem is, various microbes can be spread to other places outside the trash through vector organisms [11,12].

One of the vectors of the spread of disease is a fly. Flies are one of the most common insect groups found around humans [13–15]. Flies can carry a variety of bacteria because of their eating, developing, and mating habits in the unsanitary area [16,17]. The outbreak of humans with various pathogens occurs when flies land on food, eating utensils, vegetables, and fruit, or human body parts. [18,19]. Not surprisingly, flies are considered as the main cause of diarrhea in several countries [20,21]. Therefore, the presence of flies in less clean areas, such as in landfills, needs to be aware.

Mulyoagung is one of the areas in Malang that has a landfill. The distance between the landfill and the residential areas in Mulyoagung is only around 10 m. Also, there are fly populations scattered in the landfill area. Many fly larvae are seen in the garbage pile and produce a pungent odor. In line with conditions in a landfill, residential areas around the landfills also have flies at many locations in densely populated areas. Besides being close to the landfills, in the residential areas, there are also poultry cages and fish ponds, food stalls, kiosks, to fields. The proximity of the landfill area to residential areas and a large number of flies in the two locations could potentially disrupt the health of residents because bacteria from the landfill could be carried over to the residential areas by flies.

One of the bacteria commonly found in the body of a fly is *Escherichia coli*. The statement is based on various studies examining the existence of microbes in *Musca domestica* [19,22], *Drosophila replete* [16], and *Drosophila*

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virilis [23]. *E. coli* is included in gram-negative bacteria [24], and a member of the family of Enterobacteriaceae [25]. In general, these bacteria are found in the intestine of humans or animals [26] and going out into the environment along with the release of feces [27]. The presence of this bacterium needs to be aware because, in some cases, it can cause various diseases, from diarrhea to meningitis [25,28,29].

In Indonesia, studies on *E. coli* contamination are still often aimed at measuring water quality [30–32] because, *E. coli* is also widely known as one of the bioindicators of pollution that can provide information about certain environmental conditions [27,33,34]. On the other hand, studies on the presence of *E. coli* in various flies from various places are still rarely conducted. Similarly, studies that try to uncover the amount of *E. coli* from flies in residential areas with landfills are also rarely found. This kind of research will provide information about the potential risk of fly density on human health, as well as the risk of residential areas close to the landfills. Therefore, this study aimed to reveal whether there are differences in the number of *E. coli* found in several types of flies from landfill and residential areas in Mulyoagung, Malang.

EXPERIMENTAL METHODS

Materials

The equipment used in this study included flynet, tweezers, autoclave, ONEMED vacutainer, incubator, refrigerator, microscope, Petri dish, LAF, hot plate, 500 mL glass beaker, magnetic stirrer, centrifuge tube, glass funnel, plastic bag, analytical scales, tweezers, 3 mL syringes, markers, loopful, Bunsen burner, 100 mL Erlenmeyer flasks, and label paper. The materials used during the study included 300 mL of water tape, 1 L of sterile "WaterOne" distilled water, 9.375 g of Eosin Methylene Blue (EMB) agar, and 10 mL of 70% alcohol.

Catching flies

This research was conducted in November 2018. Catching flies was carried out four times in two weeks, from 02.00 to 05.00 pm. The flies examined in this study were house flies (*Musca domestica*), green flies (*Chrysomya megacephala*), and fruit flies (*Drosophila* sp.) collected in the residential and landfill areas of Mulyoagung, Dau District, Malang Regency. The flies were caught using a sterilized flynet beforehand using 70% alcohol. Sweeping was carried out at each location. In the flynet was given bait in the form of water tape so that the smell attracts the fly to come. The nested fly was then taken using tweezers and transferred into a vacuum container filled with sterile distilled water. The flies that have been captured were taken to the Biomedical Laboratory of the Faculty of Medicine, Universitas Muhammadiyah Malang.

Calculation of the *E. coli* number

The process of bacterial isolation used scraping or scratching (strike plate) method. Water samples containing flies were then diluted in a centrifuge tube, then shaken for 1 min (from the dilution taken 1 mL to be diluted again to some degree of dilution). The solution in the centrifuge tube was put in an incubator 37 ^{o}C for 24 h. The results of the dilution were inoculated on the EMB agar selective medium placed on a Petri dish by taking one single suspension of material containing bacteria, followed by making scratches on the agar surface. In the next process, the Petri dish which has been given a name tag was reversed and repacked. Then, the Petri dish was stored at 37 °C in an incubator for 24 h. Then, *E. coli* identification was made by direct vision. The *E. coli* colony is golden-green like metallic sheen. Calculation of the amount of colored *E. coli* used colony counter with units of CFU/mL.

