



UNIVERSITI PUTRA MALAYSIA

***CARRIAGE OF Salmonella spp. and Escherichia coli BY HOUSE
FLIES AT THREE EATERIES AROUND UNIVERSITI PUTRA
MALAYSIA (UPM) AND THREE POULTRY FARMS IN SELANGOR***

E ALVWINA JULIAN

FPV 2017 1

**CARRIAGE OF *Salmonella* spp. and *Escherichia coli* BY HOUSE
FLIES AT THREE EATERIES AROUND UNIVERSITI PUTRA
MALAYSIA (UPM) AND THREE POULTRY FARMS IN
SELANGOR**

E ALVWINA JULIAN

**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
Universiti Putra Malaysia Serdang, Selangor Darul Ehsan**

March 2017

CERTIFICATION

It is hereby certified that we have read this project paper entitled “Carriage of *Salmonella* Spp. And *Escherichia coli* by House Flies At Three Eateries around University Putra Malaysia (UPM) and Three Poultry Farms in Selangor”, by E Alvwina Julian and in our opinion it is satisfactory in terms of scope, quality and presentation as partial fulfillment of the requirement for the course VPD 4990 – Final Year Project.

DR NUR INDAH BT AHMAD

DVM (UPM), MVSC (Utrecht), PhD (Edinburgh)

Lecturer

Faculty of Veterinary Medicine

University Putra Malaysia

(Supervisor)

PROF. DR. SALEHA BT ABDUL AZIZ

DVM (UGM), Grad. Dip. Vet. Preventive Medicine (Guelph), PhD (UPM)

Lecturer

Faculty of Veterinary Medicine

University Putra Malaysia

(Co-supervisor)

ACKNOWLEDGEMENTS



I would like to express my utmost gratitude to my family for their love and support throughout my study and for giving me endless supports while conducting this project.

A million thanks to my supervisor, Dr Nur Indah Ahmad and my co-supervisor, Prof. Dr. Saleha Abdul Aziz for their kind guidance throughout the completion of this project.

Sincerest gratitude to Madam Fauziah Nordin and Dr. Saleh Jajere of Public Health Laboratory for their kindest guidance throughout the completion of my project.

Warmest gratitude to Miss Krishnammah Kuppusamy, Miss Rabiatuladawiyah Rosli and Mister Mohd. Azri Roslan of Bacteriology Laboratory for their generous help during my project.

I would also like to extend my gratitude to Mister Arrifin of Large Animal Ward for driving us for sampling at the poultry farms. Also, to all FYP-classmates in Bacteriology Lab for their advices and supports.

To my dearest roommate, Hazlini Shafie, thank you so much for your warm supports.

Lastly, to the awesome rotamates of Group 6, DVM 2017 and all lecturers and staffs of Faculty of Veterinary Medicine.

Thank you.

CONTENTS

TITLE	ii
CERTIFICATION	iii
ACKNOWLEDGEMENTS	iv
LIST OF TABLES	vi
LIST OF PLATES	vii
ABSTRACT.....	viii
ABSTRAK.....	x
1.0 INTRODUCTION	1
2.0 LITERATURE REVIEWS	3
2.1 <i>Salmonella</i> spp.....	3
2.2 <i>Escherichia coli</i>	3
2.3 House flies (<i>Musca domestica</i>).....	4
2.4 Method of house flies sampling	4
2.5 Carriage of <i>Salmonella</i> spp. and <i>Escherichia coli</i> by house flies in Malaysia.	5
3.0 MATERIAL AND METHODS	6
3.1 Sampling Site	6
3.2 Sample.....	6
3.3 Isolation of <i>Salmonella</i> spp.....	6
3.4 Isolation of <i>Escherichia coli</i>	7
3.5 Antibiotic Susceptibility Test.....	8
4.0 RESULTS	14
5.0 DISCUSSION	17
6.0 CONCLUSION AND RECOMMENDATIONS.....	22
7.0 REFERENCES	23

