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Molecular Detection of Bacterial Pathogens in Flies as Potential Vectors from Surabaya Traditional Markets

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Abstract

Flies are insects that act as mechanical vectors in the spread of disease, especially in environments with poor sanitation, such as traditional markets. Surabaya has several traditional markets with relatively poor hygiene, which poses a risk of becoming breeding grounds for flies carrying pathogens. This study aims to identify fly species and detect the presence of pathogenic bacteria, especially *Escherichia coli* and *Salmonella* spp., using the Polymerase Chain Reaction (PCR) method. This study employed purposive sampling and collected samples at five traditional market locations throughout the city of Surabaya. Morphological identification revealed that *Musca domestica* (89.78%) was the dominant species, followed by *Chrysomya megacephala* (9.43%), *Chrysomya saffranea* (0.3%), *Lucilia* sp. (0.41%), and *Sarcophaga* sp. (0.08%). The result of bacterial culture on selective media and PCR confirmation showed the presence of *Escherichia coli* (100%) in fly samples, while in SSA media, which was continued with biochemical tests, the results did not detect *Salmonella* (0%) bacteria. These findings confirm that market flies, particularly *Musca domestica*, can serve as vectors of pathogenic bacteria and have the potential to transmit diseases to humans. Therefore, monitoring and controlling fly populations in traditional markets is necessary as a preventive measure to maintain public health.

Keywords

Disease vectors, *Escherichia coli*, *Musca domestica*, Polymerase Chain Reaction, Wet market Surabaya.

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Introduction

Flies are insects that have a close relationship with the human and animal environment. Their presence is often found in areas with minimal sanitation, such as landfills, farms, and densely populated settlements. One of the important roles of flies in public health is as the mechanical vector that can transfer disease-causing agents (Salvador *et al*., 2025). Fly species such as *Musca domestica* (house fly) have been widely studied due to their ability to carry more than 130 types of pathogenic microorganisms, including bacteria, viruses, fungi, and parasites. Transmission occurs when flies touch food, saliva, food vomit, and feces (Khamesipour *et al*., 2018). In addition, other species such as *Stomoxys calcitrans, Sarcophaga* spp.*,* *Fannia* spp., and *Chrysomya megacephala* also have significant potential in spreading diseases. *S. calcitrans*, for example, is a blood-sucking fly that can transmit agents such as *Trypanosoma* spp. and *Bacillus anthracis*. Meanwhile, *C. megacephala* is known to carry worm eggs and parasitic cysts, and contributes to cases of myiasis in both humans and animals (Sakulpanich *et al*., 2023). High fly densities in locations with organic waste increase the risk of disease transmission (Sukmawati *et al.,* 2019).

Wet markets in Surabaya are the main distribution centers for various food products and non-food commodities that come from the surrounding areas. As the center of regional economic activity, the hygiene of traditional markets in Surabaya remains relatively low. One of the problems often encountered is the presence of large numbers of flies in the market area. These flies have the potential to carry pathogens that can contaminate food, reducing its quality, health, and increasing the risk of disease transmission to humans (Safitri *et al*., 2017).

The presence of flies in traditional market environments cannot be underestimated, considering their role as mechanical vectors in the spread of various infectious disease agents. Market conditions often have poor sanitation, making them ideal places for flies to breed and cause contamination, including in food sources. Therefore, it is important to investigate the potential for disease transmission from flies by identifying the dominant fly species and detecting the presence of pathogenic microorganisms, particularly bacteria, carried by these insects. The benefits of this study are to prevent and control environmental-based diseases that can threaten public health.

