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The Correlation between Bacteria and Parasite Patterns on Flies with
Prevalence of Fly Vector-Borne Disease at the Market and the
Landfill in Jember District, Indonesia

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The Correlation between Bacteria and Parasite Patterns on Flies with Prevalence of Fly Vector-Borne Disease at the Market and the Landfill in Jember District, Indonesia

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Abstract

Fly vector-borne diseases are the potential disease that can trigger an outbreak. The fly lives in a dirty place with poor sanitation, such as the market and the landfill. Some pathogens that can be transmitted by flies mechanically were bacteria such as *Escherichia coli*, *Shigella*, *Salmonella*, *Vibrio cholera* and parasites such as *Balantidium coli*, *Entamoeba histolytica* and *Giardia lamblia*. This study aimed to analyze the correlation between bacteria and parasite patterns on flies and the prevalence of fly vector-borne diseases. This research was conducted from May 2019 to March 2020. The flies samples were collected randomly at each research location, i.e., Tanjung Market and the Pakusari landfill. The bacteria and parasites identifications were carried out at the Microbiology and Parasitology Laboratory, Faculty of Medicine, University of Jember. The data of disease prevalence was collected in a cohort from the health centers around research locations. This study found three bacteria in market samples, 18 cases of fly vector-borne diseases and four bacteria in the landfill samples and 10 cases of fly vector-borne disease. The study indicated a significant correlation between bacteria and parasite patterns on flies with the prevalence of fly vector-borne diseases in the market, but no correlation in the landfill.

Keywords: Bacteria, Fly, Parasite, Vector-borne Disease.

Introduction

Fly vector-borne diseases are the potential disease that can trigger several extraordinary infectious diseases such as diarrhea, typhoid fever, dysentery and cholera. Flies are a type of vector that can carry germs on their bodies. They live close to humans and are often associated with sanitation problems. Environments with poor sanitation tend to attract flies as breeding place and

feed them, they including the market and landfill. Those areas are suspected related to the incidence and spread of infectious diseases where pathogens originate from these places.

Fly can transmit the disease to humans mechanically by carrying microorganisms attached to their bodies to such media. Studies reported the pathogens that are transmitted mechanically by flies are *Escherichia coli*, *Shigella*, *Salmonella*, *Vibrio cholera* and parasites such as *Balantidium coli*, *Entamoeba histolytica*, *Giardia lamblia*. A species of fly, *Musca domestica* can transmit pathogens such as *Campylobacter*, *E.coli*, *Salmonella sp.*, and *Shigella sp.*⁽¹⁶⁾. Previous studies on the detection of fly vector-borne diseases found the occurrence of diarrhea, dysentery, typhus and cholera in Jember District 2019⁽⁶⁾. The epidemiological study of vector-borne diseases showed the interconnection of three factors, i.e., human host, infectious agent (parasite, bacteria in

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the fly's body) and the environment where the place of the fly vector life. This study analyzed the role of flies in transmitting vector-borne diseases in Jember Regency, especially in an environment where the flies can breed and live easily, i.e., the market and the landfill.

Method

The research was conducted at the Tanjung market and the Pakusari landfill in May 2019 to March 2020. This research has received ethical clearance from the Ethical Committee of Health Research of Faculty of Dentistry, the University of Jember, with the reference number.632/UN25.8/KEPK/DL/2019. The sampling technique was carried out randomly at each location in November 2019. Researchers set a fly trap that was equipped with jackfruit bait with a strong aroma to catch flies. The caught flies were put into a sterile tube containing NaCl 30ml and marking each location and

divided into two parts. The sample was analyzed at the Laboratory of Microbiology and Parasitology, Faculty of Medicine, the University of Jember. The bacterial identification was performed by selective media culture and several biochemical tests. The identification of parasites was made by microscopical examination. The prevalence data of fly vector-borne diseases was provided in a cohort method from the health service around each research location. It was collected in the period of November-December 2019.

Research Findings: Bacterial identification of samples from the Tanjung Market found three types of bacterial patterns that are considered as *Escherichia coli*, *Salmonella sp* and *Shigella sp*. Meanwhile, the samples from the Pakusari landfill showed four types of bacterial patterns, which were suspected as *Escherichia coli*, *Salmonella sp*, *Shigella sp*, and *Vibrio cholera*, as shown in Table 1 and Table 2.