Data Analysis

After the data were collected, descriptive and inferential statistical analysis was performed. In descriptive statistical analysis, the calculation of the mean value of *E. coli* number from each fly was carried out. The mean data obtained were then presented in the form of bar graphs. In inferential statistical analysis, a two-way analysis of variance (ANOVA) test was chosen to test whether or not the influence of location and type of fly on the amount of *E. coli* was found. Before the ANOVA test was performed, the log10(x+1) transformation was undertaken. If there were factors

that had a significant effect, posthoc tests were carried out. The posthoc test used was the Least Significant Difference with a significance level of 5%.

RESULTS AND DISCUSSION

In this study, the calculation of the *E. coli* number from three types of flies originating from residential and landfill has been done. The average number of *E. coli* from each type of fly is presented in Figure 1. Based on Figure 1, the highest number of E. coli was found in *M. domestica* in the landfill region, followed by *C. megacephala* from the same location. On the other hand, the number of *E. coli* in the body of *Drosophila* sp. much smaller than the other two types of flies. Other information obtained from Figure 1 is that in all three types of flies, the number of *E. coli* from the landfill region is always more than flies from residential areas.

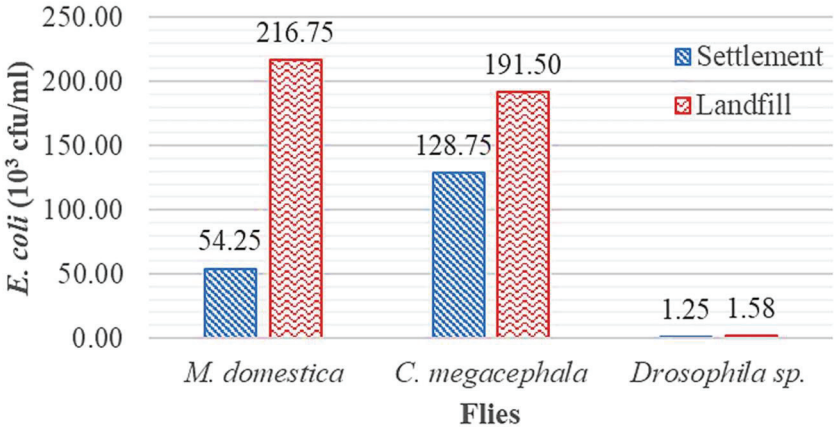

FIGURE 1. Graph of differences in the average number of *E. coli* from three types of flies from residential and landfills areas

A summary of the ANOVA test results is presented in Table 1. Based on Table 1, differences in the types of flies caused differences in the number of E. coli found [F (2.18) = 35,800, p <.001; ηp2 = 0.799]. On the other hand, location did not have a significant effect on the number of E. coli in the body of the fly [F (1.18) = 0.405, p = .333; ηp2 = .022]. In addition, the ANOVA test results informed that there was no significant interaction between the location of fly origin and the type of fly [F (2.18) = 0.206, p = .816; ηp2 = 0.022].

Investigating the role of flies around human habitation as carriers of bacteria is essential. Such investigations can be used as a basis in determining the spread of pathogenic bacteria in residential areas. In this regard, the number of *E. coli* obtained from flies from residential areas did not differ significantly from those from landfill areas (Table 1). The distance between the settlement and the landfill used as the location for the sampling of this study is only around 10 m. Such conditions increase the chance of flies from the landfill area to fly towards residential areas. These findings reinforce the role of flies as a mechanical vector carrying disease agents from sources of contamination, such as landfills, into residential areas.

Based on ANOVA test results, only the difference in fly's factor that had a significant effect on the *E. coli* number. Thus, posthoc testing only needs to be carried out on these factors. A summary of the posthoc tests results is presented in Table 2. Based on Table 2, *E. coli* found in Drosophila sp. was considerably lower than the other two flies. On the other hand, *E. coli* found in *M. domestica* did not differ significantly from *C. megachepala*.

TABLE 1. Summary of the two-way ANOVA test result: the effect of location and type of flies on the amount of *E. coli*

Source	df	Mean Square	F	Sig.	Partial Eta Squared
Fly	2	44.821	35.800	<0.01	0.799
Location	1	0.507	0.405	0.533	0.022
Interaction	2	0.258	0.206	0.816	0.022
Error	18	1.252			

Regarding the bacteria studied in this study, *E. coli* is an organism commonly found in animal feces. *E. coli* and various other pathogenic bacteria can be transmitted to humans through drinking water, food, or through human hands (smear infection). Flies can increase the contamination of these bacteria in food because flies can land on feces and then descend on human food. The statement is in line with Onwugamba *et al.,* who stated that flies eat and develop in excrement and decaying matter and can transmit enteric pathogens to humans and encourage colonization and infection [35]. The feet and wings of the fly are the two parts of the body that have the highest microbial diversity [36].

