



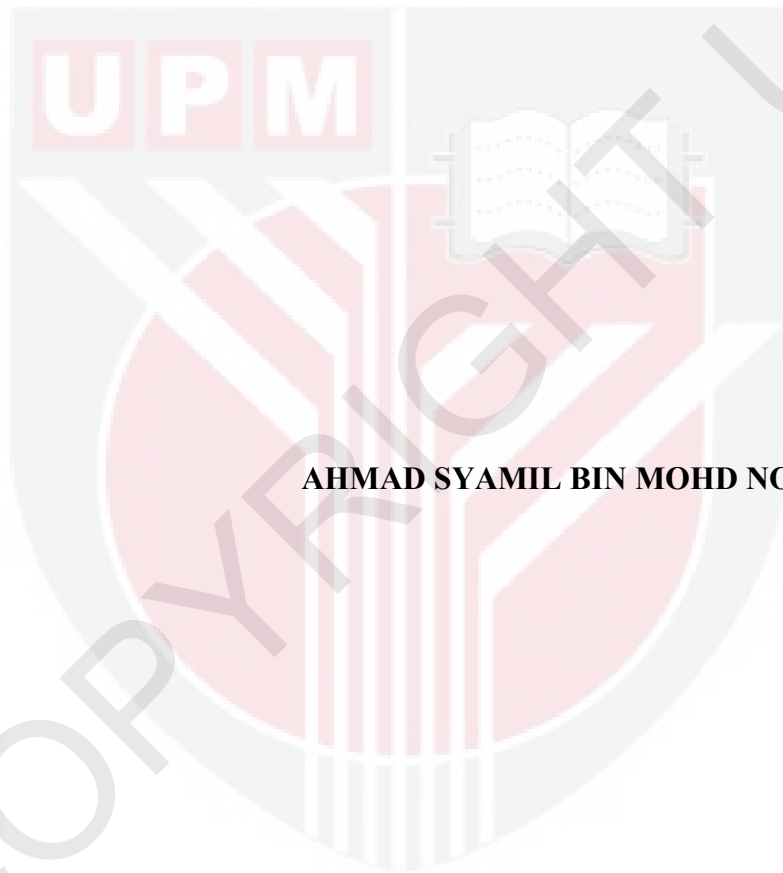
UNIVERSITI PUTRA MALAYSIA

**POTENTIAL OF HOUSEFLIES (*MUSCA DOMESTICA*) AS A VECTOR
OF *SALMONELLA* SPP. AND *ESCHERICHIA COLI* IN OPEN HOUSE
BROILER FARM IN JOHOR, MALAYSIA.**

AHMAD SYAMIL BIN MOHD NOR

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FPV 2020 53**

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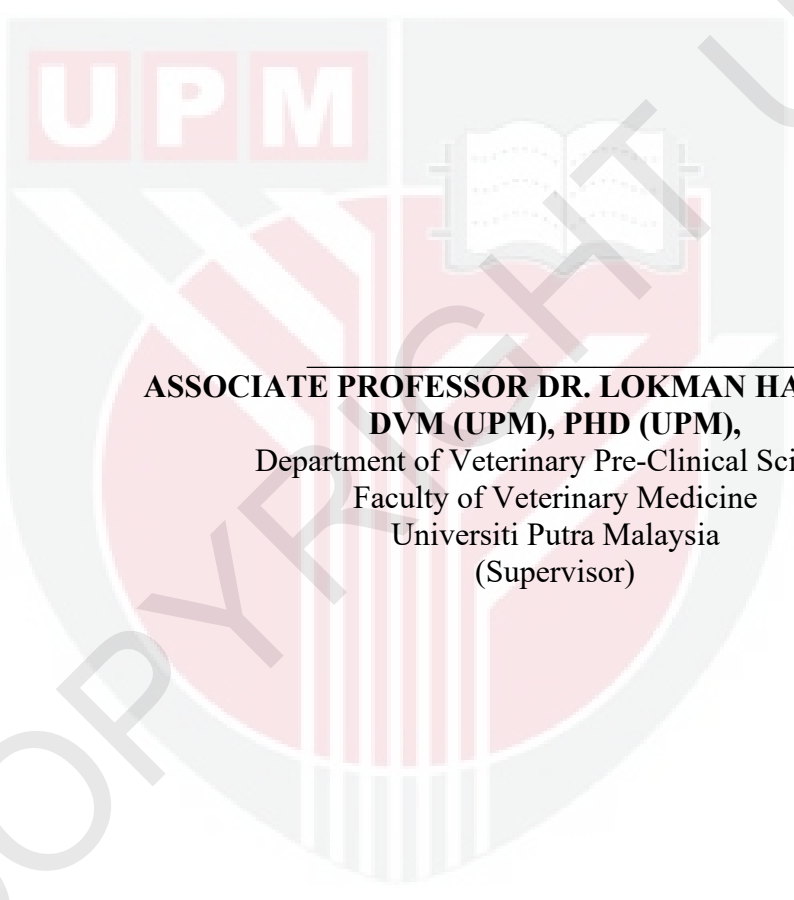
AHMAD SYAMIL BIN MOHD NOR

