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DARKLING BEETLE AS VECTOR FOR *SALMONELLA SPP*. AND *ESCHERICHIA COLI* IN OPEN HOUSE BROILER CHICKEN FARMS

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DARKLING BEETLE AS VECTOR FOR *SALMONELLA SPP.* AND *ESCHERICHIA COLI* IN OPEN HOUSE BROILER CHICKEN FARMS

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CERTIFICATION

It is hereby certified that we have read this project paper entitled "Darkling Beetle as Vector for *Salmonella spp.* and *Escherichia coli* in Open House Broiler Chicken Farms and the Effectiveness of Control Measures", by Nur Husnina Binti Abdul Wahab and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfillment of the requirement for the course VPD4999 - Final Year Project.

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DEDICATIONS

This project paper is dedicated to

My beloved family

My respectable lecturers

My friends

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Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD4999 - Projek Akhir Tahun

***DARKLING BEETLE* SEBAGAI VEKTOR UNTUK *SALMONELLA SPP.* DAN *ESCHERICHIA COLI* DALAM LADANG TERBUKA AYAM PEDAGING**

Oleh

NUR HUSNINA BINTI ABDUL WAHAB

2020

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Penyelia bersama: Prof Saleha Abdul Aziz

Kajian ini bertujuan untuk mengenal pasti kemungkinan *darkling beetle* sebagai vektor untuk *Salmonella spp.* dan *Escherichia coli* pada ayam pedaging. *Darkling beetle* (*Alphitobius diaperinus*) atau juga dikenali sebagai *lesser mealworm*, ialah salah satu perosak penting yang mempengaruhi pengeluaran unggas. Kumbang ini boleh menyebabkan kerosakan pada bahan penebat dinding rumah unggas, prestasi buruk kawanan anak ayam disebabkan oleh pengambilan kumbang dan juga kemungkinan kumbang sebagai vektor *Salmonella spp.* dan *Escherichia coli* kepada ayam pedaging. Sebanyak 30 sampel berkumpul diambil dari 5 ladang terbuka ayam pedaging dengan

6 sampel berkumpul dari setiap rumah. Pengasingan bakteria dari kumbang dilakukan dari dua lokasi, iaitu permukaan luaran dan kandungan dalaman kumbang, menjadikan jumlah sampel menjadi 60 sampel. Untuk pengasingan *Salmonella spp.*, sampel diinokulasi ke agar Brilliant Green Agar (BGA) dan agar Xylosine-Lysine-Deoxycolate (XLD). Untuk *Escherichia coli*, sampel diinokulasi ke agar Coliform Chromogenic Agar (CCA). Setelah pengasingan bakteria, pengenalan bakteria dilakukan. Daripada 60 sampel, *Salmonella spp.* tidak dapat diasingkan daripada sampel permukaan luaran, dan dapat diasingkan daripada hanya satu sampel isi dalaman, yang terdiri dari 1.7% daripada jumlah sampel. Untuk *Escherichia coli*, ia diasingkan dari dua sampel permukaan luaran dan lapan sampel kandungan dalaman, yang merangkumi 16.7% daripada jumlah sampel. Hasil ini menunjukkan bahawa ada kemungkinan rendah untuk *darkling beetle* menjadi vektor untuk *Salmonella spp.*, dan kemungkinan yang agak rendah untuk *darkling beetle* menjadi vektor untuk *Escherichia coli*.

Kata kunci: *darkling beetle*, *Salmonella spp.*, *Escherichia coli*, ayam pedaging, ladang terbuka

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD 4999 - Final Year Project

DARKLING BEETLE AS VECTOR FOR *SALMONELLA SPP.* AND *ESCHERICHIA COLI* IN OPEN HOUSE BROILER CHICKEN FARMS

By

NUR HUSNINA BINTI ABDUL WAHAB

2020

Supervisor: Assoc. Prof. Dr Lokman Hakim Idris

Co-supervisor: Prof Saleha Abdul Aziz

This study is aimed at identifying the possibility of darkling beetle as vectors for *Salmonella spp.* and *Escherichia coli* in broiler chickens. Darkling beetle (*Alphitobius diaperinus*) or also known as lesser mealworm, is one of a significant pests that affects poultry production. The beetle can cause damage to the insulating materials of walls in poultry houses, poor flock performance of the chicks caused by ingestion of the beetles and possibility of serving as a vector for *Salmonella spp.* and *Escherichia coli* to broiler chickens. A total of 30 pooled samples were taken from 5 broiler open houses with 6 pooled samples from each house. Isolation of bacteria from the beetles was done from two sites, the external surface and internal content of the beetles, making the total of