LIST OF TABLES

Table 1:	Carriage of <i>Salmonella</i> spp. by House Flies at Three Eateries in Sri Serdang and Three Poultry Farm in Selangor	14
Table 2:	Carriage of <i>Escherichia coli</i> by House Flies at Three Eateries in Sri Serdang, Selangor	15
Table 3:	Carriage of <i>Escherichia coli</i> by House Flies at Three Poultry Farms in Selangor	15
Table 4:	Reference Range for Determining Antibiotic Susceptibility	25
Table 5:	Antibiotic Susceptibility Test (AST) of samples which are tested positive for <i>Salmonella</i> spp.	26
Table 6:	Antibiotic Susceptibility Test (AST) for <i>Escherichia coli</i> isolates from Sri Serdang	27
Table 7:	Antibiotic Susceptibility Test (AST) for <i>Escherichia coli</i> isolates from Padang	28
Table 8:	Antibiotic Susceptibility Test (AST) for <i>Escherichia coli</i> isolates from Tasik	29
Table 9:	Antibiotic Susceptibility Test (AST) for <i>Escherichia coli</i> isolates from Banting	30
Table 10:	Antibiotic Susceptibility Test (AST) for <i>Escherichia coli</i> isolates from Jenderam 1	31
Table 11:	Antibiotic Susceptibility Test (AST) for <i>Escherichia coli</i> isolates from Jenderam 2	32

LIST OF PLATES

Plate 1:	10ml of Buffered Peptone Water (BPW) in a Bijou bottle	9
Plate 2:	10ml of Rappaport Vasiliadis (RV) broth in Bijou bottle before incubation	9
Plate 3:	10ml of Rappaport Vasiliadis (RV) broth in Bijou bottle after incubation	9
Plate 4:	Inoculum from Rappaport Vasiliadis (RV) broth was cultured onto Xlyose Lysine Deoxycholate XLD agar.	10
Plate 5:	Suspected white-colored <i>Salmonella</i> spp. colonies with black centers	10
Plate 6:	Triple Sugar Iron (TSI)-Left & Lysine Iron Agar (LIA)-Right	11
Plate 7:	Serum Agglutination Test (SAT) using Polyvalent 'O' antisera	11
Plate 8:	Dark blue to violet colonies of <i>Escherichia coli</i> on Chromocult agar	12
Plate 9	Pure Culture of <i>Escherichia coli</i> on nutrient agar	12
Plate 10:	0.5 McFarland turbidity standards	13
Plate 11:	Zone of Inhibition (ZOI) of antibiotics on Muller Hinton agar	13

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfillment of the course VPD 4999 – Final Year Project.

**CARRIAGE OF *Salmonella* spp. AND *Escherichia coli* BY HOUSE FLIES AT
THREE EATERIES AROUND UNIVERSITY PUTRA MALAYSIA (UPM)
AND THREE POULTRY FARMS IN SELANGOR**

By

E Alwina Julian

2017

Supervisor: Dr. Nur Indah Ahmad

Co-supervisor: Prof. Dr. Saleha Abdul Aziz

House flies are of public health concern for their high potential to be carriers of communicable diseases to both human and animals. There is an urgency to explore if the house flies can carry disease-causing bacteria in their gastrointestinal tract that can greatly amplify the risk of human and animal exposure to diseases. The aim of the present study is to study the carriage of *Salmonella* spp. and *Escherichia coli* by house flies collected from three local eateries that are frequented by students and staffs of University Putra Malaysia (UPM) and from another three poultry farms in Selangor. This study also aims to investigate the evidence of antibiotic resistance of

the isolated bacteria. A total of 60 samples of house flies were caught using sticky papers from three local eateries around UPM and three poultry farms in Selangor. The finding reveals only 3.33% of the total 60 house flies samples collected from both eateries and poultry were confirmed positive for *Salmonella* spp. The positive samples were shown to exhibit antibiotic resistance towards Amoxicillin. This study also revealed that 56.67% of the total 60 house flies samples caught from the three local eateries around UPM and three poultry farms in Selangor carry *Escherichia coli*. Most of the *Escherichia coli* isolates were found to be resistant to Amoxicillin, Streptomycin and Ceftriaxone. House flies are evidently proven to be able to carry both *Salmonella* spp. and *Escherichia coli* and these bacteria do potentially carry antibiotic resistance gene based on the resistant patterns exhibited.