A project paper submitted to the
Faculty of Veterinary Medicine, Universiti Putra Malaysia
In partial fulfillment of the requirement for the
DEGREE OF DOCTOR OF VETERINARY MEDICINE
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CERTIFICATION

It is hereby certified that we have read this project paper entitled “Potential of houseflies (*Musca domestica*) as a vector of *Salmonella spp.* and *Escherichia coli* in open house broiler farm in Johor Malaysia”, by Ahmad Syamil bin Mohd Nor and in our opinion it is satisfactory in terms of scope quality, and presentation as partial fulfillment of the requirement for the course VPD 4999 - Final Year Project.



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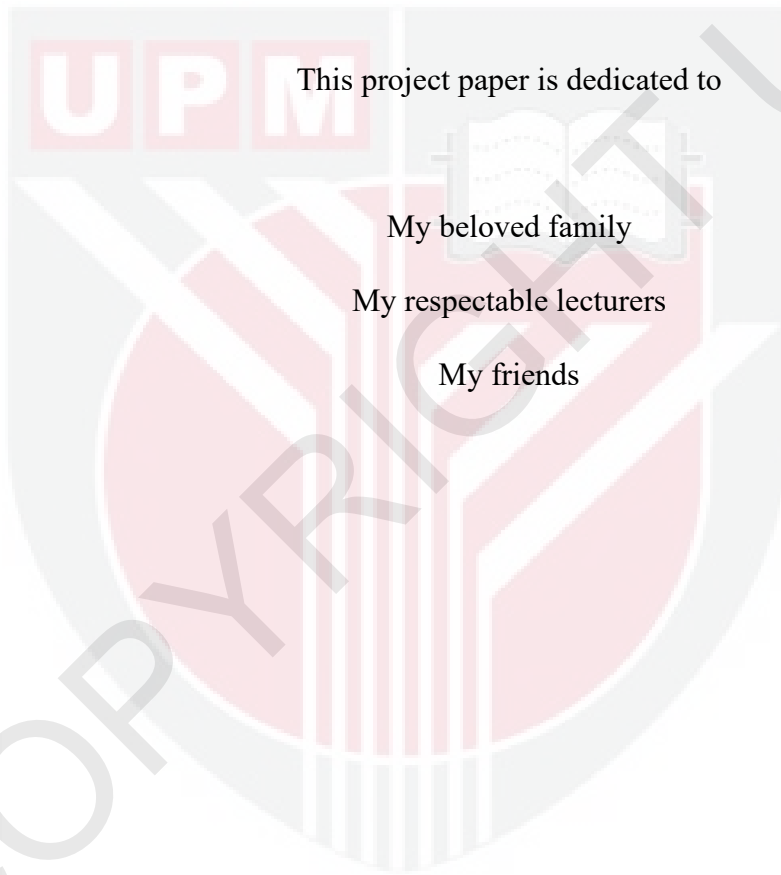
DEDICATION

This project paper is dedicated to

My beloved family

My respectable lecturers

My friends



ACKNOWLEDGMENT

I would like to give the greatest gratitude towards my final year project supervisor, Associate Professor Dr. Lokman Hakim Idris, for guiding and supervising me during my journey in completing my final year project.