samples to be 60 samples. For isolation of *Salmonella spp.*, the samples were inoculated onto Brilliant Green Agar (BGA) and Xylosine-Lysine-Deoxycolate (XLD) agar. For *Escherichia coli*, the samples were inoculated onto Coliform Chromogenic Agar (CCA). After isolation of bacteria, identification of bacteria was done. Out of 60 samples, *Salmonella spp.*, was isolated from none of external surface samples, and only one from the internal content samples, which made up 1.7% of total sample. For *Escherichia coli*, it was isolated from two external surface samples and eight internal content samples, which made up 16.7% of total sample. The results demonstrated that there is low possibility for darkling beetle to be a vector for *Salmonella spp.*, and rather low possibility for darkling beetle to be a vector for *Escherichia coli*.

Keywords: darkling beetle, *Salmonella spp.*, *Escherichia coli*, broiler chicken, open house

1.0 INTRODUCTION

Darkling beetle (*Alphitobius diaperinus*) or also known as lesser mealworm is one of the significant pests that affects poultry production especially in open house system. It can commonly be found in the cracks of poultry houses (Figure 1), under feed and water containers, near dried litter (Figure 2) and occasionally on the chickens.

Problems that are commonly associated with darkling beetles are damaged to the insulation materials of the walls and ceilings of poultry houses caused by larvae of darkling beetle (Hazeleger et al., 2008) and ingestion of darkling beetles by chicks, causing poor growth performance (Despins and Axtell, 1995). However, the major concern of infestation of darkling beetle in poultry farms is its potential as vector of various organisms. Droppings from the chickens and warm nature of the litter provide a suitable environment for the growth of a lot of pathogens, especially enteric bacteria.

This study focuses on the potential of darkling beetle as the vector for *Salmonella spp*. and *Escherichia coli*. *Salmonella spp*. And *Escherichia coli* are both gram negative bacteria and they belong to Enterobacteriaciae family. Although *Salmonella spp.* does not generally produce clinical manifestation in infected chickens, it can cause food borne disease to human upon consuming the meat of infected chickens. *Escherichia coli* on the other hand, can both potentially cause disease in chickens as well as food borne disease from ingesting contaminated meat (Hazeleger et al., 2008). This concludes that the presence of pathogens in the meat would cause the meat of affected chickens to be not safe to be consumed.

Since darkling beetle is the potential source of pathogens, control measures are normally practiced in the farm to reduce or eliminate the insects and pathogens from poultry houses. This study aims to investigate the presence of darkling beetle in broiler chicken open house system, to assess the potential of darkling beetle as a vector for *Salmonella spp.* and *Escherichia coli* and the effectiveness of control measures in the farm.