E. coli carried by flies is most likely derived from landfills. A landfill is already known as one of the main habitats of *E. coli*. In line with this statement, the presence of large amounts of *E. coli* in landfill soil has been reported in previous studies [37]. In landfills, *E. coli* will quickly grow in a pile of garbage and the waters around the trash [38,39]. In abundant quantities, E. coli will easily stick to the body surfaces of various organisms that touch or search for food in the trash. One of the organisms that are often in the trash is a fly. *E. coli* attached to the fly's body is then spread to various places where the fly perched, including in residential areas.

The insignificant number of *E. coli* in flies from landfills and residential areas is an indication of the high risk of health problems to the community. This statement is by various studies reporting that residents living in near landfills areas will be easily contaminated and exposed to various diseases [2,5]. The risk of health problems will increase if the landfill is poorly managed because it can increase the number of pathogens in the landfill [3]. These conditions are found in many developing countries, including landfills in Mulyoagung.

TABLE 2. Summary of least significant Difference test results of the influence of fly types on the *E. coli* number

Flies	*E. coli* (10^3 CFU/mL)	Notation
Drosophila sp.	0.625	a
M. Domestica	135.5	b
C. megacephala	160.125	b

Furthermore, based on Table 2, the number of *E. coli* found in *M. domestica* and *C. megacephala* was higher than that in *D. melanogaster*. The large number of *E. coli* in *M. domestica* is in line with several previous studies, which reported that this fly could be a vector of bacteria. Research in Iran reported that all *M. domestica* captured at Ahwaz Hospitals carry *E. coli* [40]. Besides *E. coli*, the report also informed the presence of *Pseudomonas* in the body of this fly. Other publications also indicated the ability of *M. domestica* as a mechanical vector of *Clostridium difficile* [41]. Studies in Thailand confirmed the ability of *M. domestica* as a mechanical vector of various bacteria, from *E. coli, Pseudomonas*, to *Salmonella* [42]. The higher number of *E. coli* in *C. megacephala* is also in line with previous research. Based on research in Thailand, along with *M. domestica, C. megacephala* also carries various species of bacteria, including *E. coli*. [42]. The results of the genomic and metagenomic analysis also confirmed the presence of various bacteria in the body of this fly [36].

On the other hand, the low number of *E. coli* found in *Drosophila* can be caused by the lower frequency of this fly perched in the trash. Even so, *Drosophila* still has the potential to carry various pathogenic bacteria. Research in Puerto Rico reported that various bacteria could be found in the body of Drosophila, where *Klebsiella* sp. was the most common bacteria found [43]. Various other bacteria could also be found in this fly, such as bacteria from the genus *Pseudomonas* and *Salmonella* [44]. *E. coli* has also been reported could be carried by Drosophila, especially in *D. repleta* [16] and *D. melanogaster* [43].

To summarize the finding, this study informed that *M. domestica, C. megacephala*, and *Drosophila* sp. could carry and distribute *E. coli* to residential areas. This finding provided empirical evidence of *E. coli* transmission by all three types of flies. Concerning the high number of *E. coli* and the risk of *E. coli* in some cases, controlling the number of flies in residential areas needs to be considered as an effort to reduce fecal contamination. Controlling the population of flies can be done in several ways, such as the use of insect net and fly traps, as well as maintaining the cleanliness of the surrounding environment. However, the most appropriate step in preventing *E. coli* contamination is to keep the residential areas away from landfill areas.

SUMMARY

In this study, an analysis of differences in the number of *E. coli* from three flies collected in the landfill and residential areas was carried out. As a result, the number of *E. coli* from flies in landfills was higher than the residential areas, although it did not have significant differences. On the other hand, different types of flies caused a significant

difference in the number of *E. coli*. *D. melanogaster* had a significantly lower number of *E. coli* than the other two types of flies. However, the location of fly collection and the types of flies did not have significant interaction. A low number of *E. coli* in *Drosophila* sp. when compared with *M. domestica* and *C. megachepala* is an exciting finding, considering that *Drosophila* is often used as an organism in various biological studies. The low bacteria will minimize the negative impact on the health of researchers who use these flies as model organisms in their research.

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