Materials and Methods

Sample Collection

The sampling method used in this study was purposive sampling. The fly sample was collected from five traditional market locations spread across the city of Surabaya, including Dukuh Kupang, Darmo, Wonokusumo, Kaliwaron LKMK, and Surya Wonokitri. The sample was collected according to a previous study with several adjustments (Wahyudi *et al.*, 2015). Floating nets are used to catch flies by swinging them for five minutes at each designated fly accumulation point in an area of 2 m^2 within the market block. The sampling locations were divided into three areas: the meat block, the vegetable block, and the outside area of the market. Sampling took place in the morning, from 7:00 a.m. to 11:00 a.m., in both dry and rainy seasons. Flies that were successfully caught with a sweep net were placed into a sample container (sample pot) and then taken to the laboratory for further analysis. The morphological identification was conducted in the laboratory of the Faculty of Veterinary Medicine at the University of Wijaya

Kusuma, Surabaya. The bacterial culture and molecular identification were carried out at the Institute of Tropical Disease, Universitas Airlangga.

Identification and Detection

Sample identification and homogenization

The collected flies were then killed using chloroform placed in a killing jar (Attaullah *et al*., 2024). The specimen preservation process was carried out using the pinning method. For small fly specimens that cannot be preserved directly by pinning, an alternative method is used by attaching the fly's body to a triangular piece of paper and then gluing it in place. Furthermore, the paper is pierced with an entomology needle on the other side for storage and further identification (Rahmawati *et al*., 2020).

After the identification process, the sample was subjected to tissue homogenization. To homogenize the sample, 50 mL of 1X phosphate buffer saline was used on the individual flies. The homogenized solution was shaken with a vortex for 1 minute at room temperature to obtain the bacteria. The homogenized tissue was filtered to obtain the supernatant.

Bacterial culture and genomic material extraction

The washing liquid was then inoculated into selective media at 37 °C, specifically EMBA (Eosin Methylene Blue Agar) (Merck, US) and SSA (Salmonella-Shigella Agar) (Merck, US), to detect the presence of bacteria (Palus *et al*., 2015). Bacterial growth consisting of 10–15 colonies was scraped from the media and suspended in TE buffer for PCR testing. DNA was extracted using the QIAamp DNA Mini Kit (Qiagen, Germany), with cell wall lysis performed by Proteinase K.

Molecular detection of the bacteria

The volume of the PCR reaction was 20 µL, containing 2x PCR Master Mix (Promega, USA), 12.5 µL forward and reverse primers (Table 1), 1 µL (10 µM) Nuclease-Free Water, 0.5 µL (Promega, USA), and DNA Template 5 µL. Initial denaturation was performed at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 1 minute, annealing at 56°C for 1 minute, and extension at 72°C for 1 minute, and final extension for 5 minutes, were set in the thermal cycler by SimpliAmp™ Thermal Cycler, ThermoFisher Scientific. Negative control (NFW) and Positive Control (positive DNA isolation results) was used for validation in the PCR process. PCR products were then visualized on 2% agarose gel electrophoresis and observed under Ultraviolet light (Kristijanto *et al*., 2023).

Table 1. Oligonucleotide primers used for PCR aplicfication (Molina *et al*., 2015)

Primer set	Sequence	Product size (bp)
yaiO	forward 5′- TGATTTCCGTGCGTCTGAATG -3′ reverse 5′- ATGCTGCCGTAGCGTGTTTC-3′	115

Results and Discussion

The identification results revealed variations in fly species across the sampling locations. The types of flies obtained from five traditional markets in Surabaya City were *Chrysomya megacephala* (n=250), *Chrysomya saffranea* (n=8), *Lucilia* sp. (n=11), *Musca domestica* (n=2381), and *Sarcophaga* sp. (n=2) (Table 2).

Various ecological factors, including temperature, humadity, precipation, altitude, light intensity, habitat type, food availability, and interactions with other organisms, influence the diversity of fly species in an area. Environmental factors and the availability of suitable habitats play an important role in determining the number and types of flies that can be found in an area (Syahputera *et al.,* 2022). Additionally, the fly population is influenced by environmental parameters such as light intensity, temperature, and air humidity. Flies are classified as ectoparasites that tend to be active in bright areas and experience decreased activity at night. Fly population growth will be optimal at temperatures between 20°C and 25°C, while the population tends to decrease if the temperature is below 10°C or exceeds 49°C. The ideal relative humidity for fly development is in the range of 90% (Fadhil *et al*., 2022).