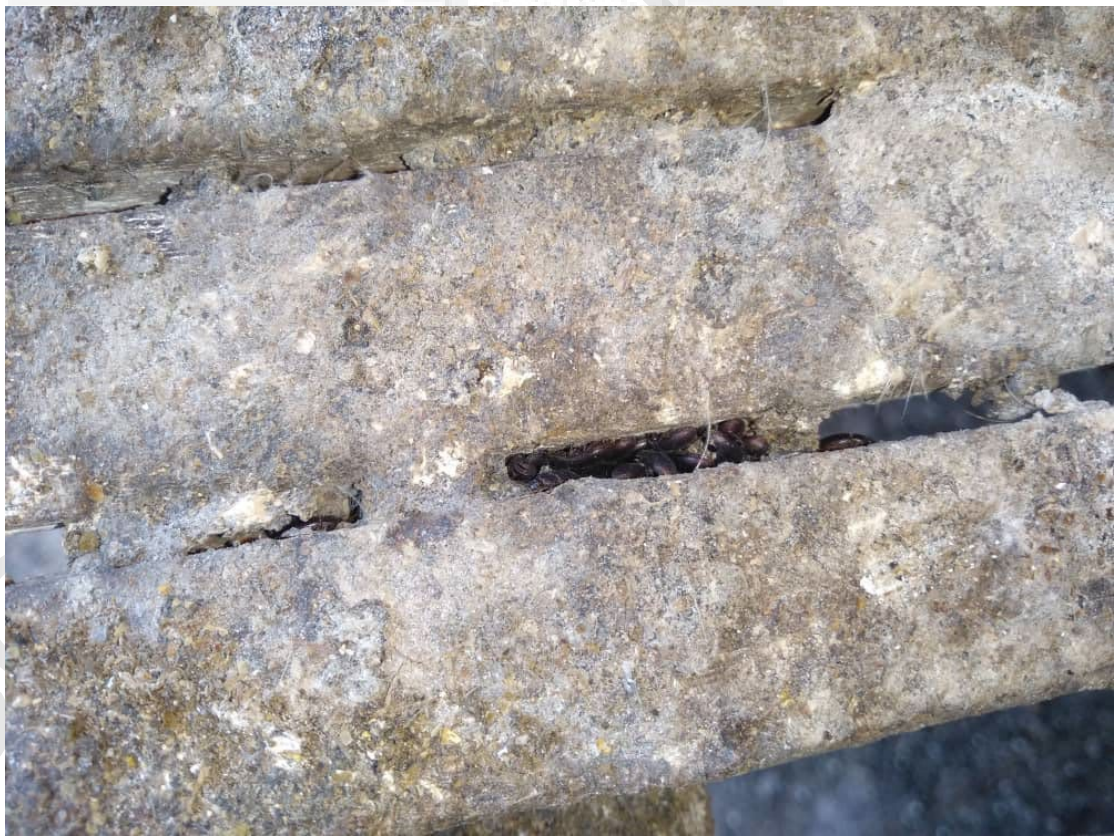

Figure 1: Darkling beetles in cracks of poultry house

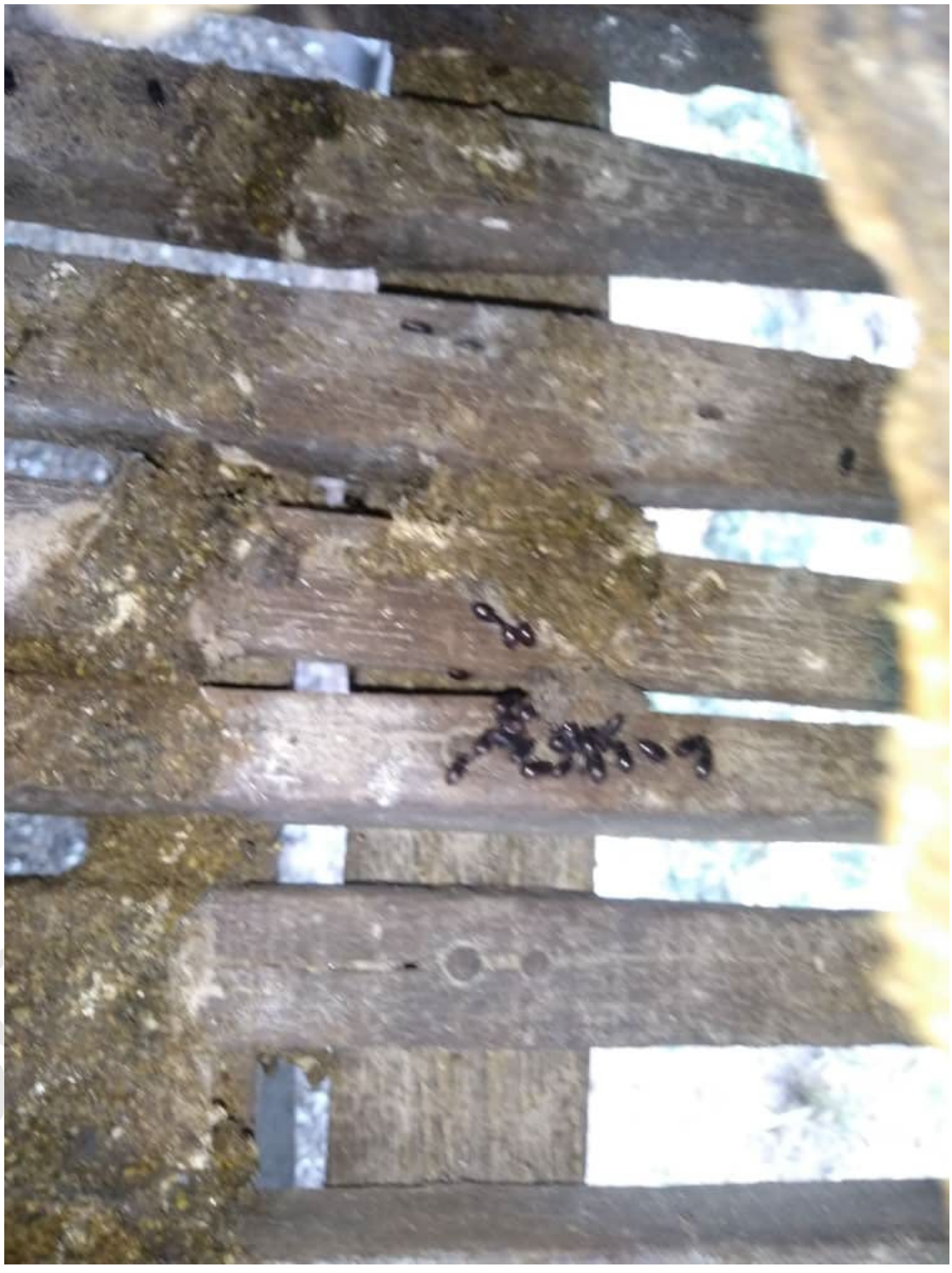

Figure 2: Darkling beetles near dried litter

2.0 LITERATURE REVIEW

2.1 Darkling beetle

Darkling beetle is found in a large population in broiler houses (McAllister et al., 1996). This beetle can be found in places where grain is stored and in poultry houses, typically in micro-habitats characterized by low relative humidity and high food availability (Salin et al., 1998) Darkling beetles scavenge for feed on manure and debris, from chicken carcasses, eggs that cracked, spilled chicken feed and maggots (Crippen & Sheffield, 2006)

The larvae and adults darkling beetle can be found in clusters in poultry litter and accumulating at localized points, for example on the ceilings of poultry houses,, under the feeders and also in nests. This distribution and fluctuations in the abundance of various developmental stages mean that fewer mealworms are moving based on local temperature to suitable locations, humidity, the presence of food and shelter, periods of substrate removal and application of insecticides (Wolf et al., 2015).

It takes about 4 to 7 days for the larvae to hatch while the development into mature adults takes about 40 to 100 days and is depending on various aspects such as ambient temperature and available quality food. The development is optimum at 30-33 degree Celsius and 90% air humidity (Dinev, 20123)

2.2 Significance of darkling beetle in poultry production

In poultry production, the presence of the beetles facilitates behavioral changes in chickens, such as scratching. The lesser mealworm may also be eaten by the chickens, causing carcass injuries, as well as causing an indirect and detrimental influence on the broiler feed intake and feed conversion. (Panzardi et al., 2019). Ingesting darkling beetles larvae in large amount can cause poor weight gain and even mortality in chicks (Despins & Axtell, 1995). Ingestion of the beetles by the chicks causing the reduce in intake of the nutritionally balanced feed, thus resulting poor weight gain (Hickman et al., 2018). By spreading beetle-containing manure on surrounding fields, the beetles are inadvertently spread to neighboring residences (Crippen & Sheffield, 2006).