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TABLE OF CONTENT

	Page no.
TITLE	i
CERTIFICATION	ii
DEDICATION	iii
ACKNOWLEDGMENT	iv
TABLE OF CONTENT	v
LIST OF TABLES	vi
ABSTRAK	vii
ABSTRACT	ix
1.0 INTRODUCTION	1
2.0 LITERATURE REVIEW	3
2.1 OPEN HOUSE BROILER FARM	3
2.2 HOUSEFLY (<i>MUSCA DOMESTICA</i>)	4
2.3 <i>ESCHERICHIA COLI</i>	5
2.4 <i>SALMONELLA SPP.</i>	6
3.0 MATERIALS AND METHODS	7
3.1 METHOD OF SAMPLE COLLECTION	7
3.2 METHOD OF ISOLATING MICROBES	7
3.3 METHOD OF IDENTIFICATION OF MICROBES	8
4.0 RESULTS	9
5.0 DISCUSSION	10
CONCLUSION	13
RECOMMENDATION	13
REFERENCES	14

LIST OF TABLES

	Page no.
Table 1: Positive isolation of <i>Escherichia coli</i> among sample pools in all five farms	9



ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD4999 - Final Year Project.

**POTENSI LALAT (*MUSCA DOMESTICA*) SEBAGAI VEKTOR KEPADA
SALMONELLA SPP. DAN *ESCHERICHIA COLI* DALAM REBAN TERBUKA AYAM
PEDAGING DI JOHOR, MALAYSIA**

Oleh

Ahmad Syamil Bin Mohd Nor

2020

Penyelia: Profesor Madya Dr. Lokman Hakim Idris

Penyelia bersama: Profesor Dr. Saleha Abd Aziz

Lalat (*Musca domestica*) ialah salah satu arthropod yang wujud di persekitaran ladang ayam pedaging. Lalat tertarik terhadap bahan organik yang reput seperti kumbahan di mana ia banyak terdapat di ladang ayam pedaging. Kumbahan mengandungi bilangan mikroorganisma dan patogen yang tinggi. *Salmonella spp.* dan *Escherichia coli* adalah mikroflora biasa dan dapat disebarkan ke ayam pedaging oleh lalat. Kajian ini adalah untuk mengetahui kehadiran *Salmonella spp.* dan *Escherichia coli* di lalat rumah hanya di ladang broiler terbuka. Sebanyak 30 sampel lalat dikumpulkan dari 5 ladang ayam pedaging yang berbeza menggunakan kaedah persampelan rawak. Satu sampel berkumpul terdiri daripada 5 lalat. Permukaan luaran dan kandungan dalaman lalat adalah tempat mengasingkan *Salmonella spp.* dan *Escherichia coli*.

Salmonella spp. dikultur ke Agar Xylose-Lysine-Deoxycholate (agar XLD) dan Agar Brilliant-Green (BGA). *Escherichia coli* dikultur ke Coliform-Chromogenic Agar (CCA). Ujian biokimia dilakukan untuk mengesahkan pengenalan bakteria. Tiada *Salmonella spp.* berjaya diasingkan dari permukaan luaran dan kandungan dalaman dari 30 sampel yang dikumpulkan. Walau bagaimanapun, *Escherichia coli* berjaya diasingkan dalam 21 dari 30 sampel yang dikumpulkan (70%) di mana 13 dari 30 sampel yang dikumpulkan (43%) diasingkan dari permukaan luaran dan 14 dari 30 sampel yang dikumpulkan (46%) diasingkan dari kandungan dalaman. Oleh itu, kebarangkalian lalat menjadi vektor penyebaran *Escherichia coli* adalah tinggi namun kebarangkalian lalat menjadi vektor penyebaran *Salmonella spp.* rendah dalam kes ini.