Table 1. Bacterial Biochemical Tests on Flies from the Tanjung Market and the Pakusari landfill

Sample Location	Bacteria	Biochemical Test					
		KIA	Indol	MIO	VP	MR	Citrate
Market	<i>E.coli</i>	S = Alk B = Acid H ₂ S = + Gas = -	-	-	-	-	+
	<i>Salmonella</i>	S = Alk B = Acid H ₂ S = + Gas = +	-	+	-	+	+
	<i>Shigella</i>	S = Alk B = Acid H ₂ S = - Gas = -	-	-	-	-	+
	<i>Vibrio cholerae</i>	S = Alk B = Acid H ₂ S = - Gas = -	-	+	+	-	+
Landfill	<i>E.coli</i>	S = Alk B = Acid H ₂ S = + Gas = -	-	-	+	-	-
	<i>Salmonella</i>	S = Alk B = Acid H ₂ S = + Gas = +	-	+	-	+	+

Sample Location	Bacteria	Biochemical Test					
		KIA	Indol	MIO	VP	MR	Citrate
	Shigalla	S = Alk B = Acid H ₂ S = - Gas = -	+	-	+	-	-
	Vibrio cholera	S = Alk B = Acid H ₂ S = - Gas = -	+	-	-	+	-

Explanation: KIA = Kligler Iron Agar; MIO = motility-indole-ornithin; MR = Methyl Red; VP = Voges Proskauer; S = slope; B = base; Alk = medium alkalis/bases (red); Acid = medium acidity (yellow); H₂S = hydrogen sulfide production.

Table 2. The Patterns of Bacteria and Parasites on Flies through Selective Media Culture Method and Microscopic Observations from Each Location

Bacteria/Parasite	Agar Media	Location		Description
		Market	Landfill	
Escherichia coli	EMB	+	+	Selective media culture
Shigalla	SS	+	+	Selective media culture
Salmonella	SS	+	+	Selective media culture
Vibrio cholera	TCBS	-	+	Selective media culture
Entamoeba histolytica		-	-	Microscopic observation
Balantidium coli		-	-	Microscopic observation
Giardia lamblia		-	-	Microscopic observation

Explanation: EMB = Eosin Methylene Blue; SS = Salmonella-Shigella; TCBS = Thiosulfate-Citrate-Bile-Sucrose

Table 3. showed that the prevalence of fly vector-borne disease around the market place was 18 cases, while from the landfill was 10 cases of fly vector-borne disease.

Table 3. The Prevalence of Fly vector-borne Disease around research Location During November – December 2019

Location	Prevalence of Fly vector-borne Disease				Total
	Diarrhea	Typhoid	Dysentery	Cholera	
Market	7	3	8	0	18
Landfill	8	2	0	0	10

Table 4. The Data Analysis on the Correlation of Bacterial and Parasite Patterns and the Prevalence of Fly Vector-borne diseases around research Location

Location	Bacterial Pattern x Disease Prevalence	Statistical analysis
Market	3*79	value = 21,000 Asymp. Sig = 0,013
Landfill	4*165	value = 14,000 Asymp. Sig = 0,082

Explanation: value = crosstab value; Asymp.sig = significance value

This study found three patterns of bacteria but no parasite around the market place. The prevalence of fly vector-borne diseases around the market was 18 cases. The statistical analysis by using Chi-Square resulted value of 21,000 and a significance value of 0,013, which was fewer than α (0,05), indicated its correlation, as shown in Table 4. Furthermore, this research found four patterns of bacteria and no parasite around the landfill. The prevalence of fly vector-borne disease around the landfill was 10 cases. The statistical test by Chi-Square showed a value of 14,000 and a significance value of 0,082, which was higher than α (0,05), implicated no correlation.

Discussion

The method to analyze the pathogen can be performed by microscopic, selective media culture, as well as serological examination. In this research, a selective media culture examination was performed to identify bacteria. The *Eosin Methylene Blue* (EMB) identified *Escherichia coli*, the *Salmonella-Shigella Agar* (SSA) identified *Salmonella sp* and *Shigella sp* and the *Thiosulfate-Citrate-Bile-Sucrose* (TCBS) selected for *Vibrio cholerae*. For each media (EMB, SSA, TBCS), we conducted several biochemical tests such as Kligler Iron Agar (KIA), Indole test, Methyl Red (MR) test, Voges Proskauer (VP) test, MIO test and citrate test on each sample location. The KIA test on each agar media (EMB, SS, TBCS) measured the ability to produce slope (S), base (B), hydrogen sulfide (H₂S) and damp as the basis to identify a particular pattern of bacteria and its growth on each media⁽⁵⁾. For the identification of the parasite on *Giardia lamblia*, *Entamoeba histolytica* and *Balantidium coli* was done through the microscopical examination by 1000x magnification.

The sample from the market showed three types of bacteria i.e., *Escherichia coli*, *Salmonella sp.*, and *Shigella sp.*, but no parasites. The result is in accordance with the research done by Safitri⁽¹⁶⁾, which has found the dominant pattern of *Escherichia coli*, *Salmonella sp.*, *Shigella sp.*, and *Staphylococcus* bacteria within the fly around markets in Surabaya. Moreover, *Escherichia coli* bacteria was a group of Coliform bacteria that could stay in the human and cattle digestive tract. In a certain amount, Coliform bacteria could cause indigestion problems like diarrhea. According to Lima et al⁽¹²⁾, *E.coli* was a pathogen that can cause diarrhea and transmitted to the human through fly mechanically. *Escherichia coli*, *Shigella spp.*, *Salmonella spp.* bacteria

around the Tanjung Market were found and derived from contaminated foodstuff, fruit and vegetable because of dust and soil exposure.