These beetles are known as mechanical vectors of various diseases such avian influenza, Marek's disease, Newcastle disease, bacterial diseases, fowl pox, coccidiosis due to their mobility, feeding habits, and prey potential (Crippen & Sheffield, 2006). Occasionally, darkling beetles feed on the flesh internal organs of dying and dead chicks where during this feeding process, they might get contaminated with avian leukosis virus (De las casas et al., 1968). Darkling beetles have high rate of reproduction and are hard to control, hence it is one of the reasons why they are thought to be the reservoir for the transmission of pathogens to the chickens in poultry houses (Crippen & Sheffield, 2006). The transmission of pathogens occurs when healthy chickens consume darkling beetles that are contaminated with the pathogens (De las casas et al., 1968)

Other than that, darkling beetles can also create economical problem where it can cause damage to the thermal insulation materials resulting from the migration of mature larvae in search for isolation pupation sites, especially when the population number is high in the house (Geden & Carlson, 2001). Beside than the larvae migration, damage of the thermal insulation material are also caused by the emerging of adults from pupae stage (Tomberlin et al., 2008)

2.3 Salmonellosis in chickens

The major causative agents of pullorum disease and fowl typhoid in poultry are *Salmonella enterica* subspecies *enterica*, with three different serovars which are *Salmonella pullorum* and *Salmonella gallinarum* (Desin et al., 2013). The chickens infected by these two serovars will have systemic disease that is manifested by anemia, hepatosplenomegaly and sometimes intestinal tract haemorrhage that can result in high mortality (Desin et al., 2013).

The most common serovar that are isolated from chickens are *Salmonella enterica* serovar enteritidis and *Salmonella typhimurium* which are non host specific and can infect a lot of animals and also human (Desin et al., 2013). The chickens infected with this serovars usually will not show any symptoms or clinicals signs and will shed the bacteria in a long period without the bacteria producing systemic disease in the chickens, except in chicks (Desin et al., 2013). Due to the nature of intermittent shedding of the bacteria, it is unreliable to sample individual birds to determine whether the bird is carrying or shedding bacteria (Gaffga et al., 2012).

Transmission of salmonella in broiler chickens can occur through vertical transmission from parent flock to day old chicks, and can also occur through horizontal transmission between the chickens (Heyndrickx et al., 2002). Inadequate disinfection and cleaning of broiler houses between the flocks, contamination through feed and poor hygiene level in the houses are the risk factors that can result in horizontal transmission between the chickens. (Heyndrickx et al., 2002)

2.4 Colibacillosis in chickens

Escherichia coli is one of normal flora of intestines. Most of the serotypes of *Escherichia coli* are non pathogenic, however some serotypes can produce lesion outside of intestines (Merck Veterinary Manual). Although both avirulent and virulent type of *Escherichia coli* can localize in intestines and outside of intestines, however the latter localization can only occur when there is presence of stressors (Dziva and Stevenes, 2008). These are due to due host defense impairment which allows the pathogens to cause disease in chickens, and these pathogens are known as Avian Pathogenic *Escherichia coli* (APEC) (Sargeant et al., 2020).

Escherichia coli infection is a disease that cause great and significant economic impact in poultry production, and it can cause variety of lesions and signs in the chickens (Dziva and Stevens, 2008). *Escherichia coli* infection in poultry can be primary and secondary infection alongside with other viral or bacterial pathogens, where the diseases is called as colibacillosis (Sargeant et al., 2020). Both larvae and mature adult of darkling beetle can harbor this pathogen long enough to be able to infect the next batch of broiler chicken (McAllister et al., 1995)

Colibacillosis in chickens can produce common lesions that can be found in chickens such as perihepatitis, airsacculitis and pericarditis and sometimes can also produce lesions such as salpingitis, coligranuloma, omphalitis, cellulitis and arthritis that are less commonly seen (Dziva and Stevens, 2008). The acute form of colibacillosis in chickens is characterized by septicaemia resulting death while the subacute form of colibacillosis is characterized by pericarditis, perihepatitis and airsacculitis (Kabir, 2010).

3.0 MATERIALS AND METHODS

3.1 Collection of Darkling Beetle

The collection of darkling beetle was done in chicken farms in Johor. 5 broiler farms were chosen for the collection. The darkling beetles are collected as pooled samples with 5 darkling beetles in one pooled sample. From each farm, 6 pooled samples were collected with one pooled sample from one house. There were a total of 30 pooled samples from all 5 farms. The darkling beetles were found most in between the cracks of wood of the houses, and some under or on feed and water containers. The darkling beetles were collected using sterile forceps and then were kept in sterile ziplock bags. Then the bags were kept in chilled containers to keep the bacteria viable during transportation.