Kata kunci: Lalat, *Salmonella spp.*, *Escherichia coli*, ayam pedaging, pemindahan

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfillment of the course VPD4999 - Final Year Project.

**POTENTIAL OF HOUSEFLIES (*MUSCA DOMESTICA*) AS A VECTOR FOR
SALMONELLA SPP. AND *ESCHERICHIA COLI* IN OPEN HOUSE BROILER FARM
IN JOHOR, MALAYSIA**

by

Ahmad Syamil Bin Mohd Nor

2020

Supervisor: Associate Professor Dr. Lokman Hakim Idris

Co-supervisor: Professor Dr. Saleha Abd Aziz

Houseflies (*Musca domestica*) is one of many arthropods that coexist in broiler farms. Houseflies are attracted to decaying organic materials such as feces that are abundant in broiler farms. Feces contains a high number of microorganisms and pathogens. In particular, *Salmonella spp.* and *Escherichia coli* are the common microflora and can be transmitted to the broilers by the house flies. This study is to determine the presence of *Salmonella spp.* and *Escherichia coli* in house flies only in open broiler farms. A total of 30 pooled samples of houseflies was collected from 5 different broiler farms using a random sampling method. One pooled sample consists of 5 houseflies. External surface and internal content of the houseflies are the site of isolating *Salmonella spp.* and *Escherichia coli*. *Salmonella spp.* was cultured

onto Xylose-Lysine-Deoxycholate Agar (XLD agar) and Brilliant-Green Agar (BGA). *Escherichia coli* was cultured onto Coliform-Chromogenic Agar (CCA). Biochemical test was done to confirm the identity of the colony. There was no *Salmonella spp.* isolated from both external surface and internal content of the 30 pooled samples. However, *Escherichia coli* was isolated in 21 out of 30 pooled samples (70%) in which 13 out of 30 pooled samples (43%) were isolated from the external surface and 14 out of 30 pooled samples (46%) were isolated from internal content. Hence, the possibilities of the houseflies to become the vector of transmitting *Escherichia coli* is high however the possibilities of the houseflies to become the vector of transmitting *Salmonella spp.* is low in this case.

Keywords: Houseflies, *Salmonella spp.*, *Escherichia coli*, broilers, transmission

1.0 INTRODUCTION

Housefly (*Musca domestica*) is one of the common arthropods that coexist in poultry farms. Decaying organic matter such as garbage, manure and feeds are what attract the houseflies (Holt et al., 2007). Decaying organic matter is an optimum place for the houseflies to feed on and to lay their eggs. Houseflies are one of the bio-security hazards and concern that they can transmit diseases in poultry farms.

Pathogens that are detrimental to the poultry health and industry are *Salmonella spp.* and *Escherichia coli*. Salmonellosis is considered one of the major public health dangers related with food-animal production (Antunes et al., 2016). Avian pathogenic *Escherichia coli* (APEC) cause airsacculitis, polyserositis, septicaemia and other mainly extraintestinal diseases in chickens, turkeys and other avian species (Dho-moulin & Fairbrother, 1999). Both pathogens are concerned to be a health hazard towards the poultry and humans.

Escherichia coli and *Salmonella spp.* are among the bacteria detected in chicken manure (Kyakuwaire et al., 2019). Pathogens adhere onto house flies' proboscis, wings, legs, and other body surfaces during their feeding process (Khamesipour et al., 2018). Both statements suggest that the houseflies can carry *Escherichia coli* and *Salmonella spp.* as a vector in a poultry farm.

Hence, the integration of what we have learned about houseflies as a possible vector of pathogens and the danger of transmitting *Escherichia coli* and *Salmonella spp.* to the poultry farm has brought the idea of this study. The aim of the study is to detect the presence of *Salmonella spp.* and *Escherichia coli* in houseflies in open house broiler farms to prove whether houseflies can be a potential vector. The hypothesis would be that there are presence of both pathogens on the houseflies.