This research found 18 cases of fly vector-borne disease from around the market. The occurrence of fly vector-borne disease was affected by several aspects as personal and environmental hygiene and environmental sanitation. The dirty environment turns any kind of pathogens to grow and attract fly. The average flight distance of some flies was about 6-9 km from the breeding place⁽⁵⁾. The population in that area has a high risk of infecting fly vector-borne diseases because bacteria can contaminate the foods and groceries. To keep the cleanliness of foodstuff, they needed to wash them with detergent to reduce the risk of pathogens such as *E. coli*, *Shigella sp.*, *Salmonella sp* to grow, which can be transmitted by the fly. The population who have a good hygiene practice would be prevented from the diseases. Furthermore, the individual with vulnerable conditions and had poor hygiene would be at risk of diseases.

The bacteria found on the flies around the landfill were *Escherichia coli*, *Salmonella spp.*, *Shigella sp.* and *Vibrio Cholera* bacteria, but no parasites. This result was in line with the previous study by Yunita⁽¹⁸⁾, which has found the *Salmonella sp.*, *Providencia*, *Escherichia* and *Vibrio* in the fly body around the Sukawintan landfill. The typhoid disease occurs due to *Salmonella sp* infection in the intestinal tract⁽¹⁸⁾. *Salmonella sp* in the landfill's flies was suspected because of the stack of organic and inorganic trash that produced leachate, which can be a suitable medium for the microorganism such as bacteria and parasite to grow. A previous study by Yunita⁽¹⁸⁾ has found fecal coliform bacteria such as *Salmonella sp.*, *Vibrio comma* and *Shigella* in leachate. The pungent smell of leachate attracted flies to come close because they like to pungent smell and dirty place⁽⁵⁾. The fly, which alighted in leachate or waste in landfills, directly carried pathogen bacteria on their bodies. The flies could fly up to 6-9 km⁽¹²⁾. The long flight distance of fly enabled them from the landfill to fly and alight in residential areas around the landfill. The fly which carried pathogen might contaminate food and water, which further can cause diseases such as typhoid fever if consumed by human.

The statistical analysis showed no significant correlation between bacteria and parasites patterns and the prevalence of fly vector-borne disease at the landfill.

The reported vector-borne disease cases from the health care centers around the landfill in specific periods showed that the population around this landfill was avoided from dysentery and cholera. The occurrence of the disease is affected by the interaction between host, agent and environment. Cholera is caused by *Vibrio cholerae*, which is transmitted through contaminated foods and drinks by the fly as a vector. However, the dead *Vibrio cholerae* can not infect and cause disease on humans. However, the bacteria live at an optimum temperature of 18-37°C⁽¹⁰⁾. *Vibrio cholerae* was assumed to be dead before it reached the host, so it would not cause disease around the landfill area. The other presumption was hygiene practice that has been implemented by the population around the landfill. The hygiene practice and healthy life behavior could prevent the individual from many kinds of infectious and transmissible diseases like diarrhea, dysentery, cholera⁽⁸⁾.

This research also detected *Shigella sp.* from the landfill sample, but there was no dysentery case reported. The dysentery is an infectious disease caused by *Shigella sp.*⁽⁴⁾, which can be transmitted by contaminated food and water⁽¹⁶⁾. When the *Shigella sp.* reaches the human digestive tract, the bacteria grow within colon epithelial cells, infect colonic mucosa and spread laterally into the surrounding cells. The infection occurs with a broad range of symptoms from asymptomatic, mild symptoms like watery diarrhea to severe symptoms like stomach cramps, nausea and vomiting, fever, anorexia and bloody feces with mucus⁽¹⁷⁾. Dysentery usually occurred in a dirty environment and poor sanitation area. The residential areas around the landfill have enough distance and space among houses, reducing the risk of fly vector-borne disease transmission in the population. It was assumed that *Shigella sp.* bacteria, which transmitted by a fly vector, did not reach the host. Thus, the population around the landfill was avoided from dysentery.

According to Ismail⁽⁹⁾, the mechanism of disease transmission occurs in several ways, i.e., the way the agent leaves the reservoir, the transmission route to reach host and the port de entry to the host. The fly vector carried pathogen (agent) after leaving the location (reservoir) and went to the residential area would be the risk to cause disease. However, as long as the pathogen carried by fly did not reach to the host or get into the human body, the interaction of disease would not exist. The prevention of infection due to bacteria could be exerted by improving personal hygiene and maintenance of clean food and drink without contamination of

pathogens carried by the fly⁽¹⁴⁾. The vector control was also an aspect that was put into a serious concern to prevent the occurrence and transmission of fly vector-borne disease⁽²⁾.

Conclusion

This research found three patterns of bacteria around the market and the landfill in Jember District during November–December 2019, i.e., *Escherichia coli*, *Salmonella sp.*, and *Shigella sp.* The most prevalent cases around the location were diarrhea and typhoid. There was a correlation between the bacterial pattern on the fly and the fly vector-borne disease. The fly was the potential mechanical vector for several vector-borne diseases. We suggested collaborating with the local government or healthcare service with aimed to plan the fly vector control program and fly vector-borne disease prevention, particularly in the area with a high prevalence of the fly vector-borne disease.

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