Keywords: house fly, *Salmonella* spp., *Escherichia coli*, antibiotic resistance

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 – Projek Ilmiah Tahun Akhir Pelajar

**PENGANGKUTAN *Salmonella* spp. DAN *Escherichia coli* OLEH LALAT DI
TIGA KEDAI MAKAN DI SEKITAR UNIVERSITY PUTRA MALAYSIA
(UPM) DAN TIGA LADANG AYAM DI SELANGOR**

Oleh

E Alwina Julian

2017

Penyelia: Dr. Nur Indah Ahmad

Penyelia bersama: Prof. Dr. Saleha Abdul Aziz

Lalat rumah menimbulkan kerisauan terhadap kesihatan awan kerana potensinya yang tinggi sebagai pembawa pelbagai penyakit terhadap manusia dan juga haiwan. Oleh sebab itu, terdapat keperluan untuk menyelidiki sama ada lalat rumah mampu mengangkut patogen di dalam salur pencernaannya. Kemampuan seumpama ini mampu meningkatkan risiko penyebaran penyakit-penyakit bawaan lalat rumah kepada manusia dan haiwan yang sering kali terdedah kepada lalat rumah. Tujuan kajian ini adalah untuk menyelidiki sama ada terdapat pembawaan *Salmonella* spp.

dan *Escherichia coli* oleh lalat rumah di kawasan-kawasan kajian iaitu tiga gerai makan di sekitar Universiti Putra Malaysia dan tiga ladang ayam di sekitar Selangor. Kajian ini juga bertujuan untuk menyiasat sama ada bakteria-bakteria tersebut memperlihatkan bukti adanya daya tahan terhadap antibiotik. Sebanyak 60 sampel terkumpul lalat rumah ditangkap menggunakan kertas lekit di kawasan-kawasan kajian. Sebanyak 3.33% daripada 60 sample tersebut membuktikan terdapat pembawaan *Salmonella* spp. oleh lalat rumah. Sampel-sampel yang positif itu turut memperlihatkan bukti terdapatnya tanda-tanda daya tahan terhadap antibiotik Amoxicillin. Sebanyak 56.67% daripada 60 sampel yang sama turut membuktikan bahawa terdapat pembawaan *Escherichia coli* oleh lalat rumah di kawasan-kawasan kajian. Sampel-sampel positif-*Escherichia coli* juga menunjukkan terdapatnya bukti daya tahan terhadap tiga jenis antibiotik yang berbeza iaitu Amoxicillin, Streptomycin dan juga Ceftriaxone. Lalat rumah sememangnya terbukti mampu membawa *Salmonella* spp. dan *Escherichia coli* dan bakteria-bakteria ini menunjukkan bukti-bukti daya tahan terhadap beberapa jenis antibiotik.

Kata kunci: lalat rumah, *Salmonella* spp., *Escherichia coli*, daya tahan antibiotic

1.0 INTRODUCTION

House fly is a well-known pest capable of transmitting numbers of diseases to both humans and animals (Keiding, 1986). House flies are non-biting arthropods that usually breed in animals manure during warm day. Thus, they can easily be found around the world. Studies conducted by Bouamama in Tangier, Morocco in 2010 revealed successful isolation of various bacteria including *Escherichia coli* from the external body of the house flies. However, among all the houseflies caught, only one sample was confirmed positive for *Salmonella* spp. This indicates low carriage of *Salmonella* spp. by house flies in Tangier, Morocco during the sampling period.