2.0 LITERATURE REVIEW

2.1 Open house broiler farm

Broiler housing system is one of many aspects of good management that has a very crucial role in ensuring production processes at the most optimum level (Muharlién et al., 2020). The type of poultry housing system employed by the proposed poultry farm is a function of the prevailing climatic conditions of the region where the farm is located (Oloyo & Ojerinde, 2020).

Broiler chickens have specific microclimate needs depending on their production and physiological development phase (Bonnet et al., 1997). Examples of microclimate in broiler houses are temperature, humidity, air movement, air velocity, air composition, and light (Kalio and Okafor, 2012). Poultry housing design plays a vital role in the determination of the internal climatic conditions of the house for optimum health, growth and productive performance of the birds (Oloyo & Ojerinde, 2020).

In the tropical area, the open poultry housing system has been identified for its simplicity, economic consequences and ease of heat generation management inside the building through natural ventilation (Daghir, 2008). Open poultry housing system is susceptible to invasion by insects, rodents, birds, and small predators that can affect chicken health, productivity and performance (Oloyo & Ojerinde, 2020). Bad hygiene around the poultry facility increases the risk of high concentrations of house flies (Loftin et al., 2014).

2.2 Houseflies (*Musca domestica*)

The housefly originated in the steppes of Central Asia, but now swarming on rural and urban populated continents, whether it is tropical or temperate country (Capinera et al., 2008). Egg, larvae, pupal, and adult stage consist in the complete cycle or other name is the metamorphosis (Bryant & Hall, 1999). The period of a complete life cycle of a housefly is seven to 10 days (Kozielska et. ak, 2006).

Houseflies can only ingest their food in liquid nature (Marshall, 2006). The mouthparts form a flexible and retractable proboscis with labellum which is the fleshy wide tip (Gullan & Cranston, 2010). In breaking down and recycling organic matter, houseflies play a significant environmental role (Mehlhorn, 2001).

The house fly (*Musca domestica*) is one of the primary insects that are related with poultry manure and this insect breeds in moist and decaying materials such as spilled feeds and in all kinds of manure (Loftin et al., 2014). House flies close association with rotting matter, garbage, and feces has long been connected to the potential for disease to spread (Holt et al., 2007). Over 100 pathogens have been identified with houseflies which are bacteria, viruses, fungi and parasites (protozoans and metazoans) (Zarrin et al., 2015).

2.3 *Escherichia coli*

Escherichia coli is a Gram-negative, facultative anaerobic, rod-shaped, coliform bacteria (Tenailon et al., 2010). Certain serogroups which are O1, O2 and O788 usually comprise Avian Pathogenic *Escherichia coli* (APEC) isolates. APEC is present in the intestinal microflora of healthy birds and most of the related diseases are secondary to environmental and host predisposing factors (Dho-moulin & Fairbrother, 1999).

Avian pathogenic *Escherichia coli* (APEC) is an aetiological agent of avian syndromic disease, characterized by fibrinous collectively called colibacillosis around the visceral organs (Kemmett et al., 2014). In the first week of age, infections with bacteria, mainly *Escherichia coli*, accounted for approximately 50% of layer flock mortality (Olsen et al., 2012). Omphalitis and/or infections of the yolk sac were recorded, with or without septicaemia (Vandekerchove et al., 2004). Airsacculitis, cellulitis, pericarditis, perihepatitis and respiratory distress are among the most commonly associated signs of colibacillosis (Barnes, 1997).

2.4 *Salmonella* spp.

Salmonella spp. are facultatively anaerobic gram-negative rod-shaped bacteria belonging to the family Enterobacteriaceae (D'Aoust & Maurer, 2007). Salmonellosis is one of the biggest causes of bacterial enteric disease in animals (Brenner et al., 2000).