3.2 Isolation of Bacteria from Darkling Beetle

Isolation of the bacteria from the darkling beetles were done on the pooled samples. From each pooled sample, the isolation was done from two sites, the surface of darkling beetles and the internal content of the darkling beetles. For isolation of bacteria from the surface of darkling beetles, a cotton swab was used to isolate the bacteria by swabbing it on all the beetles from one pooled sample. The swabbing was done thoroughly by swabbing it on the body surface, ventral part of body and legs by letting the beetles walked on the cotton swab. For isolation of bacteria from the internal content, the surface of darkling beetles from one pooled sample were first

sterilized together by shaking the darkling beetles gently in ethanol for 3 minutes. After that, the darkling beetles were picked up and let dry on sterilized mortar. Then, the darkling beetles from one pooled sample that was sterilized was crushed using the mortar. A cotton swab was then used to swab the crushed darkling beetles to obtain the internal content of the darkling beetles. For isolation of *Salmonella spp.*, both the surface and internal content sample on the cotton swab from each pool sample were inoculated in Buffered Peptone Water (BPW) first. The ratio of sample to BPW was 1 to 10. Then, the inoculated BPW were incubated in 37 degree Celsius for 24 hours. After incubation, 0.1ml of the inoculated BPW was inoculated into Rappaport-Vassiliadis (RV) broth by using the micropipette. The inoculated RV broths were then incubated at 42 degree Celsius for another 24 to 48 hours. After the incubation, a loop wire was used to inoculate RV broth onto Brilliant Green Agar (BGA) and Xylosine-Lysine-Deoxycholate (XLD) agar for the isolation of *Salmonella spp.* Both of the plates were isolated at 36 degree Celsius for 24 hours. For isolation of *Escherichia coli*, both the surface and internal content sample from the cotton swab were inoculated onto Coliform Chromogenic Agar (CCA) and then were incubated at 36 degree Celsius for 24 hours.

3.3 Identification of Bacteria

For the identification of bacteria, the colonies formed of the agar were first observed. For *Salmonella spp.*, it will form pinkish white colonies with red halos on BGA and red colonies with black precipitate on XLD agar. For *Escherichia*

coli, it will form violet to dark blue colonies on CCA. After that, subculture was done on the suspected colonies. A loop wire was used to take a little of the suspected colonies and was inoculated on nutrient agar to get subculture of the bacteria colonies. Then, biochemical tests were performed on suspected colonies from the nutrient agar. For *Salmonella spp.*, the biochemical tests used to identify *Salmonella spp*., were Triple Sugar Iron (TSI) test, Lysine Decarboxylase (LIA) test and motility test (SIM agar). For *Escherichia coli*, a positive indole test was used to identify the bacteria. One drop of COVAC reagent was dropped on the suspected colonies. After a few minutes, if the suspected colonies turned pink, it indicated the indole test result is positive

4.0 RESULTS

There were a total of 5 farms with 6 pooled samples taken from each farm, totaling up to 30 pooled samples. The samples were further divided into two parts, external surface and internal content, where the isolation of bacteria were done, making the total of samples to be 60 samples.

Farm	External content	Internal content
A	0	2
B	0	1
C	1	1
D	1	2
E	0	2
Total	2	8

Table 1: Number of samples where *Escherichia coli* was isolated from

From Table 1, it is shown that *Escherichia coli* was isolated from none of external surface samples, and 2 from internal content samples from Farm A, none of external surface samples and 1 internal content sample from Farm B, 1 external surface sample and 1 internal content sample from Farm C, 1 external surface sample and 2 internal content samples from Farm D and none from external surface sample and 2 internal content samples from Farm E. There were a total of 2 external surface samples and 8 internal content samples where *Escherichia coli* was isolated from. The

total of samples for *Escherichia coli* isolated from both external surface and internal content are 10 samples out of 60 samples, which accounts about 16.7% out of total samples.