The most dominant fly species found in this study is *Musca domestica* (house fly), followed by flies from the Calliphoridae family, including *Chrysomya megacephala*, *Chrysomya saffranea*, and *Lucilia* sp., and flies from the Sarcophagidae family. Therefore, the diversity of fly species found in the five markets is significantly influenced by the availability of organic material sources and favorable habitat conditions surrounding the market area.

Colony growth was observed in both media (Table 3). The results of this test showed that there was colony growth in the SSA media; however, further testing, including biochemical tests, did not reveal any *Salmonella* in the samples tested. Meanwhile, in the EMBA media, colonies were found with a distinctive metallic green color (Figure 1), which were then used as samples for PCR analysis. The PCR results on the E1 sample revealed the presence of a DNA band at a size of 115 bp, identical to that of the positive control sample, indicating that the sample was positive for *Escherichia coli* (Figure 2). These findings suggest that flies can act as vectors for carrying pathogenic bacteria, thereby having the potential to transmit diseases to humans, particularly when the fly population in the environment is not controlled (Gioia *et al*., 2023). The condition of most conventional markets, characterized by a poorly organized layout and high trader density, leads to the formation of dirty areas, including piles of garbage, puddles of water, and organic waste (Bertelloni *et al*., 2023). This situation supports the presence of nuisance flies and increases the risk of spreading the diseases they transmit (Pamungkas *et al*., 2023).

This study has shown that Musca domestica, or the house fly, occupies the highest number, as this fly species is most often identified as a carrier of various pathogenic agents that cause disease. The presence of this fly is often associated with gastrointestinal diseases in humans, which can occur due to the consumption of food contaminated by pathogenic bacteria. One of the behaviors of flies that contributes to the spread of disease is their tendency to land on food and drinks, allowing the transfer of harmful microorganisms to food ingredients (Yin *et al*., 2022). A previous study also reported that house flies can be important vectors in the spread of *Escherichia coli* to food, especially in urban areas such as Bangladesh, which then increases the risk of digestive infections in humans (Lindeberg *et al*., 2018). Onwugumba *et al*. (2020) found that several strains of *E. coli* carried by flies exhibited resistance to a number of major antibiotics, which adds to the complexity of the public health threat posed by this contamination.

The existence of piles of market waste that have not been immediately cleaned up, as well as the slow process of transporting waste, creates conditions that support the breeding of flies (Krystosik *et al*., 2020). This situation indirectly increases the risk of spreading contaminants from waste through the intermediary of these flies (Nayduch *et al*., 2023).

Conclusion

This study confirms that *Musca domestica* is the most dominant fly species found in five traditional markets in Surabaya, namely Dukuh Kupang, Darmo, Wonokusumo, Kaliwaron LKMK, and Surya Wonokitri. Molecular detection confirmed the presence of *Escherichia coli* in fly samples, whereas *Salmonella* spp. was not detected after biochemical testing on SSA media. These findings highlight the potential role of market flies, particularly *M. domestica*, as vectors of pathogenic bacteria and underscore the importance of continuous monitoring and control of fly populations in traditional markets as a preventive measure to protect public health.

Approval of Ethical Commission

The research protocol was approved by the Animal Care and Use Committee of the University of Wijaya Kusuma Surabaya (Approval number: 210-KKE).

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Author's Contribution

PAW: Contributed to the conceptualization, methodology, data collection, data analysis, and writing of the original draft, as well as project administration. MYK: Supervised the study, provided methodological input, validated the findings, and contributed to the review and editing of the manuscript. EPN: Involved in supervision, methodology, validation, and manuscript review and editing. Authors have read and approved the final version of the manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Data Availability Statement

The data that support the findings in this study are available for access and can be requested from the corresponding author on reasonable request.

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