Salmonella gallinarum and *Salmonella pullorum* can cause fowl typhoid and pullorum disease respectively which are two distinct septicaemic diseases largely specific to avian species. Fowl typhoid which is caused by *Salmonella gallinarum* is an acute and can be chronic septicaemic illness that usually attacks older chickens. However, Pullorum disease caused by *Salmonella pullorum* usually attacks younger chicks (Barrow & Freitas Neto, 2010).

Both morbidity and mortality are highly variable in chickens and are influenced by age, strain of bird, nutrition, flock management, concurrent diseases and route and dose of exposure. Chicks that hatched from *Salmonella* infected eggs will show signs of weakness, depression, anorexia, poor growth and soon death following 7-10 days after hatching (Shivaprasad & Barrow, 2008).

3.0 MATERIALS & METHODS

3.1 Method of sample collection

The sample (houseflies) will be collected at broiler farms in Johor. Five broiler farms will be chosen randomly. Six open broiler houses in each five broiler farms will be chosen in which houseflies' burden may vary. Five houseflies will be collected in each broiler house and will be considered as one pool sample. Two pool samples will be collected in each broiler house; one for *Salmonella spp.* isolation and one for *Escherichia coli* isolation. Houseflies will be collected by using a sterilized net and immediately transfer the flies using sterile forceps into sterile plastic (Choo et al., 2011).

3.2 Method of isolating microbes

Two sterile cotton swabs will be used to swab on the external body of each houseflies for *Salmonella spp.* and *Escherichia coli* isolation separately. Sterilize the external body part of the houseflies with 70% ethanol. Crushed the houseflies in sterile plastic bags. Two sterile cotton swabs will be used to swab the internal contents for *Salmonella spp.* and *Escherichia coli* isolation separately.

For *Salmonella spp.* isolation, swabs are placed in a bottle containing Buffered Peptone Water (Oxoid) and all bottles were incubated at 37°C for 24 hours for pre-enrichment. Each of the pre-enriched sample are then transferred into 10ml of Rappaport Vassiliadis (RV) enrichment broth (Oxoid) and will be incubated at 42°C for 24 hours. A loop of each enriched sample is then streaked onto Xylose-Lysine-Tergitol-4(XLT4) agar plate and Brilliant-Green Agar plate. Both plates will be incubated at 37°C for 24 hours.

For *Escherichia coli* isolation, swabs are streaked onto Coliform Chromogenic Agar and will be incubated at 37°C for 24 hours.

3.3 Method of identification of microbes

The agars are observed for colony growth. *Salmonella spp.* will produce red colonies with black precipitate on XLD agar and white colonies with pinkish colouration of the agar on Brilliant-Green agar. *Escherichia coli* will produce dark blue to violet colonies on CCA agar.

Biochemical tests will be performed on suspected colonies. Biochemical test for identifying *Escherichia coli* is indole test. Biochemical tests for identifying *Salmonella spp.* are triple sugar iron test (TSI), lysine decarboxylase test (LIA) and sulfide, indole, motility test (SIM).

4.0 RESULTS

Six pools of housefly samples were collected from each of the five broiler farms, thus a total of 30 housefly pooled samples were examined. The pooled samples were further divided into two part samples; external surface and internal content. Thus, a total of 60 samples were examined.

The end results of isolation of *Escherichia coli* from identification method using KOVAC's reagent test are shown in Table 1 and Table 2. 27 out of 60 samples (45%) were positive of *Escherichia coli* in which 13 was from the external surface and 14 from internal content. 21 out of 30 pooled samples (70%) were positive of *Escherichia coli* in which five are from Farm A, three are from Farm B, four are from Farm C, five are from Farm D, and four are from Farm E.

The end results of isolation of *Salmonella spp.* was from identification method using serotype method. Out of 60 samples, none were positive of *Salmonella spp.*

	External surface						Internal content					
	P1	P2	P3	P4	P5	P6	P1	P2	P3	P4	P5	P6
Far m A	+	+					+	+	+	+		+
Far m B		+	+			+						+
Far m C		+	+	+	+					+		
Far m D	+	+				+		+	+	+		
Far m E	+						+	+		+		+

Table 1: Positive isolation of *Escherichia coli* among sample pools in all five farms

5.0 DISCUSSION

The present studies found that *Escherichia coli* are isolated from the houseflies' external surface and internal content. In another study, they isolated extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* from houseflies in two of their selected broiler farms (Blaak et. al., 2014). The article supports the validity of the results that *Escherichia coli* can be obtained from the houseflies samples.