Similar studies aimed to investigate the species of bacteria harbored by house flies internally and/or externally were conducted in different countries; India (Gupta *et al.*, 2011), Iran (Kassiri *et al.*, 2012) and Iraq (Ahmed *et al.*, 2012) revealed that *Escherichia coli* were among the commonly isolated bacteria from external and/or internal of the house flies' body. However, there was no *Salmonella* spp. was isolated from either study. This suggested low occurrence of *Salmonella* spp. at all the sampling sites from the mentioned studies. However, these findings should not be overlooked as house flies can still potentially transmit *Salmonella* spp.

Based on the data acquire from the Ministry of Health Malaysia, from the year 2010 to 2015, among all the listed food borne diseases such as cholera, dysentery, food poisoning, typhoid and Hepatitis A; food poisoning was recorded having the highest incidence rate of more than 44% . This data showed that there were high numbers of food poisoning cases every year due to various causes. Hence, this study was designed to study the carriage of *Salmonella* spp. and *Escherichia coli* by house flies

at three local eateries which were frequented by students and staff of University Putra Malaysia (UPM) and three poultry farms in Selangor. In addition, this study also aimed to investigate whether the positive isolates of *Salmonella* spp. and *Escherichia coli* exhibit resistance toward several selected antibiotics.



7.0 REFERENCES

1. Ahmadu, Y. M., Goselle, O. N., & Ejimadu, L. C. Microhabitats and Pathogens of Houseflies (*Musca domestica*). *Public Health Concern. Electronic J Biol*, 12(4).
2. Barin, A., Arabkhazaeli, F., Rahbari, S., & Madani, S. A. (2010). The housefly, *Musca domestica*, as a possible mechanical vector of Newcastle disease virus in the laboratory and field. *Medical and veterinary entomology*, 24(1), 88-90.
3. Bouamama, L., Sorlozano, A., Laglaoui, A., Lebbadi, M., Aarab, A., & Gutierrez, J. (2010). Antibiotic resistance patterns of bacterial strains isolated from *Periplaneta americana* and *Musca domestica* in Tangier, Morocco. *The Journal of Infection in Developing Countries*, 4(04), 194-201.
4. Choo, L. C., Saleha, A. A., Wai, S. S., & Fauziah, N. (2011). Isolation of *Campylobacter* and *Salmonella* from houseflies (*Musca domestica*) in a university campus and a poultry farm in Selangor, Malaysia. *Tropical biomedicine*, 28(1), 16-20.
5. Gupta, A. K., Nayduch, D., Verma, P., Shah, B., Ghate, H. V., Patole, M. S., & Shouche, Y. S. (2012). Phylogenetic characterization of bacteria in the gut of house flies (*Musca domestica* L.). *FEMS microbiology ecology*, 79(3), 581-593.
6. Kassiri, H., Akbarzadeh, K., & Ghaderi, A. (2012). Isolation of pathogenic bacteria on the house fly, *Musca domestica* L.(Diptera: Muscidae), body surface in Ahwaz hospitals, Southwestern Iran. *Asian Pacific Journal of Tropical Biomedicine*, 2(2), S1116-S1119.

7. Keiding J. The housefly—biology and control. Training and information guide (advanced level). Geneva, World Health Organization, 1986 (unpublished document WHO/VBC/ 86.937; available on request from Division of Control of Tropical Diseases, World Health Organization, 1211 Geneva 27, Switzerland).
8. Macovei, L., & Zurek, L. (2006). Ecology of antibiotic resistance genes: characterization of enterococci from houseflies collected in food settings. *Applied and Environmental Microbiology*, 72(6), 4028-4035.
9. Nazni, W. A., Seleena, B., Lee, H. L., Jeffery, J., Rogayah, T. A. R., & Sofian, M. A. (2005). Bacteria fauna from the house fly, *Musca domestica* (L.). *Tropical Biomedicine*, 22(2), 225-231.
10. Tauxe, R. V. (2002). Emerging foodborne pathogens. *International journal of food microbiology*, 78(1), 31-41.
11. Ulupi, N., Sumantri, C., & Wibawan, I. W. T. (2013). Association of TLR4 gene genotype and resistance against *Salmonella enteritidis* natural infection in Kampung chicken. *International Journal of Poultry Science*, 12(8), 445.