Farm	External content	Internal content
A	0	0
B	0	0
C	0	0
D	0	1
E	0	0
Total	0	1

Table 2: Number of samples where *Salmonella spp.* was isolated from

From Table 2, it is shown that *Salmonella spp*. was isolated from none of both external surface samples and internal content samples from Farm A, none of both external surface samples and internal content sample from Farm B, none of both external surface sample and internal content sample from Farm C, none of external surface sample and 1 internal content sample from Farm D and none of both external surface sample and internal content samples from Farm E. There were a total of 0 external surface samples and only 1 internal content samples where *Salmonella spp.* was isolated from. The total of samples for *Salmonella spp.* isolated from both

external surface and internal content are only 1 sample out of 60 samples, which accounts about 1.7% out of total samples.

5.0 DISCUSSION

Darkling beetle can be commonly found in poultry houses. It can cause a variety of problems, from damage to the insulation and walls in poultry houses, to poor growth performance in chicks. According to Goodwin, M. A. and Waltman, W. D. (1996), it can also carry pathogens such as *Salmonella*, *Escherichia coli*, *Aspergillus*, *Birnavirus*, fowl poxvirus, *Paramyxovirus*, and also *Herpesvirus*. This ability can impose a great risk to the chickens as well as danger to human that are consuming the meat of affected chickens.

In this study, *Escherichia coli* was isolated from 10 out of 60 samples, which makes up about 16.7% of the total sample. However, there is no recent study to back up this data.

Only one out of 60 samples where *Salmonella spp.* is isolated from in this study, which is about 16.7% of the total sample. The low number of *Salmonella spp.* isolated from darkling beetle is probably due to Poultry Salmonella Control which is part National Salmonella Control and Eradication programme since 1999 by Department of Veterinary Service (DVS). However, according to OMAFRA website, by consuming just one darkling beetle contaminated with Salmonella can cause chickens to be infected with Salmonella.

From the results, it is concluded that darkling beetle has rather low possibility of carrying *Escherichia coli* and low possibility of carrying *Salmonella spp.* However, control measures still need to be taken to control the population of darkling beetle in farm due to the risk of infection of *Escherichia coli* and Salmonella in chickens. A study under laboratory condition was done by Jonatas Wolf et al. (2015) concluded that the combination of physical and chemical which included hydrated lime, humidity, temperature and insecticide treatment in poultry litter provided total control of darkling beetle 7 days after treatment.

Geden and Axtell (2001) mentioned that even though the beetles are susceptible to many insecticides and disinfectants, due to accumulation of dust on the treated area and surfaces, these ingredients are often not infective in killing the organisms. Other than that, according to Crippen and Sheffield (2006), the surface sterilization effectiveness is reduced due to beetle morphology which restricted the access to bacteria colonizing the beetles. This might be due to the integument of the beetles and also the fecal material covering the surface of the beetles that provide some kind of shelter for the bacteria. Furthermore, the presence of sulci or grooves on the beetles body may act as a shield for the bacteria during disinfecting programme of poultry houses.

CONCLUSION

Darkling beetle has rather low possibility of carrying *Escherichia coli* and low possibility of carrying *Salmonella spp* as a source of infection in broiler chickens. The low number of *Salmonella spp.* isolated from darkling beetle might be due to National Salmonella Control and Eradication Programme. However, control measures still need to be taken to control the population of darkling beetle in farm due to the risk of infection of *Escherichia coli* and *Salmonella spp.* in chickens.

RECOMMENDATIONS

For future studies, we highly recommend to include the study of antimicrobial sensitivity test on Salmonella spp. and *Escherichia coli* isolated from darkling beetles to know the susceptibility of these pathogens to the antibiotics.

Other than that, a study on transmission of *Salmonella spp.* and *Escherichia coli* to chickens through ingestion of contaminated darkling beetles. This can be done by manually inoculating the beetles with these pathogens and feed these beetles with the inoculated beetles.

Another recommendation for the study is to possibility of darkling beetles as a vector for other pathogens. Other pathogens that can potentially be transmitted by darkling beetles are reovirus, fowl pox virus, herpesvirus and *Campylobacter spp*.

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