The result shows that *Escherichia coli* is isolated from the external surface of the houseflies by 43 percent of the samples. This could be due to the fact that houseflies are known to have close relationships with places where there are abundance of *Escherichia coli* such as manure. Houseflies can transmit pathogens by contact with contaminated legs or mouth parts when exposed in a brief time to the contaminated environment (Antonio et al., 2004). Houseflies are well-known for their ability to become the reservoir and vectors for pathogens that relate to humans and animals due to the fact that house flies came in contact with animal manure (Sola Gines et. al., 2015). This supports that close interaction of houseflies with manure can be the source of transmission of *Escherichia coli*.

Escherichia coli was isolated from the internal content of the houseflies could be due to normal flora or even ingestion of the *Escherichia coli* by the housefly. Housefly larvae will ingest *Escherichia coli* and digest it as a food source for development (Rochon et al., 2004). Housefly may not be simple mechanical vectors of *Escherichia coli* and that this pathogen likely multiplies in the gastrointestinal tract (Kobayashi et al., 1999). The nutrient uptake by the housefly when ingesting *Escherichia coli* and also the ability of the *Escherichia coli* to

multiplies in the gastrointestinal tract of the housefly dictates that the potential of housefly to transmit *Escherichia coli* from the internal content.

However, there are no *Salmonella spp.* that have been isolated from the houseflies. Meanwhile, there are several studies found that *Salmonella spp.* can be isolated from the housefly. 4 out of 22 pool of houseflies samples were tested positive for *Salmonella spp.* (Olsen & Hammack, 2000). Moreover, there is a study found that *Salmonella spp.* fed to houseflies will reside and multiply in the fly gut (Holt et al., 2007).

There are no positive results of *Salmonella spp.* isolation may be due to successful disinfecting regime. This broiler farm company practices a very good disinfecting regime by using phenol based disinfectant plus Quaternary Ammonium Compound plus Glutaraldehyde (VIRUCID). The disinfecting regime is supported by Mueller Doblies, 2010 which state that disinfectants containing a mixture of formaldehyde, glutaraldehyde and Quaternary Ammonium Compound perform significantly better and should therefore be the first choice for disinfection of poultry premises where *Salmonella spp.* is present.

Housefly can potentially transmit *Salmonella spp.* and *Escherichia coli* in poultry farms and reducing the number of housefly burden may be a key step to eliminate the transmission of the pathogens through houseflies. The farms are using an insecticide containing Thiamethoxam to control the number of houseflies. Thiamethoxam which is a neonicotinoid insecticides, are compounds acting agonistically on insect nicotinic acetylcholine receptors (nAChR) (Nauen et al., 2003). This statement supports the appropriate use of the insecticides.

However, one study has provided a better solution for reducing the number of houseflies. The cultural plus biological plus chemical control is the most appropriate strategy

for successfully reducing housefly populations to low levels and maintaining them at low levels over time (Crespo et al., 1998).



CONCLUSION

Housefly (*Musca domestica*) can be a potential vector for *Escherichia coli* because of the close interaction with the manure and the ability for the bacteria to multiply in the houseflies in the earliest stage (larvae). However, this study cannot prove that housefly can be a potential vector for *Salmonella spp.* because there is a high chance of *Salmonella spp.* absence due to the good quality disinfectant regime done by the poultry farm against *Salmonella spp.*

RECOMMENDATION

This study can be further research in terms of finding the antimicrobial resistance of the microbes isolated. This can further discuss on the public health section.

We recommend to continue on finding the relationship between the effectiveness of various disinfectants and the specific bacteria in experimental environment.

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